Association Between Ambulatory 24-Hour Blood Pressure Levels and Brain Volume Reduction

A Cross-Sectional Elderly Population-Based Study

Sébastien Celle, Cédric Annweiler, Vincent Pichot, Robert Bartha, Jean-Claude Barthélémy, Frédéric Roche, Olivier Beauchet

Abstract—Previous literature has shown mixed results regarding the association between blood pressure levels and brain volume reduction. The objectives of this study were to determine whether high blood pressure levels were associated with focal brain volume reduction and whether high blood pressure-related focal brain volume reduction was associated with a decline in executive function performance. On the basis of a cross-sectional design, 24-hour ambulatory blood pressure measurements, as well as brain morphology from 3-dimensional magnetic resonance images, were assessed among 183 participants (mean, 65±0.6 years; 62.4% women). Average levels of systolic and diastolic blood pressures, as well as dip, pulse pressure, and mean arterial blood pressure, were used as outcomes. Cortical gray and white matter volumes were determined by automatic calculation using Statistical Parametric Mapping segmentation. Folstein’s Mini-Mental State Examination, digit span, part B of Trail Making, and Stroop tests were used to assess executive function performance. Sex, use of antihypertensive drugs, duration of hypertension, leukoaraiosis, body mass index, education level, and total brain matter volume were used as potential confounders. A significant blood pressure-related decrease in gray matter volume of the left supplementary motor areas (Brodmann area 6) and of the left superior and middle frontal gyrus (Brodmann area 8) was shown. No significant decrease was found with white matter volume. Blood pressure-related decreases in gray matter volume were significantly associated with a decline in executive function performance. The association of high blood pressure with brain volume reduction may in part explain blood pressure-related cognitive decline leading to dementia. (Hypertension. 2012;60:1324-1331.) ● Online Data Supplement

Key Words: blood pressure ▪ lobar atrophy ▪ aged ▪ cross-sectional study ▪ magnetic resonance imaging

Since the first report by Hatazawa et al1 in 1984, a growing number of epidemiological studies have shown that high blood pressure (BP) levels are associated with lower volume of regional brain tissue.1,2 Most of these previous studies used occasional clinical BP measurements for BP assessment.3-6 Compared with these few BP readings, ambulatory 24-hour BP monitoring provides numerous readings throughout the day and night.7,8 Thus, 24-hour BP gives a more sensitive measure of vessel-targeted end-organ damage, such as that observed in the brain.9

Among the 4 previous studies that used 24-hour BP monitoring to examine the association between BP levels and brain volume reduction,2,8,9,11 1 study found no association between mean values of all BP measures and focal volume reduction,11 where 3 reported a significant association of focal brain volume reduction with high mean value of systolic BP (SBP) and with high SBP sleep variability.2,8,9 No association was shown with diastolic BP (DBP).2,8,11 These mixed results underscore the need for additional information to improve our knowledge of the association between BP levels and brain volume reduction.

The association between high BP levels and low cognitive performance is now well established.12-14 Executive function (EF) performance seems to be one of the domains most sensitive to high BP levels.6,12-14 EFs are heterogeneous cognitive functions.15 According to the model by Miyake et al,15 EF may be divided into 3 main separate executive subdomains (ESDs): mental shifting, information updating, and cognitive inhibition. Little is known about the association between these specific ESDs and BP-related decreases in gray and white matter volumes.6

Previous studies of brain volume reduction have mainly used magnetic resonance imaging.1-8,11 In the last 10 years, new analysis methods for identifying and segmenting brain...
structures and functions have been developed. Despite its limitations attributable to the high variability of brain anatomy, the voxel-based morphometry (VBM) method has proved its value in identifying regional changes in brain tissue volume.\textsuperscript{16}

We had the opportunity to examine the association between 24-hour BP measurements and gray and white matter volumes in a large representative community survey of older adults, the PROgnostic indicator OF cardiovascular and cerebrovascular events (PROOF) study.\textsuperscript{17} We reported previously that high mean BP levels were associated with a decline in memory performance related to attention disorders, involving the frontal lobe among the participants of the PROOF study.\textsuperscript{13} Thus, we hypothesized that high mean BP levels could be associated with focal frontal lobe volume reduction and that high BP-related focal frontal lobe volume reduction could be associated with a decline in EF performance. The purposes of this cross-sectional study were to determine at the baseline assessment of the PROOF study whether high BP levels were associated with focal brain volume reduction and whether any significant high BP-related focal brain volume reduction was associated with a decline in EF performance.

