Original Article

N-Acetylaspartate/Creatine and Choline/Creatine Ratios in the Thalami, Insular Cortex and White Matter as Markers of Hypertension and Cognitive Impairment in the Elderly

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Our objective was to investigate the influence of hypertension on *N*-acetylaspartate (NAA) and choline (Cho) ratios in brain tissues in a community-dwelling elderly population. Brain flexibility was also evaluated with regard to the same metabolite ratios. Proton magnetic resonance spectroscopy (MRS) and the Trail Making Test (TMT) were performed in 80 subjects (75.7±4 years old) from the Three-City Study. Fifty-eight participants had hypertension. The NAA/creatine (Cr) and Cho/Cr ratios were obtained in the insular cortex, the thalami and the deep periventricular white matter. In addition, the B-A score of the TMT was evaluated. Uni- and multi-variate analyses were performed in order to examine the relationships among these data. In the insula and the thalami of the hypertensive group, NAA/Cr ratios were significantly lower (1.39±0.23 and 1.52±0.23, respectively; $p=0.01$) than those in the normotensive control goup (1.52±0.25 and 1.70±0.19, respectively; $p<0.0001$), whereas no such reduction was observed in the periventricular white matter of older hypertensive brains. Moreover, the NAA or Cho ratios were significantly correlated with the TMT B-A scores at the level of the thalami, insula and periventricular white matter. These statistical results were confirmed by the multivariate analysis. In an elderly population, hypertension leads to a reduction in NAA/Cr ratios in the insula and the thalami, possibly due to a decrease in blood flow through small perforating and cortical arteries. The TMT B-A test appears to be relevant not only for the frontal areas but also for more remote areas such as the thalami, the insula and the deep periventricular white matter. (*Hypertens Res* 2008; 31: 1851–1857)

***Key Words*: hypertension, proton magnetic resonance spectroscopy, Trail Making Test, elderly**

Introduction

There are still many unanswered questions concerning the origin and consequences of metabolic damage caused in gray and white matter during aging. A number of brain impairments may be related to aging, including brain atrophy, which has been shown to be associated with hypertension (*1*). Hypertension is also known to induce cognitive impairment. Previous studies using resting-state [^{15}O]water positron-emission tomography (PET) and ^{133}xenon CT have revealed a decrease in cerebral blood flow in many regions of the brain

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(*2*, *3*). Fujishima *et al*. (*4*) and Mentis *et al*. (*5*) have shown a decrease of glucose metabolism in the basal ganglia. *N*-Acetylaspartate (NAA) detection in brain tissue with the help of proton spectroscopy allows for an accurate assessment of neuronal functional loss (*6*) because NAA is thought to be confined to neurons and neuronal processes (*7*). NAA is also thought to be involved in brain energy metabolism since its production is coupled to energy production in brain mitochondria (*8*). The two other metabolites measured using spectroscopy are choline (Cho), which is related to cell membrane intergrity, and creatine (Cr), which is an indicator of oxidative metabolism (*9*). Proton magnetic resonance spectroscopy has been used to demonstrate the metabolic impairment in infarcted brain, as well as in the ischemic penumbra and in vascular dementia. Few spectroscopic studies have reported metabolic brain changes in cortical and subcortical gray matter of hypertensive individuals. The present study performed magnetic resonance spectroscopy (MRS) of gray matter in elderly subjects without history of stroke. Non-demented elderly subjects were included in this MRS sub-study of the Three-City Study (Dijon, Bordeaux and Montpellier, France) cohort of 9,809 non-institutionalized individuals (*10*).

Because PET and xenon CT techniques have shown low cerebral metabolism in the cortex, the thalami and the white matter of hypertensive patients (*2–4*), MRS analysis was concentrated on these regions. In addition, the insular cortex, a key site of limbic autonomic integration that is also involved in the circadian blood pressure regulation (*11–13*), was included in the analysis.

Methods

Population Selection and Medical Data Collection

In Dijon, participants were first examined at home through an interview and cognitive testing. They were subsequently invited to the study examination center to complete the other parts of the study protocol; those who refused or were unable to visit the center received a second visit at home. Data were collected during a face-to-face interview using a standardized questionnaire administered by trained psychologists or nurses. Blood pressure was measured twice at home during the interview by a trained lay interviewer after the subject had rested for at least 5 min in a seated position, using an appropriately sized cuff placed on the right arm and a validated digital electronic tensiometer (OMRON M4; OMRON Corp., Kyoto, Japan). The mean of the two measures was used for the statistical analyses.

