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RESEARCH ARTICLE

# Magnetic resonance markers of bilateral neuronal metabolic dysfunction in patients with unilateral internal carotid artery occlusion

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## Abstract

**Objectives** To evaluate cerebral hemodynamic, metabolic and anatomic changes occurring in patients with unilateral occlusion of the internal carotid artery (ICA).

**Materials and methods** Twenty-two patients with unilateral occlusion of ICA and twenty age and sex matched healthy subjects were included in the study. Single voxel proton magnetic resonance spectroscopy ($^1$H-MRS) of the centrum semiovale, semi-automated hippocampal volumetry in T1-weighted scans and transcranial Doppler examination (TCD) with calculation of Breath Holding Index (BHI) were performed in both groups. Metabolic, anatomic, and hemodynamic features were compared between the two groups.

**Results** The *N*-acetylaspartate (NAA)/choline (Cho) ratio was significantly lower in both hemispheres of enrolled patients compared to controls ($p = 0.005$ for the side with occlusion, $p = 0.04$ for the side without occlusion). The hippocampus volume was significantly reduced bilaterally in patients compared to healthy subjects ($p = 0.049$). A statistically significant difference in BHI values was observed between the side with occlusion and without occlusion ($p = 0.037$) of the patients, as well as between BHI values of the side with occlusion and healthy volunteers ($p = 0.014$).

**Discussion** Patients with unilateral ICA occlusion have reduced NAA/Cho ratio in the white matter of both hemispheres and have bilateral atrophy of hippocampus. The alteration of hemodynamics alone cannot explain these changes.

**Keywords** Internal carotid artery · Magnetic resonance spectroscopy · Choline · *N*-Acetylaspartate · Hippocampus

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## Introduction

Cerebrovascular disease is the third leading cause of death in developed countries [1]. Approximately 85% of strokes are ischemic and about 20% of these originate from large artery stenosis [2, 3]. Occlusion of the internal carotid artery (ICA) is a known risk factor for stroke in the ipsilateral hemisphere [4]. The overall risk of subsequent stroke in the presence of carotid occlusion is 5–7% per year and the risk of stroke ipsilateral to the occluded carotid artery is 2–6% per year [5], increasing to 10–20% per year in the subgroup of patients with significant impairment in cerebrovascular hemodynamics [6, 7].

A widely accepted and easily reproduced method of evaluating hemodynamic status in patients with carotid occlusion is the assessment of cerebral vasomotor reactivity by measuring cerebral blood flow (CBF) with transcranial Doppler (TCD) sonography. The examination can be performed in physiological conditions and repeated after a stress challenge (hypoventilation, administration of $CO_2$ or acetazolamid) in order to evaluate the residual capacity of the cerebrovascular system maintaining adequate perfusion [8]. However, this method alone has a low specificity, as it cannot distinguish between reduced CBF caused by the hemodynamic effects of arterial occlusion and compensatory physiological reductions in CBF caused by reduced metabolic demands of a chronically damaged tissue [9]. Integration of a method capable of revealing metabolic and anatomical changes may help to discern these conditions.

Proton magnetic resonance spectroscopy ($^1$H-MRS) is a non-invasive method of measuring in vivo concentrations of several intracerebral metabolites in order to investigate metabolic alterations associated with various cerebral diseases [10]. Since metabolic changes in white and grey matter play an important role in the cascade of events leading to stroke, the option of non-invasively detecting these changes may also help understand the complex pathophysiologic mechanisms underlying this disease and potentially identify high-risk patients for future stroke [11–13].

Multimodal integration of different diagnostic methods is therefore mandatory in order to throw light on the wide spectrum of anatomic, hemodynamic and metabolic changes occurring in patients with cerebrovascular disease and further studies investigating relationships between these diagnostic modalities are welcome. The main goal of this study was to compare results of TCD examination, single voxel quantitative $^1$H-MRS and semi-automated MR hippocampal volumetry in a group of patients with unilateral carotid occlusion with a matched cohort of healthy subjects. Concentrations of *N*-acetylaspartate (NAA), choline (Cho), creatine (Cr) and lactate (Lac) and their ratios were compared in the affected and contralateral hemisphere. Afterwards, they were also compared with healthy subjects, and correlated with TCD results and hippocampal volume measurements.

## Materials and methods

A total of 36 patients with unilateral occlusion of the ICA were enrolled in this study between March 2016 and June 2017. The diagnosis of ICA occlusion was confirmed by CT angiography. Occlusion was defined as complete obliteration of the vessel without detectable contrast enhancement distally. Exclusion criteria were: a modified Rankin score higher than 1, more than 50% contralateral ICA stenosis on CT angiography, the presence of any concurrent disease of the central nervous system, a history of smoking, alcohol or drug abuse, a body mass index higher than 30. After selection, a total of 22 patients were included in the study (16 male and 6 female subjects, mean age $62 \pm 7$). The occlusion was asymptomatic in 8 patients, the remaining subjects suffered a brain ischemic event 6 months prior to enrolment or earlier (minor stroke event in 5 patients, transitory ischemic attack in 9 patients). No patient showed a degree of disability higher than 1 according to the modified Rankin Scale (mRS = 0 in 17 subjects, mRS = 1 in 5 subjects). Eight subjects showed some degree of contralateral carotid stenosis (20% $N = 2$, 30% $N = 2$, 40% $N = 3$, 50% $N = 1$). Regarding comorbidities, nineteen patients suffered from hypertension, 12 patients had hypercholesterolemia, 8 patients showed diabetes and 6 patients had a history of ischemic heart disease. A group of 20 healthy controls age- and sex-matched were recruited for this study as well (10 male and 10 female subjects, mean age $63 \pm 8$). In the control group, carotid arteries were examined by Ultrasound to reduce radiation burden in healthy volunteers. Exclusion criteria for the control group were: more than 50% ICA stenosis on any side, a history of stroke or other neurologic disorders, smoking or drug abuse, a body mass index higher than 30. In the control group, 7 subjects had carotid stenosis under 50% on either side (20% $N = 3$, 30% $N = 2$, 40% $N = 2$). Comorbidities were distributed as follows: hypertension in 7 subjects, hypercholesterolemia in 5 subjects, diabetes in 2 subjects, ischemic heart disease in 1 subject. For all the diagnostic modalities, only one hemisphere per control subject was included in the analysis ($N = 20$) to match the size of the patient subgroups: ipsilateral ($N = 22$) and contralateral ($N = 22$) hemisphere in respect to the side of occlusion. In the 7 control subjects with carotid stenosis, attention was paid to select the hemisphere ipsilateral to the carotid artery without any degree of stenosis, otherwise the left hemisphere was arbitrarily used. The main demographic and neurological features of the patient group are shown in Table 1.

