Differential effects of mild central hypovolemia with furosemide administration vs. lower body suction on dynamic cerebral autoregulation

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Ogawa Y, Aoki K, Kato J, Iwasaki K. Differential effects of mild central hypovolemia with furosemide administration vs. lower body suction on dynamic cerebral autoregulation. J Appl Physiol 114: 211–216, 2013. First published November 29, 2012; doi:10.1152/japplphysiol.00741.2012.—Diuretic-induced mild hypovolemia with hemoconcentration reportedly improves dynamic cerebral autoregulation, whereas central hypovolemia without hemoconcentration induced by lower body negative pressure (LBNP) has no effect or impairs dynamic cerebral autoregulation. This discrepancy may be explained by different blood properties, by degrees of central hypovolemia, or both. We investigated the effects of equivalent central hypovolemia induced by furosemide administration or LBNP application on dynamic cerebral autoregulation to test our hypothesis that mild central hypovolemia due to furosemide administration enhances dynamic cerebral autoregulation in contrast to LBNP. Seven healthy male subjects received 0.4 mg/kg furosemide and LBNP, with equivalent decreases in central venous pressure (CVP). Dynamic cerebral autoregulation was assessed by spectral and transfer function analysis between beat-to-beat mean arterial blood pressure (MAP) and mean cerebral blood flow velocity (MCBFV). CVP decreased by ~3–4 mmHg with both furosemide administration (~26 mg) and LBNP (approximately ~20 mmHg). Steady state MCBFV remained unchanged with both techniques, whereas MAP increased significantly with furosemide administration. Coherence and transfer function gain in the low and high frequency ranges with hypovolemia due to furosemide administration were significantly lower than those due to LBNP (ANOVA interaction effects, \(P < 0.05\)), although transfer function gain in the very low frequency range did not change. Our results suggest that although the decreases in CVP were equivalent between furosemide administration and LBNP, the resultant central hypovolemia differentially affected dynamic cerebral autoregulation. Mild central hypovolemia with hemoconcentration resulting from furosemide administration may enhance dynamic cerebral autoregulation compared with LBNP.

hemocoherence; intravascular dehydration; lower body negative pressure; cerebral blood flow velocity; transfer function analysis

THE OCCURRENCE OF orthostatic hypotension, or presyncope, or both is usually indicative of central hypovolemia (2, 12, 14), which is caused by several conditions, such as dehydration, hemorrhage, or postural change. Many investigators in the field of pathophysiology have studied the effects of central hypovolemia on circulatory regulation in relation to orthostatic intolerance, or presyncope, or both (2, 12–14, 22, 26).

In previous studies, administration of diuretics (16), lower body negative pressure (LBNP) (15, 26), and head-up tilt (HUT) (20) have been used to simulate central hypovolemia. However, the effects of central hypovolemia on cerebral autoregulation are controversial (15, 16, 20, 26). Our previous study reported that mild hypovolemia due to furosemide administration improves cerebral autoregulation (16), whereas several previous studies reported that central hypovolemia by mild lower body suction does not alter cerebral autoregulation (15, 26). Moreover, the severe hypovolemia induced by ~50 mmHg lower body suction or 80° HUT that leads to presyncope is likely to impair cerebral autoregulation (20, 26). This discrepancy may be explained by different blood properties (viscosity, density of red blood cell, etc.), or by degrees of central hypovolemia induced by the various techniques, or both. Administration of diuretics induces a reduction in plasma volume (i.e., hemoconcentration). On the other hand, LBNP or HUT distributes blood to the lower body, simulating central hypovolemia without hemoconcentration. Thus hemoconcentration may be one of the key factors related to altered cerebral autoregulation during mild central hypovolemia. However, no studies have compared the effects of equivalent central hypovolemia resulting from furosemide administration and LBNP or HUT on cerebral autoregulation.

We therefore evaluated dynamic cerebral autoregulation during equivalent central hypovolemia induced by furosemide administration and LBNP to test our hypothesis that mild hypovolemia has different effects on dynamic cerebral autoregulation, depending on the presence or absence of hemoconcentration, and that mild central hypovolemia due to furosemide administration enhances dynamic cerebral autoregulation in contrast to LBNP.