Methods

Brain Imaging and Neuropsychological Assessment

A total of 183 participants from the PROOF study\textsuperscript{11} were included in the current study (see online-only Data Supplement for description of participant’s selection). All participants had 3D T\textsubscript{1}-weighted magnetic resonance imaging at 1.0 Tesla to measure gray matter and white matter volumes, and fluid-attenuated inversion recovery imaging to rate leukoaraiosis using the Fazekas standardized scale (see online-only Data Supplement). VBM was performed with Statistical Parametric Mapping version 8 to classify voxels into gray and white matter (see online-only Data supplement material for description of image analysis). Neuropsychological tests were used to probe several aspects of cognitive function at baseline before BP measurements (see online-only Data supplement).

BP Measurement

Noninvasive and validated arterial BP was assessed by ambulatory 24-hour BP monitoring using the auscultatory method (Diaysis Integra, Novacor, Rueil-Malmaison, France) on a weekday, starting early in the morning.\textsuperscript{18} Measurements were taken from the nondominant arm, every 15 minutes during the day and every 30 minutes at night. Patients were instructed to adhere to their normal daily activities and regular sleeping hours. In addition, clinical SBP and DBP measures were performed. BP was measured twice by a physician with a mercury sphygmomanometer after the patient had been lying down for 15 minutes. BP was measured in the lying position for convenience at the end of a 15-minute continuous BP recording for Bureoreflex assessment. The clinical value retained was the mean of the 2 consecutive measurements. Average values of clinical SBP and DBP, 24-hour SBP and DBP, awake SBP and DBP, sleep SBP and DBP, Dip (ie, dip in BP calculated from the formula: Dip=[(SBPAwake BP)], pulse pressure calculated from the formula (pulse pressure=24-hour SBP−24-hour DBP), and mean arterial BP (MAP) calculated from the formula MAP=(24-hour SBP+2x24-hour DBP)/3, were used for data analysis.\textsuperscript{19,20}

Covariates

Sex, use of antihypertensive drugs, leukoaraiosis (Fazekas score),\textsuperscript{21} body mass index, cardiovascular risk factors (ie, use of antihypertensive drugs, type 2 diabetes mellitus, duration of hypertension, smoking, alcohol use, and hypercholesterolemia), education level, and total brain matter volume were examined as potential confounders in data analysis. Antihypertensive drugs were defined by the use of ≥1 of the following drug therapies: renin-angiotensin inhibitor agents, β-blocking agents, diuretics, calcium channel blockers, and central antihypertensive agents. The use of antihypertensive drugs was combined into a single Yes versus No category. Type 2 diabetes mellitus was considered to be present in case of the use of oral antidiabetic drugs. The smoking status was defined as current or former smoker versus nonsmoker. Alcohol consumption was combined into the 2 following categories, regular versus nonregular consumer of beer, wine, or spirits. Hypercholesterolemia was defined as a total cholesterol level >6.5 mmol/L or use of lipid-lowering drugs or both. Education level was assessed with the number of years at school categorized in 4 levels (level 1≤5 years, level 2=6–8 years, level 3=9–11, and level 4≥12). Participants were separated into 2 groups based on the presence (Fazekas scale score ≥2) or absence (Fazekas scale score <2) of significant leukoaraiosis.\textsuperscript{21}

Data Analyses

Participants’ baseline characteristics were summarized using means and SDs or frequencies and percentages, as appropriate. First, comparisons between participants included in this study and the other participants of the PROOF study with regard to the clinical characteristics (Table 1). There was no significant difference between the participants included in this study and the other participants of the PROOF study according to the Fazekas scale in the participants separated into 2 groups based on the presence/absence of significant leukoaraiosis (Fazekas scale score ≥2) or absence (Fazekas scale score <2) of significant leukoaraiosis.\textsuperscript{21}

Results

There was no significant difference between the participants included in the current study and the other participants of the PROOF study with regard to the clinical characteristics (Table 1).

Table 2 shows the prevalence of leukoaraiosis stratified by class according to the Fazekas scale in the participants separated into 2 groups based on use of antihypertensive drugs (ie, renin-angiotensin inhibitor agents, β-blocking agents, diuretics, calcium channel blockers, and central antihypertensive agents). No significant difference was shown between participants using antihypertensive drugs and those who did not.