**Table 1** Demographic, neurological and clinical features in the patient group. TIA stands for Transitory Ischemic Attack

| | Sex | Age | Side of ICA occlusion | Clinical manifestation | Modified Rankin Scale | Hypertension | Hypercholesterolemia | Diabetes | Ischemic heart disease |
|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 62 | R | Stroke | 1 | Yes | Yes | No | No |
| 2 | M | 68 | L | Asymptomatic | 0 | No | No | no | Yes |
| 3 | F | 70 | L | TIA | 0 | Yes | Yes | No | Yes |
| 4 | M | 53 | R | TIA | 0 | Yes | Yes | No | No |
| 5 | F | 72 | L | TIA | 0 | Yes | Yes | No | No |
| 6 | F | 48 | R | TIA | 0 | Yes | No | No | No |
| 7 | M | 54 | L | TIA | 0 | No | No | No | No |
| 8 | F | 63 | R | Asymptomatic | 0 | Yes | No | No | No |
| 9 | M | 49 | L | Stroke | 1 | Yes | Yes | No | No |
| 10 | M | 67 | R | Stroke | 1 | Yes | No | Yes | Yes |
| 11 | M | 59 | L | Stroke | 1 | Yes | Yes | Yes | No |
| 12 | M | 64 | R | Asymptomatic | 0 | Yes | Yes | Yes | No |
| 13 | M | 61 | R | Stroke | 1 | Yes | No | Yes | Yes |
| 14 | M | 62 | L | Asymptomatic | 0 | Yes | Yes | Yes | No |
| 15 | F | 66 | R | TIA | 0 | Yes | Yes | Yes | Yes |
| 16 | M | 63 | R | TIA | 0 | No | No | No | No |
| 17 | M | 74 | L | Asymptomatic | 0 | Yes | Yes | Yes | No |
| 18 | M | 60 | R | TIA | 0 | Yes | Yes | Yes | No |
| 19 | M | 62 | L | Asymptomatic | 0 | Yes | No | No | Yes |
| 20 | M | 71 | R | Asymptomatic | 0 | Yes | No | No | No |
| 21 | F | 60 | L | Asymptomatic | 0 | Yes | Yes | No | No |
| 22 | M | 64 | L | TIA | 0 | Yes | No | No | No |

## TCD ultrasound examination

The patients were examined using a TCD ultrasound device with a 2 MHz probe (Toshiba Nemio, Toshiba, Tokyo, Japan). The following parameters were measured via the thin bone of the temporal region on middle cerebral arteries of both sides: peak systolic velocity, mean flow velocity, end diastolic velocity, resistance index, pulsatility index. These parameters were measured thrice during normal calm breathing and thrice after breath-holding for the maximal possible time in an alternating manner. There was always a minimum 2-min pause between the breath-holding and the following resting measurements. The total breath-hold time was noted. The measured parameters were then used to calculate an average breath holding index (BHI) separately for each hemisphere [14] using the following formula: $\frac{\Delta V}{t}$ where $\Delta V$ is the percentage increase in middle cerebral artery blood flow velocity occurring during breath-holding and $t$ is the total breath-hold time.

## MR semi-automated hippocampal volumetry

The input MR data for volumetry was acquired on 3 T Signa HDxt MR scanner (GE Healthcare, Waukesha, WI) using a body coil for transmission and 8-channel phased-array receiver head coil for reception. Product BRAVO sequence (which is magnetization prepared T1-weighted 3D gradient echo) with parameters TR/TI/TE = 10.876/450/4.588, voxel size $0.4688 \times 0.4688 \times 1$ mm, was used. Hippocampal volume was measured by means of Freesurfer software [15]. Automatic measurement was performed as the initial step, with manual correction by a single operator using all three anatomical planes simultaneously in ITK-SNAP software [16]. The anterior border of the hippocampus was determined by the alveus, the posterior part by the lateral ventricle (fornix in full length in coronal planes). Superior and lateral borders were defined by cerebrospinal fluid (CSF). White matter of the parahippocampal gyrus separated from grey matter of the hippocampus created the inferior border. The inferior medial boundary was drawn by cutting the subiculum from the cortex of the parahippocampal gyrus, the superior medial by CSF of the cisterna ambiens. Sagittal planes were used to identify precise hippocampal borders [17] and uncertain regions, where separation from adjacent brain structures was less clear, were checked in coronal plane. Typically, demarcation of hippocampal borders was more challenging in the anterior part (separation from the amygdala) and in the posteromedial region, which

is adjacent to posterior thalamic areas. Ten subjects were measured twice to assess intra-operator variability. Anatomical boundaries were compared to an overview provided by Konrad et al. [18]. An example of manual correction of the primary automated output volume is given in Fig. 1.