METHODS

Subjects. The institutional review board of Nihon University School of Medicine (Itabashi-ku, Tokyo, Japan) approved this study. Procedures adhered to the tenets of the Declaration of Helsinki. All study participants provided written, informed consent and a medical history, and were screened via physical examination that included electrocardiography (ECG), and arterial blood pressure and cerebral blood flow (CBF) velocity measurements. Exclusion criteria included failure to obtain CBF velocity signals in the middle cerebral artery by transcranial Doppler ultrasonography. We investigated seven healthy, normotensive men with a mean age of 22 yr (range, 20–24 yr), a mean height of 173 cm (range, 164–179 cm), and a mean weight of 66 kg (range, 58–79 kg). Prior to the experiments, all subjects fasted for at least 2 h and refrained from heavy exercise and consumption of caffeinated or alcoholic beverages for at least 12 h.

Instrumentation. All experiments were performed in the morning. Subjects lay supine in a comfortable bed in an environmentally controlled experimental room at an ambient temperature of 23–25°C. ECG, pulse oximeter, and nasal cannula for monitoring end tidal carbon dioxide (ETCO2) (Life scope BSM-5132; Nihon Kohden, Tokyo, Japan) were applied. Arterial blood pressure was continuously measured in the radial artery using tonometry with a noninvasive arterial blood pressure monitor at the level of the heart on a beat-to-beat basis, and calibrated by intermittent arterial blood pressure measurement.
measured using the oscillometric method with a sphygmomanometer cuff placed over the brachial artery (JENTOW 7700; Colin, Aichi, Japan). CBF velocity in the middle cerebral artery was continuously measured by transcranial Doppler ultrasonography (WAKI: Atrys Medical, St. Genislaval, France). A 2-MHz probe was placed over the temporal window and fixed at a constant angle, with a customized probe holder with polymer made to fit individual facial bone structures and the ear (10) by an experienced technician. When careful attention is paid to probe placement, excellent reliability of CBF velocity measured by transcranial Doppler ultrasonography has been reported (1). A central catheter [First PICC catheter 18 gauge(4F) 1.35 mm × 65 cm, Becton Dickinson, Franklin Lakes, NJ] was peripherally inserted via an antecubital vein up to the level of the superior vena cava, for measurement of central venous pressure (CVP), for drug administration, and for blood collection. The catheter length was equal to the external distance from the antecubital fossa to the sternoclavicular joint plus an additional 3 cm. The catheter was connected to a pressure transducer (DX-312, Becton Dickinson) placed at the level of the heart. Each waveform of continuous arterial blood pressure, CBF velocity, ECG, and CVP was recorded at a sampling rate of 1 kHz using commercial software (Notocord-hem 3.3; Notocord, Paris, France) on a personal computer.

Protocols. On the first experimental day, baseline data for 6 min was measured after at least 30 min of quiet rest. Furosemide (0.4 mg/kg) was administered to reduce plasma volume. This dose of furosemide was determined to induce a ~10% reduction in plasma volume, on the basis of our previous studies (12, 19). When urorhesis thesis arose, subjects walked to the lavatory and urinated, after which the assorted monitors were reapplied. Subjects were allowed to urinate for, at most, up to 90 min following furosemide administration. Hypovolemia data for 6 min were measured after an additional 15 min of quiet rest in the supine position. Complete blood count, including hematocrit and hemoglobin concentration, was measured before and after administration of furosemide. The complete blood count during hypovolemia was obtained by averaging two values, one before and one after measurement of 6-min hypovolemia data.

At a minimum of 7 days after the furosemide experiment, the LBNP experiment was performed. LBNP that provided steady suction was used to induce central hypovolemia without hemococoncentration. The magnitude of the suction was adjusted to produce an equal reduction in CVP as that of the furosemide experiment. Thus equivalent reductions in central blood volume were defined by equal changes in CVP. After recording baseline data for 6 min after at least 30 min of rest, lower body suction was applied for 7 min and the last 6 min of data were collected for analysis. The LBNP was terminated if signs or symptoms of presyncope, such as nausea, sweating, light-headedness, bradycardia, or hypotension (sustained systolic blood pressure <80 mmHg) developed.