Subjects with diabetes mellitus, hyperlipidemia, carotid stenosis, or a history of cerebrovascular accident were excluded. A sample of 80 subjects from Dijon who had volunteered for the Three-City Study agreed to participate in the MRS sub-study. Within this sub-sample of 80 volunteers (women/men: 47/33), 58 (women/men: 31/27) were receiving antihypertensive treatment or had two measurements over 140 mmHg for the systolic blood pressure or 90 mmHg for the diastolic blood pressure. Body-mass index (BMI) was calculated for each participant as weight in kilograms divided by the square of height in meters. The mean (±SD) duration of hypertension in the hypertensive group was 18.4±12 years. The normotensive group was composed of the remaining 22 participants (women/men: 15/7) who had normal blood pressure (<140/90 mmHg) and were not receiving blood pressure lowering drugs. The study protocol was approved by the ethical committee of Dijon University Hospital, and written informed consent for inclusion was obtained from all subjects.

Neuropsychological Evaluations

Participants were given the Mini-Mental State Examination (MMSE) (*14*) to assess global cognitive functions and the Trail Making Test (TMT) (*15*) to investigate sub-cortical dysfunction such as sequence alternation, cognitive flexibility, visual search, motor performance, and executive function (*16*). Part A of the test evaluated visuospatial ability by asking the subject to connect numbers from 1 to 25 in ascending order; part B was used to test executive function by asking the subjects to alternate numbers and letters in ascending order. The variable calculated was the difference between the times required to perform parts A and B. This switch score is free from the influence of processing speed (*16*) and has high test-retest reliability (*17*).

A high score for the trail making B – A (TMT B-A) indicated a poor performance.

Magnetic Resonance Techniques

A 1.5-T whole-body magnetic resonance (MR) imager (Magnetom Vision; Siemens, Erlangen, Germany) with a standard head coil was employed in this study. The imaging protocol included axial inversion recovery (turbo spin-echo; T_E 14/85 ms, T_R 5,000 ms, T_I 200 ms, slice thickness 5 mm) as well as axial T_2-weighted (turbo spin-echo; T_E 16/98 ms, T_R 4,400 ms, slice thickness 3 mm) and 3D T_1-weighted scans (coronal MP RAGE; T_R 9.7 ms, T_E 4 ms, flip angle 10°, T_I 600 ms, slice thickness 1 mm). After standard MRI, the basal ganglia plane was selected for chemical shift imaging (CSI) based on the point-resolved spatially localized spectroscopy (PRESS) technique (T_E=135 ms, T_R=1.5 s, slice thickness 15 mm). Over a field of view of 240 mm, 16×16 partitions were acquired, giving voxel dimensions of 15×15×15 mm (3.4 mL). Before spectra acquisition, water suppression was achieved by applying chemical-shift–selective saturation pulses. The CSI data were processed using the spectroscopy analysis package jMRUI v2.2 for Java (Magnetic Resonance User Interface: http://www.mrui.uab.es/). The residual water resonance was removed using the HSVD filter routine. Peak detection and quantitation were performed in the time domain using a VARPRO-like algorithm called AMARES

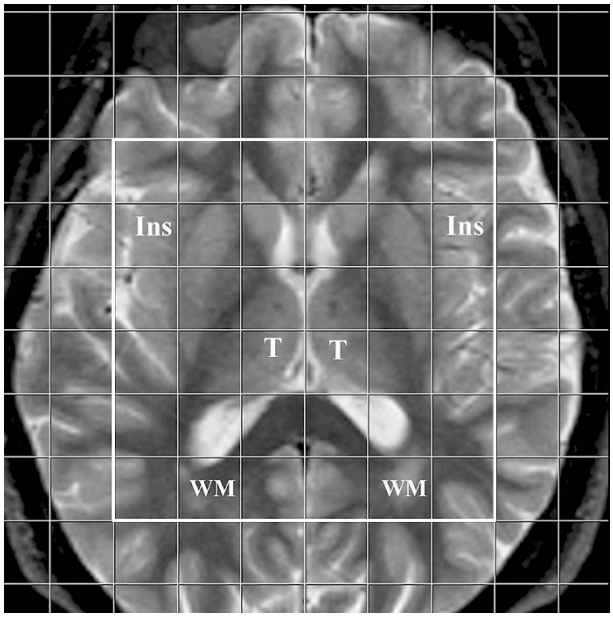

Fig. 1. *Axial T_1-weighted MRI (turbo spin-echo inversion recovery) through the basal ganglia plane. The large white square indicates the position of the PRESS volume of interest, and letters depict the location of chemical shift imaging voxels for the thalami (T), the insular cortex (Ins) and the parieto-occipital trigone of the periventricular white matter (WM).*