## $^{1}$H-MRS study

Single voxel $^{1}$H MRS was performed on the same 3 T MR scanner and by the same coil by means of standard single-voxel product PROBE-P point-resolved spectroscopy sequence (GE Healthcare) with water suppression. Using T1-weighted images as anatomical reference, two cuboid volumes of interest (VOI) were placed symmetrically to the midline plane along the long axis of the centrum semiovale of right and left hemisphere excluding the lateral ventricles and minimizing grey matter inclusion (Fig. 2). Meticulous attention was paid to exclude areas of altered T1 signal, in order to not include possible postischemic lesions. Mean volume of the VOI in the whole cohort was $14.8 \pm 1.5$ ml, average dimensions of the VOI were: $50.1 \pm 2.6 \times 19.7 \pm 1.1 \times 15.0 \pm 0.8$ mm. The shimming was done by automatic built-in procedure. The quality of automatic shim process was evaluated by monitoring the FWHM (full width at half-maximum) outputs from measured VOI as an indicator of the linewidth of unsuppressed water signal. Shim settings were manually refined by adjusting first-order shim currents to improve shim quality as assessed by the resulting FWHM in the corresponding adjustment user interface. The following parameters were used: TR/TE = 1500/144 ms, $\tau$ (delay between 1st and 2nd excitation pulse) = 12.768 ms, number of signal averages = 128, 8-step phase cycle, vector size = 4096, spectral width = 5000 Hz. Three chemical shift-selective saturation pulses (CHESS [19]) with flip angles different to 90° adapted to T1 of water and timing of sequence were used for water suppression (WET scheme = water suppression enhanced through T1 effects [20]). Four outer volume suppression (OVS) bands parallel to the VOI borders were placed at a distance of approximately 3 cm from the VOI limits to minimize lipid contamination. LCModel [21] was used to combine signals from individual coil elements, for eddy-current correction [22] and for estimating concentrations of brain metabolites. The estimation was based on modelling of the acquired MR signal as linear combination of individual metabolite basis-set signals. The basis-set signals for LCModel were obtained by quantum–mechanical simulation using SIMPSON simulation software [23]. LCModel basis set included the following metabolites

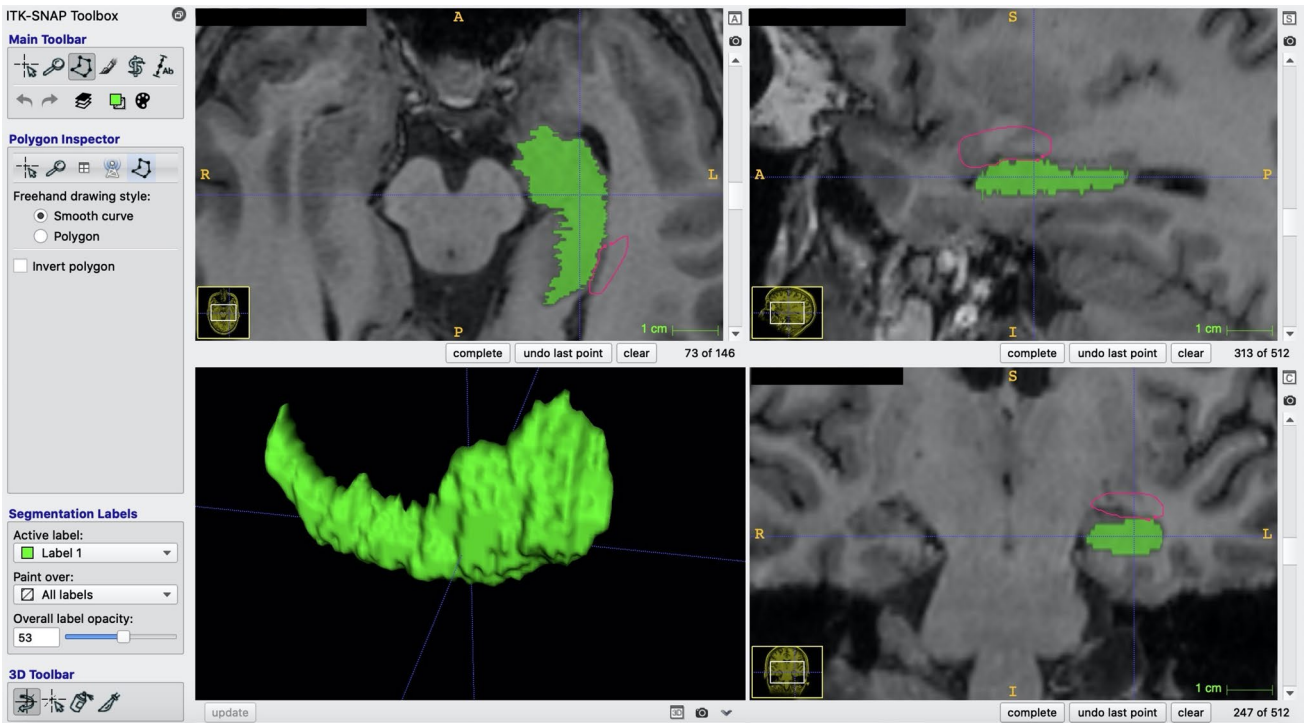


**Fig. 1** An example of manual correction with ITK-SNAP software of the primary hippocampal volume automatically selected by Freesurfer software. From left upper panel proceeding clockwise the axial, sagittal and coronal view are shown. A 3D rendering of the hippocampal volume is pictured in the left lower panel. Anatomical borders of the hippocampus were primarily verified in the sagittal plane (slice by slice), the coronal plane was used to discern uncertain areas. Primary volume in green. Manually drawn line contouring an area to exclude in pink