Data analysis. Six minutes of continuous arterial blood pressure and CBF velocity waveforms were used to estimate dynamic cerebral auto-regulation during spontaneous respiration of room air. Averaged values of steady state mean arterial blood pressure (MAP), mean CBF velocity (MCBFV), heart rate (HR), and CVP over 6 min were obtained. In addition, cerebrovascular resistance was expressed as cerebral vascular resistance index (CVRi), where CVRi = MAP/MCBFV.

Values of respiratory rate, arterial oxygen saturation (SpO2) and ETCO2 were manually recorded every minute. The values at seven time points (0, 1, 2, 3, 4, 5, and 6 min) during this period were averaged and used to calculate each subject’s individual data.

Beat-to-beat values of MAP and MCBFV were obtained by integrating signals within each cardiac cycle using a personal computer and Notocord-hem 3.3 software for spectral and transfer function analyses. Using previously validated algorithms (9, 25), MAP and MCBFV beat-to-beat data were then linearly interpolated and resampled at 2 Hz. The time series of the data were first detrended with third-order polynomial fitting. Fast Fourier transform and transfer function analyses were performed using a Hanning window on 256-point segments with 50% overlap. This process resulted in five segments over the 6 min of data. These data were then analyzed using DADSip software (DSP Development, Cambridge, MA). The spectral power of MAP variability and MCBFV variability, mean value of transfer function gain, phase, and coherence function were calculated in the very low (0.02–0.07 Hz), low (0.07–0.20 Hz), and high (0.20–0.35 Hz) frequency ranges (Fig. 1). These ranges were specifically selected to reflect different patterns of the dynamic pressure-flow relationship (9, 25). A coherence function (strength of association) between 0 and 1 reflects the linear relationship between MAP and MCBFV. When coherence function was greater than 0.5, transfer function gain and phase were used as interpretable indices. Phase reflects the temporal relationship between the two variables. Transfer function gain (magnitude of transfer) reflects the ability of the distal cerebral arterioles to buffer changes in MCBFV induced by transient changes in MAP at different frequencies. A small gain indicates that any given change in pressure leads to a small change in flow, implying enhanced autoregulation.

In addition, the percentage change in plasma volume from baseline to hypovolemia was calculated using the Dill formula (7). Also, the arterial oxygen content (CaO2) was calculated as CaO2 = [136 × (hemoglobin concentration) × % O2 hemoglobin (SpO2)/100 + 0.0031 × arterial oxygen tension (PaO2)], where PaO2 was substituted with 100 mmHg.

Further, percentage changes in cerebral oxygen transport after furosemide administration were estimated on the supposition that cerebral oxygen transport is proportional to CaO2 multiplied by MCBFV.

Statistical analysis. Variables were compared using two-way repeated measures ANOVA with Stage (baseline and hypovolemia) × Technique (furosemide and LBNP). The effect of the interaction was considered the most relevant for determining the effects of technique. To determine where significant differences occurred, a Student-Newman-Keuls post hoc test was used for all pair-wise comparisons. Hematocrit, hemoglobin concentration, and the values of CaO2 calculated in the furosemide experiment were compared using the paired t-test. A value of \( P < 0.05 \) was considered statistically significant. Analyses were performed using SigmaStat software (Systat Software, Chicago, IL) on a personal computer. Data are presented as mean ± SEM.

RESULTS

In the furosemide experiment, data were obtained at an average of 64 ± 14 min (range: 48–89 min) after administration of the drug. Hematocrit increased from 24.2 ± 0.9% to 45.3 ± 1.0% (\( t_6 = 5.840, P = 0.001 \)) and hemoglobin concentration increased from 14.1 ± 0.2 g/dl to 15.2 ± 0.3 g/dl (\( t_6 = 7.852, P < 0.001 \)). The estimated percentage change in plasma volume was –11.9 ± 1.5%. The calculated CaO2 increased from 18.9 ± 0.3 ml/dl to 20.5 ± 0.4 ml/dl (\( t_6 = 7.896, P < 0.001 \)) and cerebral oxygen transport increased by 9 ± 4%. In the LBNP experiment, the mean degree of lower body suction was –20 ± 2 mmHg.