As reported in Table 3, a significant decrease in gray matter volume of the left supplementary motor area (Brodmann area BA6) was associated with an increase in the mean value of 24-hour SBP (P=0.003; Figure 1), 24-hour DBP (P=0.035), awake SBP (P=0.004), sleep SBP (P=0.015), and MAP (P=0.003). A significant decrease in gray matter volume of the left superior frontal gyrus was...
Table 1. Comparison of Baseline Characteristics Between the Subset of Participants (n=183) and Other Participants From the PROOF Study (n=828)

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>VBM+ (n=183)</th>
<th>VBM− (n=828)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>68/115</td>
<td>332/496</td>
<td>0.73</td>
</tr>
<tr>
<td>Age, mean±SD, y</td>
<td>65.3±0.6</td>
<td>65.8±2.3</td>
<td>0.12</td>
</tr>
<tr>
<td>BMI, mean±SD, kg/m²</td>
<td>25.3±3.3</td>
<td>25.3±4.1</td>
<td>0.92</td>
</tr>
<tr>
<td>Duration of hypertension, mean±SD, y</td>
<td>9.9±7.5</td>
<td>10.8±7.5</td>
<td>0.34</td>
</tr>
<tr>
<td>Use antihypertensive drugs, n (%)†</td>
<td>61(33.3)</td>
<td>301(36.4)</td>
<td>0.45</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus, n (%)</td>
<td>11 (6)</td>
<td>48 (5.8)</td>
<td>0.95</td>
</tr>
<tr>
<td>Smoking, n (%)‡</td>
<td>44 (24)</td>
<td>207 (25)</td>
<td>0.69</td>
</tr>
<tr>
<td>High total cholesterol, n (%)§</td>
<td>66 (36.1)</td>
<td>300 (36.2)</td>
<td>0.89</td>
</tr>
<tr>
<td>Alcohol daily use, n (%)</td>
<td></td>
<td></td>
<td>70 (38.3)</td>
</tr>
<tr>
<td>| Education level¶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD, y</td>
<td>11.2±3.3</td>
<td>11.0±3.0</td>
<td>0.06</td>
</tr>
<tr>
<td>Level 1, n (%)</td>
<td>18 (10)</td>
<td>62 (9)</td>
<td>0.23</td>
</tr>
<tr>
<td>Level 2, n (%)</td>
<td>71 (39)</td>
<td>329 (40)</td>
<td>0.45</td>
</tr>
<tr>
<td>Level 3, n (%)</td>
<td>52 (28)</td>
<td>226 (27)</td>
<td>0.29</td>
</tr>
<tr>
<td>Level 4, n (%)</td>
<td>42 (23)</td>
<td>211 (24)</td>
<td>0.28</td>
</tr>
<tr>
<td>MMSE score (/30), mean±SD</td>
<td>28.7±1.4</td>
<td>28.5±1.5</td>
<td>0.31</td>
</tr>
<tr>
<td>Executive subdomains performance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit span score, mean±SD#</td>
<td>7.9±2.6</td>
<td>8.3±2.8</td>
<td>0.16</td>
</tr>
<tr>
<td>TMTB score, mean±SD**</td>
<td>100.7±49.2</td>
<td>106.4±51.1</td>
<td>0.20</td>
</tr>
<tr>
<td>Ratio of Stroop score, mean±SD††</td>
<td>2.2±0.6</td>
<td>2.2±0.6</td>
<td>0.93</td>
</tr>
<tr>
<td>Clinical blood pressure measures, mm Hg‡‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mean±SD</td>
<td>141.7±14.7</td>
<td>143.1±19.1</td>
<td>0.42</td>
</tr>
<tr>
<td>DBP, mean±SD</td>
<td>86.6±9.5</td>
<td>87.7±10.5</td>
<td>0.20</td>
</tr>
<tr>
<td>24-h ambulatory blood pressure measures, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-h SBP, mean±SD</td>
<td>119.1±11.4</td>
<td>119.2±13.9</td>
<td>0.91</td>
</tr>
<tr>
<td>24-h DBP, mean±SD</td>
<td>76.1±7.5</td>
<td>76.1±8.0</td>
<td>0.97</td>
</tr>
<tr>
<td>Awake SBP, mean±SD</td>
<td>123.6±14.7</td>
<td>123.7±14.4</td>
<td>0.88</td>
</tr>
<tr>
<td>Awake DBP, mean±SD</td>
<td>78.9±8.2</td>
<td>78.9±8.3</td>
<td>0.97</td>
</tr>
<tr>
<td>Sleep SBP, mean±SD</td>
<td>106.1±11.4</td>
<td>106.3±15.3</td>
<td>0.84</td>
</tr>
<tr>
<td>Sleep DBP, mean±SD</td>
<td>67.9±7.9</td>
<td>68.9±8.9</td>
<td>0.66</td>
</tr>
<tr>
<td>Dip SBP, mean±SD</td>
<td>0.139±0.077</td>
<td>0.139±0.079</td>
<td>0.99</td>
</tr>
<tr>
<td>PP, mean±SD</td>
<td>42.8±10.9</td>
<td>43.0±9.7</td>
<td>0.82</td>
</tr>
<tr>
<td>MAP, mean±SD</td>
<td>90.1±8.8</td>
<td>90.2±9.2</td>
<td>0.87</td>
</tr>
</tbody>
</table>