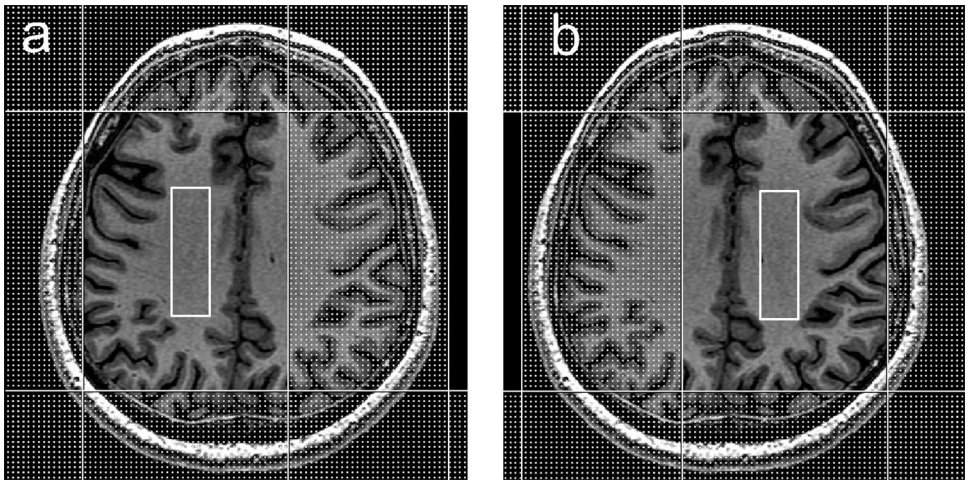


**Fig. 2** Position of the VOI along the long axis of the centrum semiovale of the right (**a**) and left (**b**) hemisphere. The dotted areas represent outer-volume suppression bands

(quantum mechanically simulated): glycerophosphocholine (GPC), phosphocholine (PCho), phosphocreatine (PCr), creatine (Cr), glutamine, glutamate, inositol, scyllo-inositol, lactate (Lac), *N*-acetylaspartate (NAA), *N*-acetylaspartylglutamate (NAAG), taurine, guanine. Lipids and macromolecules were analytically simulated inside LCModel. The following standard simulated lipids and macromolecules setting were used: Lip09, MM09, Lip20, MM20, MM12, MM14, MM17. The fitting range was 0.2–4.2 ppm. After some preliminary testing, zero and first order phasing was slightly constrained by imposing the following parameters: sddegz = 3 (limiting zero order phasing), sddegp = 1 (limiting first order phasing). Eddy current correction and water scaling were on. Overall signal to noise ratio of the measured spectra (as estimated by LCModel) was 17.8 ± 5.0. The preliminary analysis of the LCModel results of all subjects showed strong positive correlation between concentrations of alanine, lipids at 1.3 ppm and Lac due to the fact that Lac signal is of opposite amplitude with respect to other model signals in the region. This led to ill determination of the fit and systematic overestimation of Lac concentration in some subjects. Lipid resonances at 1.3 ppm and alanine were therefore removed from the original basis-set to improve accuracy of Lac concentration estimation. Unsuppressed water signal was acquired with 16 averages (2 × 8-step phase cycles) and used as internal standard for metabolite concentration estimation. The calculated concentrations were corrected for individual water content and T1/T2 relaxation of water in white matter/grey matter/CSF based on the methodology described in Gasparovic et al. [24]. Since the study of Gasparovic was performed at 1.5 T, the T2 relaxation times of water in white and gray matter were derived from Posse et al. [25], which used a 3 T protocol adopting the values from Wansapura et al. [26] and Ethofer et al. [27]. Namely, the following values were used for grey matter, white matter, CSF respectively: T1 relaxation times 1470, 1060, 3000 ms, T2 relaxation times 110, 74, 200 ms. The white matter/grey matter/CSF volume fractions of the VOI, needed for the correction, were determined by co-registration of 3D high-resolution BRAVO MR images segmented in SPM8 [28] and spectroscopic VOI by home-made software written in Java language. Finally, the concentrations of total Cho (GPC + PCho), total Cr (Cr + PCr) and total NAA (NAA + NAAG) were corrected for T1 and T2 relaxation times by using literature values [27, 29, 30]. This correction was not applied to Lac since, to the best of our knowledge, no in-vivo T1 and T2 relaxation times of Lac have been published thus far. Furthermore, other effects occur on J-coupled systems during volume localization MRS, which affect lactate signal intensity [31] and cannot be easily accounted for. Due to these facts, Lac concentrations remained in institutional units. An example of spectrum on the side of occlusion before and after LCModel fitting is shown in Fig. 3.

## Statistical methods

The dataset consisted of continuous variables (e.g. BHI, NAA). Correlation analysis was based on calculation of Spearman rank correlation coefficient (Rs). Given the continuous character of the variables, comparison of data in the following three groups: (a) patients, ipsilateral side; (b) patients, contralateral side; and (c) healthy volunteers (controls) was performed by Kruskal–Wallis test. If the result of Kruskal–Wallis test was significant ($p < 0.05$), Mann–Whitney *U* test was used to analyse differences between individual groups. In the patient group, further subgroup analysis was performed in a similar fashion comparing symptomatic versus asymptomatic subjects and patients with versus without contralateral stenosis. Normality of data distribution was tested by Kolmogorov–Smirnov and Shapiro–Wilk test. All statistical analyses were performed at significance level of alpha = 5% and all the statistical tests used were two-sided. Data was analysed using statistical software package STATISTICA v. 10 (StatSoft Inc., USA).