(Advanced Method for Accurate, Robust and Efficient Spectral fitting of MRS data with use of prior knowledge). The resonances quantified in each metabolite spectrum were the NAA peak at 2.02 ppm, the Cr peak at 3.02 ppm, and the Cho peak at 3.20 ppm. The use of a long T_E (135 ms) in this CSI sequence has the advantage of providing a spectrum dominated by the three peaks of interest (NAA, Cho, and Cr), with little contribution from other, faster relaxing metabolites that have a tendency to clutterthe profile in shorter T_E acquisition. The NAA and Cho data were expressed as metabolite ratios with respect to the Cr peak for the gray matter of the thalami, the insular cortex, and the parieto-occipital trigone of the periventricular white matter (Fig. 1).

Statistical Analyses

Metabolite ratios in each location (thalami, insula and white matter) were compared between hypertensive and normotensive subjects using an analysis of variance. We used univariate analysis to identify the variables that predicted changes in brain metabolic ratios. We then carried out multivariate analysis, including the parameters with a significance level ≤0.20 (*18*). Uni- and multivariate tests took into account the correlated nature of the data by using generalized estimating equations or mixed models (*19*).

Because of the plausible influence of age on the TMT B-A (*16*), we looked for this relationship by performing a univariate linear regression between the two variables. Owing to the non-normal distribution of age, BMI, NAA/Cr and Cho/Cr ratios, log-transformed versions of these variables were used. This procedure also stabilized the variance.

Statistical analysis was performed using STATA for Windows (version 9.0) (STATA Corp LP, College Station, USA). For all tests, a p value of <0.05 was considered statistically significant.

Table 1. Demographic Characteristics of the Participants

Subject characteristics	Values*
Age, years	75.8±4.1 (66–84)
Women/men	47/33
Body mass index, kg/m^2	25.4±3.6 (19–37)
SBP, mmHg	149±24.1 (93–261)
DBP, mmHg	84.6±11.2 (61–131)
With hypertension†	
n (%)	58 (72)
Women/men	31/27
Mean duration, years	18.4±12.0 (3–53)
Use of antihypertensive drugs	39
Without hypertension, *n* (%)	22 (28)
Women/men	15/7
Tobacco use, *n* (%)	
Never	51 (63)
Smoker or ex-smoker	29 (36)

*Mean±SD and range, if applicable. †SBP≥140 or DBP≥90 mmHg or use of antihypertensive drugs. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Results

Subject characteristics are shown in Table 1.

In the Thalami

In the thalami of the hypertensive group, the mean NAA/Cr ratio was 1.52±0.23 *vs*. 1.70±0.19 in normotensive subjects ($p<0.0001$). According to the univariate analyses, NAA/Cr was significantly correlated ($p<0.05$) with hypertension and the TMT B-A. A weaker non-statistically significant correlation ($0.05<p<0.20$) was also observed between this metabolite ratio and sex or duration of hypertension. However, NAA/Cr was not found to be associated with BMI, MMSE, age or smoking.

Four variables (hypertension, TMT B-A, sex and duration of hypertension) were chosen for the multiple regression analysis, of which only hypertension ($\beta=-0.124\pm0.032$ [SEM], $p<0.0001$) and TMT B-A ($\beta=-0.0008\pm0.0002$, $p=0.002$) remained statistically significant for NAA/Cr.

In the univariate analysis, Cho/Cr was significantly correlated with sex. A weaker correlation was also observed with

TMT B-A. However, no association was seen between Cho/Cr and hypertension, BMI, MMSE, or age. In the multivariate analysis that included sex and TMT B-A, only sex ($\beta=-0.093\pm0.033$, $p=0.005$) remained statistically significant for Cho/Cr.