Table 1 shows the average values of steady state hemodynamics, respiratory conditions, and spectral analysis. CVP decreased with furosemide administration or LBNP application (significant main effect of Stage, \( F_{1,6} = 50.385, P < 0.001 \)). Steady state MCBFV did not change with furosemide administration or LBNP. MAP increased after furosemide administration (significant main effect of Stage, \( F_{1,6} = 8.751, P = 0.025 \)). CVRi, HR, and respiratory rate did not change. SpO2 with furosemide administration was slightly lower than with LBNP (significant main effect of Technique, \( F_{1,6} = 8.647, P = 0.026 \)). Changes in ETCO2 showed slight but different effects between furosemide administration and LBNP (significant interaction effect, \( F_{1,6} = 8.127, P = 0.029 \)).

Figure 1 shows group-averaged transfer function analysis between MAP and MCBFV. The spectral power of MAP variance for each subject's individual data, statistical analysis variables, and the values of CaO2 calculated for the furosemide experiment were compared using the paired t-test. A value of \( P < 0.05 \) was considered statistically significant. Analyses were performed using SigmaStat software (Systat Software, Chicago, IL) on a personal computer. Data are presented as mean ± SEM.
variability, MCBFV variability, and phase in all the frequency ranges were not different between furosemide administration and LBNP. Coherence function in the low and high frequency ranges showed different changes with furosemide administration and LBNP (low frequency: significant interaction effect, $F_{1,6} = 19.398, P = 0.005$; high frequency: significant interaction effect, $F_{1,6} = 6.945, P = 0.039$), the indices with furosemide administration being significantly lower than with LBNP (Fig. 2A). Transfer function gain in the low and high frequency ranges also showed different changes with furosemide administration and LBNP (low frequency: significant interaction effect, $F_{1,6} = 6.448, P = 0.044$; high frequency: significant interaction effect, $F_{1,6} = 16.373, P = 0.007$), the indices with furosemide administration being significantly lower than those with LBNP (Fig. 2B). In the very low frequency range, coherence showed different changes with furosemide administration and LBNP (significant interaction effect, $F_{1,6} = 7.502, P = 0.034$), although the values were below or near 0.5. Transfer function gain, on the other hand, did not change in this frequency range.

**DISCUSSION**

The main finding of the present study was that although the decreases in CVP were equivalent with furosemide administration and LBNP, changes in transfer function gain and coherence with MAP variability and MCBFV variability were significantly different with the two central hypovolemic techniques studied. Furosemide administration resulted in a smaller transfer function gain and coherence in the low and high frequency ranges compared with LBNP. These results suggest that mild central hypovolemia due to furosemide administration can enhance dynamic cerebral autoregulation in contrast to LBNP.

In the present study, furosemide administration induced intravascular dehydration that caused an ~11% reduction in circulating plasma volume, whereas LBNP led to reduced central blood...
Changes in steady state hemodynamics, respiratory conditions, and spectral power and transfer function indices

Table 1. Changes in steady state hemodynamics, respiratory conditions, and spectral power and transfer function indices