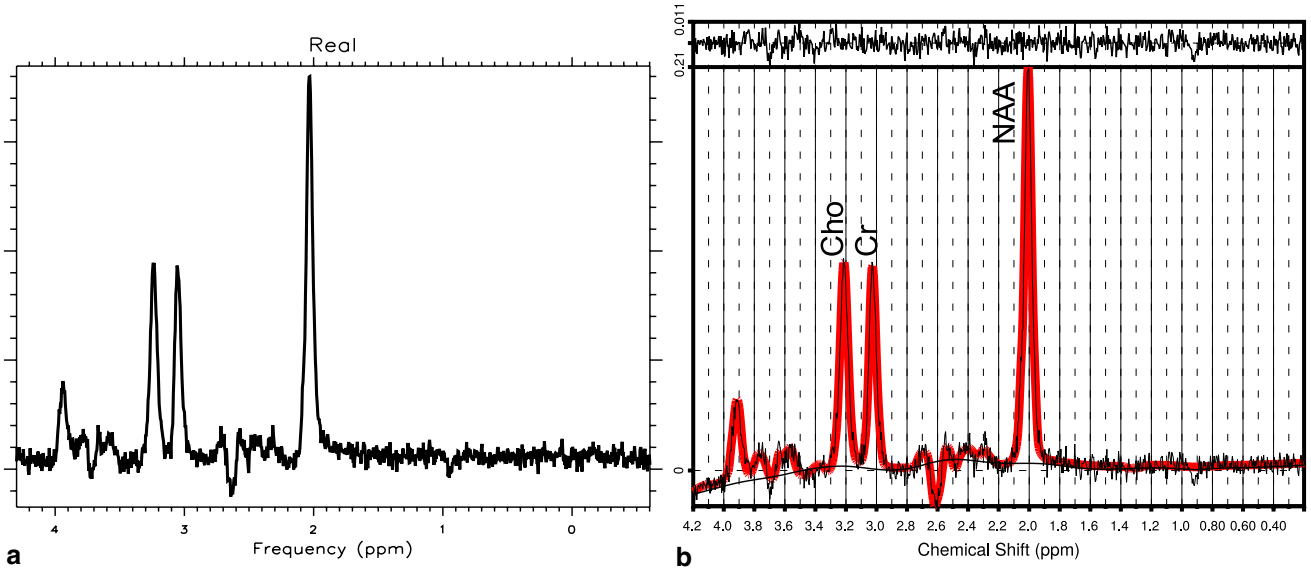


**Fig. 3** An example of spectrum on the side of ICA occlusion before (**a**) and after (**b**) LCModel fitting. In (**a**) the only processing step applied to the measured spectrum was "PROBE-P" reconstruction, which comprises phase correction and combination of signal from coil channels without zero-filling or apodization. In (**b**) note the baseline output from LCModel (smooth black line) and the curve after fitting (red line); the spiked line on the top represents the difference between the fitted line and the measured signal. The three most prominent peaks represent (from left to right)—choline compounds (Cho), creatine (Cr), *N*-acetylaspartate (NAA)

## Results

All patients underwent brain $^{1}$H-MRS and MR hippocampal volumetry. Complete TCD examination with calculation of BHI for both hemispheres was performed in 19 patients. Bilateral measurement was technically not possible in 3 patients because of a lacking temporal bone window. These patients were still included in the 1H-MRS and volumetric analysis to maximize the statistical strength of the MR results. The values of BHI, main metabolite concentration and hippocampal volume of patients and healthy controls are shown in Table 2.

A statistically significant difference in BHI values was observed in patients between the side with occlusion ($0.56 \pm 0.48$) and without occlusion ($0.79 \pm 0.54$) $p = 0.037$, as well as BHI values between the side with occlusion and healthy volunteers ($0.82 \pm 0.47$), $p = 0.014$. BHI results in

**Table 2** Comparison of brain vasoreactivity, metabolic features and hippocampal volumetry in both hemispheres of patients and in control group

| | Side with occlusion | | | | Side without occlusion | | | Healthy controls | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | Mean ± SE | Min/Max | %SD | Mean ± SE | Min/Max | %SD | *N* | Mean ± SE | Min/Max | %SD |
| BHI | 19 | 0.56 ± 0.48*† | 0.08/1.91 | | 0.79 ± 0.54 | 0.09/2.86 | | 20 | 0.82 ± 0.47 | 0.12/2.24 | |
| NAA[a] | 22 | 17.2 ± 5.0* | 9.3/27.9 | 1.8 | 18.1 ± 5.7 | 9.8/28.3 | 1.7 | 20 | 20.4 ± 2.8 | 13.4/25.4 | 1.6 |
| Cho[a] | 22 | 3.2 ± 0.6 | 1.9/4.2 | 2.9 | 3.0 ± 0.8 | 1.4/4.4 | 2.8 | 20 | 3.0 ± 0.4 | 2.0/3.9 | 2.7 |
| Cr[a] | 22 | 12.1 ± 2.9 | 3.7/16.6 | 2.5 | 12.3 ± 3.9 | 4.5/18.1 | 2.4 | 20 | 13.5 ± 1.7 | 9.4/16.2 | 2.6 |
| Lac[a] | 22 | 0.2 ± 0.1 | 0.0/0.4 | 61.3[c] | 0.2 ± 0.1 | 0.0/0.5 | 54.2[c] | 20 | 0.1 ± 0.1 | 0.0/0.4 | 63.7[c] |
| NAA/Cho | 22 | 5.7 ± 0.9* | 3.5/6.9 | | 5.9 ± 0.7* | 4.1/6.8 | | 20 | 6.3 ± 0.6 | 5.1/7.4 | |
| Vol-HIP[b] | 22 | 3287.4 ± 409.6* | 2634/4214 | | 3285.2 ± 416.9 | 2619/4224 | | 20 | 3325 ± 349.8 | 2694/4279 | |

