ONLINE SUPPLEMENT
ASSOCIATION BETWEEN AMBULATORY 24-H BLOOD PRESSURE LEVELS AND BRAIN VOLUME REDUCTION: A CROSS-SECTIONAL ELDERLY POPULATION-BASED STUDY

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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.
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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).
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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>
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<tbody>
<tr>
<td>Whole-brain</td>
<td></td>
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<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>
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<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>
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<tr>
<td>Left supplementary motor area</td>
<td>0.34 ± 0.04</td>
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<tr>
<td>Left superior frontal gyrus</td>
<td>0.50 ± 0.07</td>
</tr>
<tr>
<td>Left middle frontal gyrus</td>
<td></td>
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</tbody>
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