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hypovolemia</th>
<th>LBNP</th>
<th>Hypovolemia</th>
<th>ANOVA</th>
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<tbody>
<tr>
<td></td>
<td>CVP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td>n.s.</td>
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<tr>
<td>Baseline Hypovolemia</td>
<td>6.4 ± 0.6</td>
<td>2.7 ± 0.7†</td>
<td>6.9 ± 0.6</td>
<td>2.1 ± 0.6†</td>
<td>P = 0.029</td>
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<tr>
<td>ΔCVP (mmHg)</td>
<td>−3.6 ± 0.3</td>
<td></td>
<td>−3.8 ± 0.3</td>
<td></td>
<td>n.s.</td>
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<tr>
<td>MCBFV (cm/s)</td>
<td>65 ± 5</td>
<td>65 ± 5</td>
<td>69 ± 5</td>
<td>67 ± 4</td>
<td>n.s.</td>
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<tr>
<td>MAP (mmHg)</td>
<td>75 ± 2</td>
<td>83 ± 2†</td>
<td>74 ± 2</td>
<td>77 ± 2</td>
<td>n.s.</td>
</tr>
<tr>
<td>CVRi (mmHg·cm⁻¹·s⁻¹)</td>
<td>1.2 ± 0.2</td>
<td>1.4 ± 0.1†</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>n.s.</td>
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<tr>
<td>HR (beats/min)</td>
<td>53 ± 1</td>
<td>55 ± 2</td>
<td>50 ± 1</td>
<td>55 ± 2</td>
<td>n.s.</td>
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<tr>
<td>Resp-R (breath/min)</td>
<td>13 ± 1</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
<td>12 ± 1</td>
<td>n.s.</td>
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<tr>
<td>SpO₂ (%)</td>
<td>97 ± 0</td>
<td>97 ± 0</td>
<td>98± 0†</td>
<td>98± 0†</td>
<td>n.s.</td>
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<tr>
<td>ETCO₂ (mmHg)</td>
<td>41 ± 0</td>
<td>42 ± 0</td>
<td>43 ± 0</td>
<td>41 ± 1</td>
<td>P = 0.029</td>
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<tr>
<td>VLF₉₅MAP (mmHg²)</td>
<td>3.66 ± 0.85</td>
<td>5.94 ± 1.43</td>
<td>4.11 ± 0.97</td>
<td>3.93 ± 0.74</td>
<td>n.s.</td>
</tr>
<tr>
<td>LF₉₅MAP (mmHg²)</td>
<td>1.73 ± 0.44</td>
<td>2.00 ± 0.44</td>
<td>1.21 ± 0.22</td>
<td>1.92 ± 0.39</td>
<td>n.s.</td>
</tr>
<tr>
<td>HF₉₅MAP (mmHg²)</td>
<td>0.14 ± 0.06</td>
<td>0.15 ± 0.04</td>
<td>0.17 ± 0.09</td>
<td>0.14 ± 0.03</td>
<td>n.s.</td>
</tr>
<tr>
<td>VLF₉₅ (cm²/s²)</td>
<td>4.29 ± 1.01</td>
<td>0.56 ± 0.05</td>
<td>4.57 ± 1.10</td>
<td>3.69 ± 0.78</td>
<td>n.s.</td>
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<tr>
<td>LF₉₅ (cm²/s²)</td>
<td>1.76 ± 0.37</td>
<td>1.29 ± 0.26</td>
<td>1.40 ± 0.39</td>
<td>2.07 ± 0.50</td>
<td>n.s.</td>
</tr>
<tr>
<td>HF₉₅ (cm²/s²)</td>
<td>0.18 ± 0.07</td>
<td>0.15 ± 0.04</td>
<td>0.28 ± 0.15</td>
<td>0.26 ± 0.08</td>
<td>n.s.</td>
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</table>

Values are means ± SE. LBNP, lower body negative pressure; ANOVA, analysis of variance; CVP, central venous pressure; ΔCVP, changes in central venous pressure; MCBFV, mean cerebral blood flow velocity; MAP, mean arterial pressure; CVRi, cerebral vascular resistance index; HR, heart rate; Resp-R, respiratory rate; SpO₂, arterial oxygen saturation; ETCO₂, end-tidal carbon dioxide pressure; VLF₉₅MAP, very low frequency component of the mean blood pressure variability; LF₉₅MAP, low frequency component of the mean blood pressure variability; HF₉₅MAP, high frequency component of the mean blood pressure variability; VLF₉₅, very low frequency component of MCBFV variability; LF₉₅, low frequency component of the MCBFV variability; HF₉₅, high frequency component of the MCBFV variability. * P < 0.05 (vs. each baseline), †P < 0.05 (vs. furosemide).

In this study, we attempted to achieve equal changes in central blood volume by inducing equal reductions in CVP, because CVP is a clinically reliable index for estimation of venous return or central blood volume. However, we could not prove that changes in hematocrit and hemoglobin equate to changes in central blood volume. Furosemide administration and LBNP produced similar mild central hypovolemia that caused a CVP reduction of approximately 3–4 mmHg in the present study. Further, steady state MCBFV did not change with furosemide administration or LBNP, and the values were not different between these techniques. The present results were consistent with previous studies that evaluated steady state MCBFV during mild central hypovolemia (16, 26). Despite the equivalent reduction in CVP and unchanged steady state MCBFV between furosemide administration and LBNP, transfer function gain and coherence function in the low and high frequency ranges showed significantly different changes between these techniques. These results suggest that equivalent mild central hypovolemia due to furosemide administration and LBNP have different effects on dynamic cerebral autoregulation with unchanged steady state CBF.