* Data show the between-group comparison based on independent samples t test or χ² test, as appropriate.
† Data show the use of renin-angiotensin inhibitor agents, β-blocking agents, diuretics, calcium channel blockers, or central antihypertensive agents.
‡ This was defined as current or former smoker versus nonsmoker.
§ This was defined as a total cholesterol level >6.5 mmol/L or use of lipid-lowering drugs or both.
¶ This was combined into 2 categories, regular versus nonregular consumer of beer, wine, or spirits.
‖ This was assessed with the number of years at school and categorized in 4 levels (level 1≤5 years, level 2=6–8 years, level 3=9–11, and level 4≥12).
# This included the standardized total number of digits that a participant was able to absorb and recall in correct forward serial orders after hearing them.
** This shows the time to connect the dots as quickly as possible for 25 consecutive targets on a sheet of paper part B of trail making test (ie, alternated numbers and letters).
†† This shows the ratio score of Stroop test (no interference [color]/interference [color−word] with [color] corresponding with time to name color and [color−word] corresponding with time to name the color of incongruent color words).
‡‡ BP was measured twice by a physician with a mercury sphygmomanometer after the patient had been lying down for 15 minutes, and the clinical value retained was the mean of the 2 consecutive measurements.
Table 2. Prevalence of Leukoaraiosis Stratified by Class Following Fazekas Classification in Sample of Studied Participants Separated Into 2 Groups Based on the Use of Antihypertensive Drugs (n=183)

<table>
<thead>
<tr>
<th>Fazekas Classification of Leukoaraiosis</th>
<th>Use Antihypertensive Drugs*</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 0, n (%)</td>
<td>Yes (n=78)</td>
<td>No (n=105)</td>
</tr>
<tr>
<td>Class 1a, n (%)</td>
<td>28 (35.8)</td>
<td>35 (33.3)</td>
</tr>
<tr>
<td>Class 1b, n (%)</td>
<td>13 (16.7)</td>
<td>15 (14.3)</td>
</tr>
<tr>
<td>Class 2, n (%)</td>
<td>22 (28.2)</td>
<td>39 (37.1)</td>
</tr>
<tr>
<td>Class 3, n (%)</td>
<td>13 (16.7)</td>
<td>13 (12.4)</td>
</tr>
</tbody>
</table>

*Data show the use of renin-angiotensin inhibitor agents, β-blocking agents, diuretics, calcium channel blockers, or central antihypertensive agents.
†Comparison was based on \( \chi^2 \) test.

Blood Pressure and Brain Volume Reduction

This population-based study of elderly participants showed that high BP levels were associated with smaller gray matter volume in the supplementary motor area (BA6), regardless of the type of BP measures, whereas in the superior frontal gyrus (BA8), only high 24-hour and awake SBPs were associated with smaller gray matter volume. In addition, high-sleep SBP and DBP were significantly associated with smaller gray matter volume in the middle frontal gyrus (BA8). These BP-related decreases in gray matter volume in supplementary motor areas were significantly associated with decline in all ESDs performance, whereas BP-related decreases in gray matter volume in superior and middle frontal gyrus were only associated with a decline in mental shifting performance. All BP-related decreases in gray matter volume were shown on the left side. No significant BP-related decrease in white matter volume was found.

Previous studies of the effect of BP on brain tissue volume reduction have been conflicting, with some reports suggesting a significant association of high BP levels with brain volume reduction consistent with the current study and others showing no effect.1–5,11 These mixed findings may be attributable to a number of methodological factors including the cutoff value.