SE stands for standard error, %SD are the estimated standard deviations (Cramer–Rao lower bounds) expressed in percent of the estimated metabolite concentrations

[a]NAA, Cho, Cr and Lac concentrations are expressed in mmol/kg. Lac concentrations were not corrected for relaxation

[b]Hippocampal volume is expressed in $mm^3$

[c]Null values were eliminated before calculation of %SD ($N = 4$ side with occlusion, $N = 5$ side without occlusion, $N = 7$ healthy controls)

*$p < 0.05$ vs control subjects

†$p < 0.05$ vs side without occlusion

the hemisphere without occlusion showed no statistically significant difference in comparison with healthy controls ($p = 0.810$).

Concerning MRS measurements, FWHM outputs from LCModel after shimming correction were $7.28 \pm 1.53$ Hz at the occlusion side, $7.15 \pm 1.53$ Hz contralaterally and $7.03 \pm 1.53$ Hz in control subjects with no statistically significant difference between groups ($p = 0.595$–$0.974$). Regarding cerebral metabolites, Cho, Cr, NAA, Lac and their ratios were statistically tested. No statistically significant difference was observed in NAA values between the side with occlusion ($17.2 \pm 5$ mmol/kg) and without occlusion ($18.1 \pm 5.7$ mmol/kg), $p = 0.493$ of the patient group. There was a significant difference in NAA values between the side with occlusion in the patient group and NAA levels of the healthy volunteers ($20.4 \pm 2.8$ mmol/kg), $p = 0.009$. The difference between NAA levels in patients on the side without occlusion and healthy volunteers did not reach statistical significance ($p = 0.08$). No statistically significant difference in NAA/Cho values was observed in patients between the side with occlusion ($5.7 \pm 0.9$ mmol/kg) and without occlusion ($5.9 \pm 0.7$ mmol/kg), $p = 0.329$. A statistically significant difference was observed between NAA/Cho values at the side with occlusion in patients and healthy volunteers ($6.3 \pm 0.6$ mmol/kg), $p = 0.005$, and between NAA/Cho values at the side without occlusion in patients and healthy volunteers, $p = 0.04$. Lac, Cho, Cr and their ratios showed no statistically significant difference between the affected and contralateral side, nor in comparison with healthy controls. Similarly, NAA/Cr or Cho/Cr values did not show statistically significant difference in the three groups. Three examples of spectra registered on the side of ICA occlusion, contralaterally and in a healthy subject are shown in Fig. 4.

Concerning the results of semi-automated hippocampal volumetry, intra-operator variability was 3%. The values were found to be significantly lower in the hemisphere with occlusion ($3287 \pm 410$ mm$^3$) compared to healthy volunteers ($3325 \pm 350$ mm$^3$), ($p = 0.049$). No significant difference of hippocampal volume values was observed in patients between the side with occlusion and the side without occlusion ($3285 \pm 417$ mm$^3$), ($p = 0.954$). The difference between hippocampal volume in the contralateral side from occlusion and healthy volunteers showed a trend, approaching statistical significance ($p = 0.051$).

Correlation analysis between BHI and $^1$H-MRS parameters and hippocampal volume did not show any significant relationship. No significant difference was found between patient subgroups (symptomatic versus asymptomatic and patients with versus without contralateral stenosis).

## Discussion

This study investigates a group of patients with unilateral occlusion of the ICA, who were asymptomatic or suffered a minor stroke in the past without residual disability. As expected, the BHI was significantly decreased in patients on the side of carotid occlusion. This result confirmed hemodynamic impairment of the ipsilateral hemisphere. The integration of metabolic $^1$H-MRS and anatomic MR data revealed a more widespread impairment also involving the contralateral side.

The main spectroscopic alteration in patients with an ICA occlusion was found to be a significant reduction in NAA concentration on the side of the ICA occlusion compared to healthy controls and a significant reduction of NAA/Cho ratio in both hemispheres of the patient group compared to controls. Van der Grond et al. found the NAA/Cho ratio to be significantly reduced in the hemisphere ipsilateral to carotid occlusion due to higher Cho concentrations, but no difference in NAA values [12]. The discrepancy can be explained by a different methodology: the authors estimated metabolite intensities as pure peak heights, while this study evaluated quantitative MRS results. In the present study, the NAA was