In the Insular Cortex

The mean (±SD) NAA/Cr ratio in the insular cortex was 1.39±0.23 in hypertensive subjects and 1.52±0.25 in the normotensive subjects ($p=0.01$). According to the univariate analyses, NAA/Cr was significantly correlated ($p<0.05$) with hypertension and BMI. A weaker correlation ($0.05<p<0.20$) was observed between this metabolite ratio and TMT B-A. No association was observed between NAA/Cr and MMSE, age, sex, duration of hypertension, or smoking.

Three variables (hypertension, BMI and TMT B-A) were chosen for the multiple regression analysis in which only hypertension ($\beta=-0.087\pm0.036$ [SEM], $p=0.015$) and TMT B-A ($\beta=-0.0006\pm0.0002$, $p=0.015$) remained statistically significant for NAA/Cr.

In the univariate analysis, Cho/Cr was significantly correlated with sex. A weaker correlation was observed with BMI, whereas no association was seen between Cho/Cr and hypertension, MMSE, TMT B-A, age, or smoking. No variables remained statistically significant for Cho/Cr after their integration into the multivariate model analysis.

In White Matter

According to the univariate analyses, only a weak correlation ($0.05<p<0.20$) was observed between NAA/Cr and hypertension, TMT B-A and BMI. The NAA metabolite ratio was not correlated with sex, MMSE, age, duration of hypertension, or smoking. Unsurprisingly, no variables attained statistical significance for NAA/Cr after their integration into the multivariate model analysis.

In the univariate analysis, Cho/Cr was significantly correlated with TMT B-A ($p=0.02$). A weak correlation ($0.05<p<0.20$) was also observed with MMSE and sex. On the other hand, no association was seen between Cho/Cr and hypertension, BMI, age, or smoking. In a multivariate analysis including sex, MMSE and TMT B-A, only sex ($\beta=-0.081\pm0.038$ [SEM], $p=0.035$) and TMT B-A ($\beta=0.0006\pm0.0002$, $p=0.009$) remained statistically significant for Cho/Cr.

Table 2 summarizes the results of the neuropsychological evaluations and the spectroscopy, and Table 3 compiles the results of the multiple regression analyses of explanatory variables for the NAA/Cr and Cho/Cr ratios.

TMT B-A *vs.* Age

It is worth noting that the TMT B-A variable was not sensitive to the effects of aging ($p=0.422$).

Table 2. Results of Neuropsychological Evaluations and Spectroscopy

Neuropsychological evaluations and spectroscopy	Values*	
	NAA/Cr	Cho/Cr
MMSE score	28.03±1.34 (23–30)	
Trail Making Test (B − A), s	60±45 (1–223)	
Thalami		
Hypertension	1.52±0.23	1.09±0.18
No hypertension	1.70±0.19	1.09±0.14
Women	1.53±0.22	1.04±0.15
Men	1.61±0.28	1.13±0.17
Insula		
Hypertension	1.39±0.23	0.90±0.17
No hypertension	1.52±0.25	0.93±0.13
Women	1.46±0.30	0.91±0.18
Men	1.45±0.25	0.95±0.18
White matter		
Hypertension	1.68±0.30	1.16±0.25
No hypertension	1.78±0.31	1.16±0.20
Women	1.71±0.26	1.15±0.19
Men	1.80±0.42	1.29±0.27

*Mean±SD. MMSE, Mini-Mental State Examination; NAA, *N*-acetylaspartate; Cr, creatine; Cho, choline.

Discussion

The relationship between hypertension and lower NAA/Cr in the thalami and the insular cortex has been demonstrated in a subgroup of the Three-City Study. Hypertension, found in 65% of all subjects in the Three-City Study (*11*), is the main risk factor for cerebral small vessel disease (*20*). Cerebral small vessel disease induces silent ischemia in both the perforating arteries of the thalami and the leptomeningeal or cortical vessels of the insula. The long-term functional outcome of cerebral small vessel disease is characterized by a significantly increased risk of cognitive impairment (*21*). This might be explained by the fact that, in the presence of mild or severe arteriosclerosis/lipohyalinosis, there is simultaneous involvement of thalamic and cortical small vessels as shown in autopsy brains by Thal *et al*. (*22*). Moreover, previous studies using PET (*3*, *4*) have confirmed a reduction in the regional cerebral blood flow in the cortex and the thalami of elderly hypertensive brains. Thus, the decrease in the NAA ratio in the hypertensive group may be a consequence of hypoperfusion.