Cerebral autoregulation maintains a relatively constant blood flow to the brain in response to sustained changes in perfusion pressures (17). The steady state relationship between pressure and flow, assessed by measurement of the CBF response to gradual changes in cerebral perfusion pressure, is referred to as static cerebral autoregulation (17, 23). In contrast, dynamic cerebral autoregulation reflects the ability of cerebral arterioles to buffer changes in CBF induced by rapid changes in pressure. Further, dynamic cerebral autoregulation has a frequency-dependent property that is assessed by transfer function analysis (9, 25). Coherence function (strength of association) is generally higher at a relatively higher frequency range (>0.07 Hz), reflecting that fluctuations in flow are more dependent on relatively faster oscillations in pressure. Also, transfer function gain (magnitude of transfer) is generally higher at a relatively higher frequency range, indicating that any given oscillation in pressure leads to greater fluctuations in flow. Thus dynamic cerebral autoregulation in the low and high frequency ranges has a relatively low autoregulatory ability compared with that in the very low frequency range. However, although transfer function indices in the high frequency range are affected by respiratory conditions, transfer function in the high frequency range also reflects simple, passive transmission of pressure to flow (5, 25), signifying alteration in the cerebral vascular state.

In the present study, transfer function gain and coherence function in the low and high frequency ranges were smaller after furosemide administration compared with LBNP. Therefore, the present results documented that mild hypovolemia due to furosemide administration leads to a smaller magnitude of linear transfer and lower linear dependence of CBF fluctuation on MAP oscillations compared with LBNP. On the other hand, transfer function gain in the very low frequency range was not altered in this study. It is possible that the small changes in central hypovolemia had no noticeable effects on transfer function gain, because dynamic cerebral autoregulation is most effective in the very low frequency range. On the contrary, because autoregulation in the low and high frequency ranges is less effective than in the very low frequency range, they would be altered by even mild hypovolemia.

There is a key report regarding the effect of dehydration on dynamic cerebral autoregulation (18). In that study, chronic dehydration was induced by complete fluid restriction for 7 h together with oral ingestion of 40 mg furosemide, and dynamic cerebral autoregulation was evaluated under controlled breathing (0.25 Hz). That study revealed that steady state MCBFV decreased significantly, and coherence function was <0.5 in the low frequency range, omitting the calculation of transfer function gain. On the other hand, the present study induced acute dehydration by intravenous administration of 0.4 mg/kg furosemide, and evaluated dynamic cerebral autoregulation under spontaneous respiration. We found that steady state MCBFV remained unchanged, and coherence function was...
Therefore, we believe that no significant hemoconcentration occurred during the short-term, low-grade LBNP in the present study, and that the physical properties of blood were probably different during furosemide administration and LBNP application. Hemoconcentration generally leads to an increase in CaO₂; in the present study, both CaO₂ and cerebral oxygen transport increased after furosemide administration. Therefore, it is possible that the differential changes in oxygen supply to the brain between the two techniques may be partly related to the differential alterations of dynamic cerebral autoregulation, although the present study did not attempt to elucidate the specific mechanisms behind the changes in dynamic cerebral autoregulation. To confirm this speculation, however, future studies measuring cerebral oxygenation using near infrared spectroscopic topography are needed. It is also possible that different changes in blood viscosity can influence dynamic cerebral autoregulation. Under furosemide administration, increased blood viscosity resulting from apparent hemoconcentration probably augmented shear stress in the cerebral arterioles. Augmentation of shear stress, which increases the release of endothelial factors, may improve the responses of cerebral arterioles to rapid changes in pressure, implying improvement of dynamic cerebral autoregulation in the low and high frequency ranges, although the relationship between blood viscosity and cerebral circulation is controversial (3, 24). Moreover, the augmented cerebrovascular resistance due to increased blood viscosity may lead to decreases in transfer function gain because there is an inverse relationship between cerebrovascular resistance and transfer function gain (21). However, the estimated cerebrovascular resistance with the two techniques did not show significant changes in the present study.