Discussion

BP-related decrease in left superior frontal gyrus gray matter volume was also associated with lower score on part B of trail making test (\( P=0.006 \) for SBP, \( P=0.008 \) for awake SBP, \( P=0.043 \) for MAP). Finally, BP-related decrease in left middle frontal gyrus gray matter volume was associated with lower score on part B of trail making test (\( P=0.015 \) for sleep SBP and \( P=0.002 \) for sleep DBP) (Table 4). No significant associations were found between BP-related decrease in gray matter volume and Mini-Mental State Examination score.

Table 3. Localization of High Blood Pressure-Related Decreases in Gray Matter Volume According to 24-h Blood Pressure Measures Adjusted for Baseline Characteristics of Participants (n=183)

<table>
<thead>
<tr>
<th>Type of Blood Pressure Measures</th>
<th>Gray Matter Localization</th>
<th>MNI Coordinates</th>
<th>Cluster Size†</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h SBP</td>
<td>L Supplementary motor area</td>
<td>6, 9, 5, 51</td>
<td>543</td>
<td>0.003</td>
</tr>
<tr>
<td>24-h DBP</td>
<td>L Superior frontal gyrus</td>
<td>8, −12, 39, 40</td>
<td>322</td>
<td>0.023</td>
</tr>
<tr>
<td>Awake SBP</td>
<td>L Supplementary motor area</td>
<td>6, −9, 0, 60</td>
<td>206</td>
<td>0.035</td>
</tr>
<tr>
<td>Sleep SBP</td>
<td>L Superior frontal gyrus</td>
<td>8, −10, 39, 42</td>
<td>360</td>
<td>0.018</td>
</tr>
<tr>
<td>Sleep DBP</td>
<td>L Middle frontal gyrus</td>
<td>8, −40, 29, 43</td>
<td>290</td>
<td>0.013</td>
</tr>
<tr>
<td>MAP</td>
<td>L Middle frontal gyrus</td>
<td>8, −26, 17, 58</td>
<td>253</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>L Superior frontal gyrus</td>
<td>6, −9, 0, 58</td>
<td>486</td>
<td>0.003</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure calculated from the formula: (SBP + 2 × DBP)/3; L, left; MNI, Montreal Neurological Institute; x, y, and z coordinates in millimeters where x=right (+) to left (−), y=anterior (+) to posterior (−), and z=superior (+) to inferior (−). Data were expressed in number of voxels.
§Uncorrected P values for cluster were derived from linear regression model showing the association between decrease in volume of gray matter (dependent variable) and blood pressure measure adjusted on sex, duration of hypertension, use of antihypertensive drugs, body mass index, leukoaraiosis, and total brain matter volume; Psignificant (ie, <0.05) indicated in bold.
used to define hypertension, the most suitable BP assessment method, the technique used to assess brain volume reduction, and the control of confounders, such as age, sex, or total brain matter. In the current study, all these parameters were carefully controlled allowing observation of preferential localization of BP-related brain volume reduction in the frontal lobe and in particular in 2 Brodmann areas that were the supplementary motor areas (BA6) and the superior and middle frontal gyrus (BA8). The involvement of these areas has been reported in previous studies.\textsuperscript{5,7,23,24} For instance, Gianaros et al\textsuperscript{6} reported a similar high SBP-related decrease in gray matter in supplementary motor areas and the superior frontal gyrus. Furthermore, similar to Gianaros et al,\textsuperscript{6} we found a preferential localization of high BP-related decrease in gray matter volume in the left hemisphere. We also found that the association between BP and gray matter volume depends on the type of BP measurement. Although the volume of the left supplementary motor area was affected by high BP, regardless of the type of BP measures, a BP-related decrease in gray matter volume of the left superior frontal gyrus was shown only for 24-hour and awake SBP.

The association of high BP with brain volume reduction in humans is supported by several animal models that have shown that BP-related brain volume reduction is caused by a decrease in cerebral blood flow, capillary abnormalities, and an alteration of the integrity of the blood-brain barrier.\textsuperscript{24–27} All these BP-related cerebral changes promote neuronal death and microglial and macrophage formation that may contribute to brain volume reduction.\textsuperscript{24–27} The specificity of BP-related focal volume reduction to the frontal cortex
observed in the current study may be related to ischemic effects that are supplied by the middle and anterior cerebral arteries. 