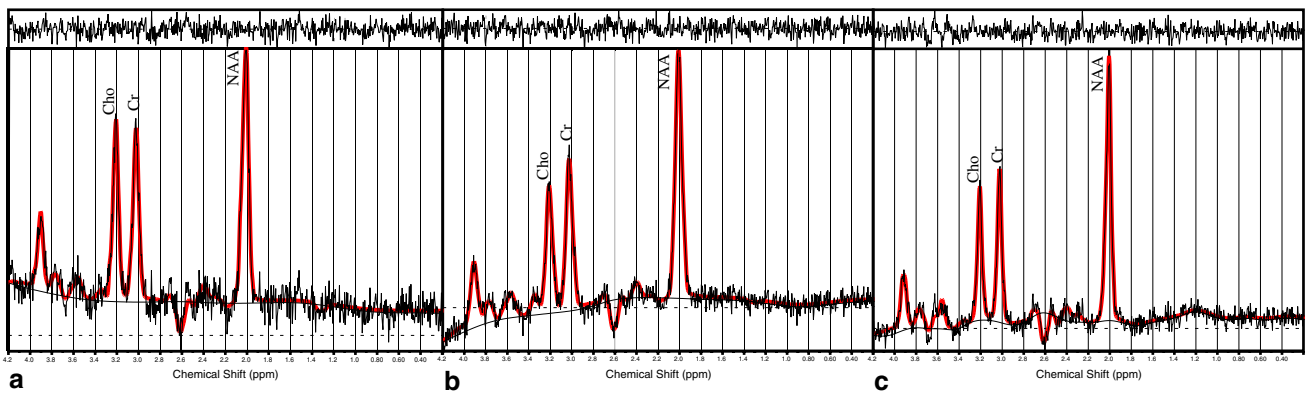


**Fig. 4** Typical spectrum on the side of ICA occlusion (**a**), on the contralateral side (**b**) and in healthy control (**c**)

significantly decreased on the side of ICA occlusion; the contralateral decrease did not reach a significant difference compared to healthy subjects, however demonstrated a trend ($p = 0.08$). The fact that the difference in NAA concentrations between the patients on the side without occlusion and the controls did not reach statistical significance may be the result of a small number of enrolled subjects.

Even though Cho concentration was not significantly higher in any of the hemispheres of patients in comparison with controls, the NAA/Cho ratio was significantly lower in both hemispheres. According to Zhang et al. NAA and NAA/Cho ratio was significantly lower only in the ipsilateral side of the ICA occlusion [32]. A different methodology may explain the discrepancy with the present results. The authors compared and statistically analyzed the relative metabolite concentrations calculated as metabolite under-the-peak areas ratio. In the present study the absolute concentration of each metabolite was obtained and used for statistical testing.

Due to the fact, that NAA is generally considered a neuroaxonal marker and Cho a marker of cell turnover or myelin damage, the results of this study suggest the presence of a neuronal dysfunction in both hemispheres of patients with ICA occlusion without significant active cell destruction. The decrease in NAA concentrations without significant increase of Cho are most likely the result of generalized mitochondrial dysfunction in the neurons of both hemispheres, rather than active neuronal loss, since NAA is synthesized in mitochondria [33]. These results suggest that the metabolic impairment of brain tissue in cases of chronic occlusion of a main arterial cerebral trunk may not be limited to the affected side but could also involve the contralateral hemisphere. Multiple factors can contribute to this bilateral affection. Firstly, the fact that unilateral occlusion of the main vessel for brain blood supply may cause impoverishment of the contralateral hemisphere via a steal phenomenon must be taken into account [34]. In the presence of occlusion on one side, blood flow is partly redirected from the healthy hemisphere to the affected side, subtly reducing supply to the hemisphere contralateral to occlusion. While this hemodynamic compensation may not be detectable at TCD examination, it may have an influence on brain metabolism of both hemispheres. Furthermore, the patho-physiologic mechanism leading to ischemic stroke in patients with ICA occlusion could involve a combination of macroscopic hemodynamic factors, which result in decreased perfusion on the affected side detectable by TCD examination, as well as microembolic or microangiopathic factors, which affect both hemispheres. Using this theory, it can be assumed that systemic cerebrovascular risk factors in the patient group might have contributed to the impairment of the contralateral hemisphere as suggested by Walker et al. [35]. In other words, chronic occlusion of a major cerebral vessel and the consequent hemodynamic impairment of the ipsilateral hemisphere may be the macroscopic expression of a more generalized small vessel disease affecting both hemispheres. This hypothesis is further supported by Van der Grond et al., who found the mean NAA/choline ratio to be slightly lower in the asymptomatic hemisphere of patients with unilateral ICA lesion compared to healthy controls [12]. In their study the difference was not statistically significant, this may be due to a different method of estimation of metabolite signal intensities, as mentioned above. Similarly, in a cohort of patients before and after endarterectomy Saito et al. found the NAA/Cr ratio in the contralateral cerebral hemisphere was higher in patients with postoperatively improved cognition than in those with postoperatively unchanged or impaired cognition, suggesting bilateral metabolic damage in the presence of unilateral stenosis correlating with cognitive function [36].

Rutgers et al. performed quantitative $^{1}$H-MRS measurements in symptomatic patients with ICA occlusion and found NAA significantly reduced on the side of vascular pathology compared to the contralateral hemisphere [37]. As previously mentioned, in the present study there was no significant difference in NAA between hemispheres. This discrepancy may be due to different inclusion criteria: Rutgers et al. enrolled symptomatic patients after a completed stroke which occurred 6 months or less before enrollment, whereas over one third of the patients in the present study were asymptomatic and the remainder suffered a minor neurological event 6 months or more prior to the $^{1}$H-MRS examination. It can be assumed that shortly after an ischemic event, pronounced unilateral metabolic changes on the side of the lesion may prevail and thus overshadow more subtle bilateral impairment. These changes may eventually become detectable in the chronic phase, as well as in asymptomatic patients. Accordingly, Rutgers et al. found no significant inter-hemispheric difference in NAA concentration in the subgroup of three patients, which had symptoms of retinal ischemia without a hemispheric lesion. In line with these results, no significant differences were found between symptomatic and asymptomatic patients in the present study. The timing of enrolment likely played a major role: symptomatic patients were included in a chronic phase of the disease after a relatively long time from the symptomatic event, thus being, from a clinical and metabolic point of view, similar to asymptomatic patients. The sample size surely limits the power of this subgroup analysis.