**Limitations.** The primary limitation of the present study is the potential effect of changes in arterial CO₂ on the results. The cerebral arteriole is sensitive to changes in arterial CO₂, affecting changes in steady state MCBFV and dynamic cerebral autoregulation. Although arterial CO₂ was not measured in the present study, changes in ETCO₂ were slightly but significantly different between furosemide administration and LBNP. Although the present change in ETCO₂ might not be clinically relevant (5), we performed an additional experiment that investigated the effects of slight changes (~2 mmHg) in ETCO₂ on dynamic cerebral autoregulation in seven subjects (five of the original seven subjects and two additional subjects). When ETCO₂ decreased from 38 ± 0.6 mmHg to 36 ± 0.8 mmHg with controlled breathing, none of the indices of transfer function analysis changed (e.g., transfer function gain in the low frequency range from 1.14 ± 0.1 cm·s⁻¹·mmHg⁻¹ to 1.10 ± 0.1 cm·s⁻¹·mmHg⁻¹), despite a slight but significant decrease in steady state MCBFV from 70 ± 5 cm/s to 67 ± 5 cm/s. Therefore, the slight difference in ETCO₂ between furosemide administration and LBNP would have had little effect on the present results of dynamic cerebral autoregulation. Moreover, we speculated that increases in dead space resulting from changes in the position of the diaphragm by LBNP might be responsible for the slight difference in ETCO₂ between furosemide administration and LBNP because there were no differences in steady state MCBFV between the two techniques in the present study.

There are some other limitations to the present protocol. The changes in cerebral circulation during LBNP were obtained 1 min after the start of lower body suction, whereas furosemide administration probably augmented shear stress in the cerebral arterioles.

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**Fig. 2.** Group-averaged transfer function analysis in the low frequency range between mean arterial pressure and mean cerebral blood flow velocity after furosemide administration and during lower body negative pressure (LBNP). BL, baseline; HYPO, hypovolemia. *P < 0.05; significant interactions between furosemide administration and LBNP (ANOVA); #P < 0.05 (post hoc test).
administration data were measured ~1 h after administration of the drug. The different duration of central hypovolemia may have influenced the present results. To avoid this influence, changes in cerebral circulation during prolonged lower body suction should be measured. Another limitation was the small sample size; however, we could not obtain a larger sample size because insertion of the central catheter twice was a heavy burden for subjects. However, post hoc powers ($\alpha = 0.05$) in CVP, transfer function gain in the high frequency range, and coherence function in the low frequency range were 1.00, 0.910, and 0.951, respectively, whereas values of transfer function gain in the low frequency range and coherence function in the high frequency range were below 0.80. Hence, the main finding of the different effects on dynamic cerebral autoregulation between furosemide administration and LBNP are not likely to have been affected by the small sample size.

Central hypovolemia is often caused by either pooling of blood in the lower part of the body (postural change) or volume loss (dehydration, hemorrhage), leading to orthostatic hypotension, or presyncope, or both (2, 12–14, 22, 26). The elderly, in particular, often have central hypovolemia, either due to pooling of blood (orthostatic hypotension, postprandial hypotension) or volume loss (dehydration, use of diuretics). One previous study reported that the prevalence of orthostatic hypotension, postprandial hypotension, and carotid hypersensitivity in the elderly is ~50% (13). Occurrence of these hypertensive and presyncope syndromes may partly and importantly relate to altered cerebral autoregulation with or without hemoconcentration. We speculate that the risks of presyncope may be higher in conditions without hemoconcentration than in those with dehydration, due to the lack of compensatory cerebral autoregulation.

**Conclusion.** We investigated the effects of mild central hypovolemia induced by furosemide administration and LBNP on dynamic cerebral autoregulation, and discovered that mild central hypovolemia due to furosemide administration and LBNP have different effects on dynamic cerebral autoregulation despite equivalent decreases in CVP and unchanged steady state CBF. Mild central hypovolemia with hemoconcentration might contribute to enhanced dynamic cerebral autoregulation in contrast to that without hemoconcentration.

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**AUTHOR CONTRIBUTIONS**

K.I. conceived and designed the research; Y.O., K.A., J.K., and K.I. performed experiments; Y.O. analyzed data; Y.O. and K.I. interpreted results of the experiments; Y.O. prepared the figures and drafted the manuscript; Y.O., K.A., J.K., and K.I. edited the manuscript; Y.O., K.A., J.K., and K.I. approved the final version of the manuscript.

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