Similarly, preferential BP-related left focal volume reduction suggests an asymmetry in the organization of the autonomic nervous system of the vessels with a greater sensitivity of the left hemisphere compared with the right one. Indeed, it was reported previously that SBP tended to be higher and less stable in patients with acute ischemic injury may have a worse prognosis. The reason for the association between SBP and smaller supplementary motor area gray matter volume observed in the current study remains unclear. Indeed, some previous studies found an association between brain volume reduction and SBP but not DBP, whereas others found an association with DBP but not SBP. 

BP-related decreases in gray matter volume in the frontal lobe were associated with EF impairment. More specifically, we showed for the first time that BP-related decrease in supplementary motor area volume was independently associated with focal frontal volume reduction. 

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Table 4.  Multiple Linear Regression Models Showing the Associations Between Relevant High Blood Pressure-Related Change in Cluster Size (Dependent Variable) and Executive Subdomain Performance (Independent Variables) Adjusted for Sex and Education Level (n=183)
The current study combined brain magnetic resonance imaging with the VBM method to characterize brain volume reduction. Furthermore, a cluster-level P values approach was used, which was previously discouraged in VBM analyses because it is nonstationary (cluster P level depends on local smoothness, and VBM data are known to exhibit non-stationarity), but this approach is now acceptable in the latest versions of Statistical Parametric Mapping 8 v4290. The use of cluster-level analysis using P values that are uncorrected for multiple comparisons is a limitation of the current study. However, we had a preconceived hypothesis regarding the frontal area; therefore, it is possible to examine this region using uncorrected P statistics. The limitations of the method are described in the online-only Data Supplement.

In conclusion, high BP levels were significantly associated with focal frontal volume reduction involving left supplementary motor gray matter and superior and middle frontal gyrus gray matter, whereas no BP-related decrease in white matter volume was found in the studied sample of participants. Furthermore, the findings indicate that focal brain volume reduction is associated with a decline in ESDs performance. Further research is needed to corroborate these findings.

**Perspectives**

We suggest that the association of high BP with brain volume reduction provides new perspective about our understanding of BP-related cognitive decline leading to dementia. Because high BP could contribute to neurodegenerative lesions of dementia, such as Alzheimer disease, confirming and characterizing BP-related brain volume reduction shown in the current study may be helpful in understanding the process of cerebral volume reduction in neurodegenerative dementia.

**Acknowledgments**

The PROOF study group thank all persons who took part in this study, Dr Catherine Thomas-Antérion, Delphine Maudoux, Judith Kerleroux, and Arnaud Garcin (Saint-Etienne University Hospital, Saint-Etienne, France) for their expert help in data acquisition and interpretation. S. Celle has full access to all study data and takes responsibility for the integrity of the data and the accuracy of the data analyses. F. Roche, V. Pichot, and J.-C. Barthélémy were responsible for study concept and design. S. Celle, F. Roche, and J.-C. Barthélémy were responsible for acquisition of data. O. Beauchet, C. Annweiler, F. Roche, J.-C. Barthélémy, V. Pichot, and S. Celle were responsible for analysis and interpretation of data. S. Celle, C. Annweiler, and F. Roche drafted the article. O. Beauchet, R. Bartha, and J.-C. Barthélémy were responsible for critical revision of the article for important intellectual content. F. Roche and J.-C. Barthélémy obtained funding. S. Celle contributed statistical expertise. F. Roche and J.-C. Barthélémy contributed administrative, technical, or material support; F. Roche and J.-C. Barthélémy supervised the study.

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**Disclosures**

None.

**References**


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### Novelty and Significance

**What Is New?**

- BP-related decreases in gray matter volume of the left supplementary motor areas (Brodmann area 6) and of the left superior and middle frontal gyrus (Brodmann area 8) are significantly associated with decline in executive subdomains performance.

**What Is Relevant?**

- High BP levels are long known to generate cerebrovascular damage corresponding to ischemic and hemorrhagic lesions of white and gray matter. The current study demonstrates that high BP levels are also associated with focal frontal atrophy and that this BP-related brain atrophy is associated with decline in executive subdomains performance.

**Summary**

- High BP levels were associated with brain volume reduction involving the left prefrontal cortex. This BP-related decrease in gray matter volume was significantly associated with a decline in executive subdomains performance.
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ASSOCIATION BETWEEN AMBULATORY 24-H BLOOD PRESSURE LEVELS AND BRAIN VOLUME REDUCTION: A CROSS-SECTIONAL ELDERLY POPULATION-BASED STUDY

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Short title: Blood pressure and brain volume reduction
Blood pressure and brain volume reduction

Celle, S et al.