Measurement of NAA concentration may be clinically significant, due to its possible association with cognition [38]. The reduction in NAA concentration has been related to impairment of cognitive function, which is described in patients with a symptomatic ICA occlusion [39]. Furthermore, a correlation has been already demonstrated between the volume of hippocampus and cognitive functions [40, 41] related to chronic hypoperfusion [42]. In accordance with

these studies, there was a statistically significant reduction of hippocampal volume in patients with ICA occlusion in comparison to healthy controls. Similarly to NAA changes, hippocampal volumes were lower in both hemispheres without significant differences between the affected and the contralateral side. Indeed, the difference between hippocampal volume in the contralateral side from occlusion and healthy volunteers approached statistical significance ($p = 0.051$). In accordance with the results of the present study, Avelar et al. found significant atrophy in grey and white matter of both hemispheres in a cohort of 25 patients with severe carotid stenosis/occlusion in comparison with controls [43]. These bilateral anatomical changes, together with the metabolic alterations previously described, suggest that the altered hemodynamics on the affected side cannot entirely explain the complex pathological events occurring in patients with chronic occlusion of ICA.

No correlation between NAA concentration and cerebrovascular reactivity expressed by BHI was found in this study. This relationship has been previously investigated by other authors. Rutgers et al. did not find a significant association between NAA values and cerebrovascular reserve capacity evaluated by $CO_2$ inhalation test [37]. Lytghoe et al. also found no correlation between these factors [44]. Tsuchida et al. found a positive correlation between regional CBF measured by PET and the NAA/Cr ratio [45]. Their conclusions may differ from the results of the present study because the authors examined patients in an early stage of the ischemic disease, within 12 weeks of symptom onset. In these cases, metabolic features are expected to correlate more significantly with CBF. In the present study, no correlation between cerebral hemodynamics and NAA concentration in the chronic phase of ICA occlusion was found. This also suggests the presence of a pathological mechanism acting globally on the entire brain tissue, rather than unilaterally on the occluded side.

Among the four brain metabolites included in this analysis, estimation of Lac concentration was certainly the most challenging from a technical point of view. While possible interference with lipids resonating at 1.3 ppm and alanine was minimalized by removing them from the original basis-set, chemical shift displacement artifacts remained an issue and may have played a confounding role in detection of Lac signal. Considering that the VOI position was computed using reference frequency 2.7 ppm and given the parameters of the refocusing pulses, the J-coupled Lac resonances at 1.33 and 4.09 were displaced of a distance approximately 7% (12% respectively) the size of the VOI in opposite direction, complicating the task of Lac estimation. Also, signal shape of Lac and J-coupled metabolites in volume localized MRS is inherently distorted due to differential refocusing of its coupled partners, as described by Yablonskiy et al. [46] Furthermore, absolute concentrations of Lac in brain tissue are of one order of magnitude lower than those of NAA, Cho and Cr. For all of the above, error measures for Lac in this study are significantly higher than for other metabolites and interpretation of results from Lac quantification has to be considered with great caution. In the present series, patients with ICA occlusion showed no significant change in Lac concentrations in cerebral white matter on the ipsilateral hemisphere compared to the asymptomatic side and control subjects. It has been shown, that the concentration of Lac tends to increase in the white matter of subjects after an ischemic event [11–13]. The common feature of these studies is that the patients were enrolled in an earlier stage after the onset of symptoms, during which acute transition from oxidative metabolism to anaerobic glycolysis is expected in the ischemic area. In this phase, cerebral Lac is likely to be increased, whereas in a more chronic stage it is expected to decrease due to normalization of CBF and quick wash-out of higher levels of hyper-produced molecules. Few publications exist demonstrating the presence of Lac even in a chronic phase of cerebral hypoperfusion. Examples include Zhang et al. who report a peak of Lac in nearly 20% of patients with chronic stenosis or occlusion of ICA [32], or Bakker et al. who report a percentage reaching 35% [47]. There was no significant difference in Lac concentrations between patients and healthy controls in the present study. This discrepancy may be due to a different methodological approach: in both cited papers the authors registered the presence or absence of an inverted double peak at 1.33 ppm with a signal-to-noise ratio higher than two, without calculating absolute Lac concentrations. In this study, quantitative $^1$H-MRS measurements of the absolute concentrations of Lac were performed and used for statistical analysis.

In conclusion, this study shows that patients with unilateral occlusion of the ICA present with bilateral reduction of NAA/Cho ratio in the white matter and bilateral hypotrophy of the hippocampus. Altered hemodynamics alone cannot explain the metabolic and anatomical changes occurring in both hemispheres of patients with unilateral ICA occlusion. Indeed, the present study has certain limitations. Mainly, the relatively small number of patients inherently weakens the strength of statistical analysis. Secondly, approximately one third of patients in this cohort showed some degree of carotid stenosis (under 50%) on the contralateral side. In a clinical scenario, carotid stenosis under 50% is considered irrelevant, as it is not associated with a higher risk of stroke [48]. In a research set though, where subtle metabolic and anatomic changes were detected with a highly sensitive 1H-MRS and volumetric protocol, this difference between patient and control groups, where the hemisphere chosen for analysis was ipsilateral to a carotid artery without any degree of stenosis, may have had an influence on the results. Furthermore, chronic hemodynamic impairment of brain tissue in patients may have caused changes in water content, T1 or

T2 relaxation times of metabolites or water, thus potentially affecting metabolite quantification. Finally, the observational character of the study without prospective follow up does not allow us to draw any conclusions about the prognostic value of the collected variables. Further prospective studies investigating relationships between impaired brain hemodynamics, metabolic changes, anatomic features and the risk of stroke are required to potentially identify high-risk patients and tailor preventive measures.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Joint Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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