Another possible interpretation for the differences of NAA/Cr between the hypertensive and normotensive subjects is that hypertension is noxious with respect to cerebral white matter and contributes to leukoaraiosis (*23*). Hence, hypertension might alter the neuronal network linking cortical areas and deep gray nuclei. Myelin damage within the white matter is responsible for physiological dysfunction in the subcortical

Table 3. Results of the Multivariate Analysis of Explanatory Variables for NAA/Cr and Cho/Cr

	NAA/Cr		Cho/Cr	
	β	p	β	p
Hypertension	Thalami		—	
	−0.124±0.032	<0.001		
	Insula			
	−0.087±0.036	0.015		
Trail Making Test (B − A)	Thalami		White matter	
	−0.0008±0.0002	0.002	0.0006±0.0002	0.009
	Insula			
	−0.0006±0.0002	0.015		
Sex	—		Thalami	
			−0.093±0.033	0.005
			White matter	
			−0.081±0.038	0.035

Data are mean±SEM. NAA, *N*-acetylaspartate; Cr, creatine; Cho, choline.

and cortical gray matter (*24*, *25*), as revealed by electrical (EEG) (*26*) and metabolic (PET (*27*) and MRS (*28–30*)) studies. The disruption of the neuronal signal may, in turn, induce an alteration in the metabolism of both the thalami and the insular cortex.

In a previous retrospective study of 59 patients with cortical middle cerebral artery stroke, we found that NAA/Cho in normal contralateral cerebral tissue was lower in patients who had a history of hypertension (*31*). The correlation with age and sex disappeared with multivariate analysis. The previous findings are in accordance with those of the present study and emphasize the role of hypertension in neuronal loss in the brain.

Age is a possible cause of gray-matter impairment both in the cortex and in the subcortical areas. It has previously been shown that increasing age predicts a lower cortical and subcortical gray-matter volume in the thalami and insula (*32*). As a consequence, the decrease of NAA/Cr in the hypertensive group in the present study may be partially explained by age-dependent gray-matter atrophy. However, a relationship between age and NAA/Cr was not established by the univariate analysis in the present study, nor has it been demonstrated in other studies (*9*, *33*, *34*).

We found that insular NAA/Cr and periventricular white-matter Cho/Cr ratios were independently associated with the TMT B-A. The TMT is a sensitive indicator of executive function (*35*) and therefore a good estimator of frontal lobe function. Interestingly, functional MRI performed during the trail making procedure has shown not only frontal activity but also a bilateral insular activation (*36*). Several studies have shown that white-matter integrity was related to TMT performance (*26*, *37*, *38*). All these findings reinforce the role of the insular cortex and the periventricular white matter in TMT performance. In this sub-population of the Three-City Study, all subjects were younger than 85 years (range: 66–84 years), and we did not observe any effect of age on the TMT B-A score. This result is consistent with the work of Hashimoto *et al*. (*39*) and Hester *et al*. (*40*), who found no significant difference between groups in this age bracket.

The present study found that a reduced NAA/Cr ratio in both the thalami and the insular cortex seems to be associated with both hypertension and TMT performance. Since the insular cortex mediates arterial blood pressure (*11–13*), it is legitimate to ask whether metabolic disorders in the insula of hypertensives are a cause or a consequence of the hypertension. Unfortunately, the cross-sectional study design and the antihypertensive drugs taken by members of the cohort prevent us from formulating a direct response to this question. The insular cortex is also involved in TMT performance, and the metabolic alteration of this brain region may be an important marker of aging in the brain. Because Cho/Cr in white matter is associated with TMT performance, but not with hypertension, there may be different metabolic patterns associated with chronic hypertension and the impairment of executive cognitive function. Alteration of cognitive function may require the cumulative effects of neuronal loss in gray matter and of reduced connectivity represented by the white matter. Sijens *et al*. (*41*) observed that a decrease of Cho was only seen in the brains of elderly women. This may be related to our finding that sex was related only to the Cho/Cr ratio. The hypothesis put forward by Sijens *et al*. (*41*) regarding the drop of Cho in women is a loss of myelin lipids, which is reported to be particularly severe in female brains after the age of 70 years (*42*).

In summary, hypertension causes a reduction of NAA ratios in the insular cortex and in the thalami of the community-dwelling elderly, but this neuronal marker is not affected in periventricular white matter. The TMT B-A results are not related to age in this study, but this switch test is associated with a metabolic pattern similar to that induced by hypertension. Interactions between hypertension and alteration of cognitive function may be complex and deserve further

longitudinal studies of patients during the aging process.

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