METHODS

Participants
The sampling and data collection procedures of the PROOF study have been described elsewhere in detail (1,2). The PROOF study is a community-dwelling observational prospective cohort study designed to evaluate the prognostic value of autonomic nervous system activity levels on fatal and non-fatal cardiovascular and cerebrovascular events. In summary, from 2001 to 2002, 3,983 eligible subjects born between January 1, 1934 and September 30, 1936, and registered on the electoral lists of Saint-Etienne (a mid-sized town in Eastern France) were contacted by mail. The volunteers returned a phone call to manifest their intention to participate in the study and to evaluate exclusion criteria. Exclusion criteria were previous myocardial infarction, previous stroke, heart failure, atrial fibrillation, insulin-dependent diabetes mellitus, cardiac pace-maker, any disease limiting life expectancy below 5 years, contraindication to brain MRI, living in an institution and intention to move within the next two years. The cardiovascular exclusion criteria used in this study allowed in part controlling a potential interaction in the association between BP levels and cognitive performance. Among the 3,983 eligible participants, 2,660 (66.8%) participants gave no answer, 443 (11.1%) refused to participate, and 49 (0.01%) were considered as ineligible. The sample was completed by the participation of spouses of volunteers (n=48; 0.01%) and a few volunteers participants recruited via PROOF association (n=132; 3.3%). A total of 1,011 participants (25.4%) were included after having given their written informed consent. The subset of participants included in the current study corresponded to the 183 participants who received a three dimensions (3D) MRI in 2002 allowing volumetric measurements of brain structures. All included participants underwent a complete clinical examination by physicians at Saint-Etienne University Hospital. Information was gathered about cardiovascular risks, the use of antihypertensive drugs and anthropometry measurements. Body mass index (BMI) was calculated as weight/height² in kg/m².

Brain magnetic resonance imaging volumetry
Images of the brain were acquired on average 122±40 days after completing the 24-h BP monitoring, clinical examination and cognitive assessment. The MRI protocol used has been previously described (3). In summary, images were acquired on a Siemens 1.0 Tesla scanner. For each participant, a 3D T1-weighted image set (MPRAGE) was acquired with the following parameters: TR = 1,900 ms, TE = 3.95 ms, TI= 1,100 ms, FOV = 256 X 256, 88 slices per volume with a voxel size of 2mm X 2mm X 2mm. T2-weighted (24 slices of 5.5mm, TR = 6,620 ms, TE = 123 ms, FOV = 173 X 230, pixel size: 1.5 X 0.9 X 5.5 mm, turbo factor = 23) and FLAIR (24 slices of 5.5mm, TR = 9,000 ms, TE = 102 ms, TI= 2,200 ms, FOV = 230 X 173, pixel size: 0.9 X 0.9 X 5.5mm) images were also acquired in the same MRI session. The cortical gray matter and white matter volumes, expressed in cm³, were calculated from the segmented images and used for analysis. An experienced radiologist rated leukoaraiosis using the Fazekas standardized scale divided in four levels from zero defining the absence of leukoaraiosis to 3 corresponding to severe leukoaraiosis (4). In contrast to a previous study (3), voxel-based morphometry (VBM) analysis was performed with Statistical Parametric Mapping (SPM) version 8 using unified segmentation to classify voxels into gray and white matter (5) and DARTEL registration (6). To create a study-specific template, 61 data-sets without visible pathology (leukoaraiosis, lacunas) were selected. The DARTEL procedure allows the creation of this template by iteratively registering segmented images to their own mean. All 183 segmented images were then registered to this template using DARTEL, and spatially normalized to the MNI template. As registration may shrink or enlarge brain areas, a post-processing step called modulation was applied to the images.
Blood pressure and brain volume reduction

Celle, S et al.

Finally images were smoothed by an 8-mm FWHM kernel. The smoothing kernel size was chosen as a compromise to validate assumptions on the general linear model while maintaining the ability to detect relatively small volume changes.

The total brain matter (TBM) volume used in our analysis was composed of the sum of gray matter and white matter volume. TBM volume included the following brain structures: left and right frontal, temporal, parietal and occipital lobes, thalamus, caudate, putamen, globus pallidus and cerebellum. The quantification of the different volume measures (i.e., gray matter, white matter and CSF) were segmented by applying the segmentation procedure provided by SPM. The segmentation algorithm assigned each voxel to gray matter, white matter or CSF. Table S1 shows the mean values and standard deviations of the different brain volumes.

Cognitive assessment

Neuropsychological tests to probe several aspects of cognitive function were performed at baseline a couple of days before blood pressure measurements. Firstly, global cognitive efficiency was evaluated with Folstein’s Mini-Mental State Examination (MMSE) (7). Folstein’s Mini-Mental State Examination (MMSE) is a 30-point test that assesses orientation in time and space, instantaneous recall and short-term memory, attention and calculation ability, and language and visual-constructive ability. Secondly, ESD were evaluated according to Miyake's model that distinguishes mental shifting from information updating and cognitive inhibition (8). We used the standardized digit span test for the evaluation of information updating (9), the part B of Trail Making Test (TMTB) for the evaluation of mental shifting (10), and the Stroop Color-Word test for the evaluation of cognitive inhibition (11). The digit span test examines the ability to recall a sequence of numbers forward in corrected order immediately after its presentation. The Trail Making Test part B (TMTB) requires a participant to alternatively connect numbers and letters (1, A, 2, B, etc.) with a total of 25 consecutive targets on a sheet of paper. The goal is to perform the test as quickly as possible, and the time taken to complete it is used as the primary performance metric. The Stroop Color-Word test is composed of three boards: board 1 shows color names written in black ink; board 2 shows color rectangles; board 3 shows color names written in color ink. Board after board, the participant must either read, or name the colors as quickly as possible, from right to left going to the following line at the end of each line. The test is performed in the following order, with the following instructions: color naming (board showing color rectangles), reading of color names (board showing color names written in black) and interference situation (board showing color names written in color). The number of words and colors found within 45 seconds is measured. The total number of digit recalls in correct order, the TMTB time expressed in seconds and the ratio of Stroop score (i.e., (“No Interference” [Color]) / (“Interference” [color - word])) were used as outcomes; increased scores of TMTB and ratio of Stroop score, and decreased score of digit span corresponded to a decline of ESD performance.

Ethic

The study was conducted in accordance with the ethical standards set forth in the Helsinki Declaration (1983). The Saint-Etienne Ethical Committee, France approved the entire study protocol.

Limitations

The main limitations of the current study are its cross-sectional design and the low resolution of the MRI scans. First, the cross-sectional design prevented causal inferences. For instance, in contrast to our hypothesis, a scenario of reverse causation may be considered and it is possible that brain volume reduction leads to high blood pressure. Second, in the current study, the resolution of MRI scans was low (i.e., 2mm x 2mm x 2mm), which may lead to substantial partial volume effects, and thus impact the image segmentation on the boundaries.
or on the gray matter/white matter interface. As our voxels were large, small decreases in gray matter volume may be missed in our analyses. Third, VBM has recently been criticized because of segmentation and normalisation defects. Segmentation of brain into gray and white matter is a major difficulty. Indeed, partial volume effects at the boundary between gray and white matter as well as mislabelling are two limitations of segmentation. Of note, the use of SMP8 unified segmentation, which is based on a generative model and thus performs better than previous versions, may minimize this limitation. Recently, Klein et al. (4) showed major differences between registration algorithms. We tried to avoid this problem by using DARTEL, a fluid deformation algorithm capable of precisely realigning brain structures: DARTEL was one of the four highest-ranking registration methods in an evaluation of 14 non-linear deformation algorithms (12).
Blood pressure and brain volume reduction

Celle, S et al.

References
# Blood pressure and brain volume reduction

Celle, S et al.

## Table S1. Mean values and standard deviations of the different brain volumes

<table>
<thead>
<tr>
<th>Brain volume expressed in cm³</th>
<th>Mean value ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-brain</td>
<td></td>
</tr>
<tr>
<td>Gray matter</td>
<td>639.8 ± 54.8</td>
</tr>
<tr>
<td>White matter</td>
<td>523.2 ± 54.2</td>
</tr>
<tr>
<td>Gray + white matter</td>
<td>1163.0 ± 105.6</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td></td>
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<tr>
<td>Gray matter</td>
<td>152.9 ± 14.3</td>
</tr>
<tr>
<td>White matter</td>
<td>162.3 ± 19.2</td>
</tr>
<tr>
<td></td>
<td>0.60 ± 0.07</td>
</tr>
<tr>
<td>Left supplementary motor area</td>
<td>0.34 ±0.04</td>
</tr>
<tr>
<td>Left superior frontal gyrus</td>
<td>0.50 ±0.07</td>
</tr>
<tr>
<td>Left middle frontal gyrus</td>
<td></td>
</tr>
</tbody>
</table>