Choline and Creatine Are Not Reliable Denominators for Calculating Metabolite Ratios in Acute Ischemic Stroke

Susana Muñoz Maniega, PhD; Vera Cvoro, MD; Paul A. Armitage, PhD; Ian Marshall, PhD; Mark E. Bastin, DPhil; Joanna M. Wardlaw, MD

Background and Purpose—Choline and creatine are commonly used as denominators for other metabolites in ischemic stroke spectroscopy, assuming that they do not change. We investigated their concentration variation over time after stroke.

Methods—Choline and creatine concentrations were measured by proton MR spectroscopic imaging in 51 patients at 5 times up to 3 months after stroke.

Results—Choline and creatine levels changed significantly in the ischemic region. Choline was significantly reduced during the first 2 weeks after stroke onset (P=0.034). Creatine was significantly reduced during the whole period of the study (P=0.011).

Conclusion—Choline and creatine concentrations are not reliable denominators for metabolite ratios in acute stroke because their levels vary significantly in ischemic brain regions. (Stroke. 2008;39:2467-2469.)

Key Words: acute ischemic stroke ■ MR spectroscopy ■ metabolic ratios

Previous studies of ischemic stroke used choline and creatine to calculate ratios of NAA or lactate to compare affected and healthy tissue in individuals or between different subjects. This is based on the assumption that the concentrations of choline or creatine do not change significantly during ischemia. The aim of the present article was to establish whether choline and creatine could be used as reliable denominators for other brain metabolites in ischemic stroke by investigating how their levels varied within the ischemic lesion over time.

Materials and Methods
Choline and creatine were measured longitudinally after acute stroke at 5 fixed time points up to 3 months after onset. Patients with total or partial anterior circulations syndromes underwent MRI as soon as possible after admission (maximum of 24 hours); patients with hemorrhage were excluded. The local research ethics committee approved the study and written informed consent was obtained.

MRI included axial T2-weighted fast spin-echo, axial diffusion tensor MRI, and proton MR spectroscopic imaging. Scanning was repeated at 4 to 7 days, 10 to 14 days, and 1 and 3 months, taking care to place the MR spectroscopic volume-of-interest in the same position relative to the ischemic lesion on each occasion. Raw image data were processed in C, and FLIRT (www.fmrib.ox.ac.uk/fsl) was used to remove bulk patient motion and eddy-current induced artifacts from the diffusion-weighted image volumes by registering them to the first T2-weighted volume. Average diffusion-weighted image was obtained from the 6 diffusion-weighted images acquired for each slice. Follow-up scans and MR spectroscopic imaging data were also registered to the first T2-weighted volume. Details of the acquisition parameters and data processing methods have been published previously.

The brain slice from the averaged diffusion-weighted image volume on which the MR spectroscopic volume-of-interest had been placed was windowed on a fixed signal intensity to optimize diffusion signal contrast between normal and abnormal tissue. A voxel grid (5×5 voxels or 4.7×4.7 mm) was superimposed over the image (Figure 1A) and a neuroradiologist blinded to all other data classified each voxel as abnormal or normal tissue according to its appearance on the first scan (averaged diffusion-weighted image).

Metabolite concentrations were extracted for abnormal and normal voxels from the MR spectroscopic imaging grid (Figure 1B). Average values of choline and creatine concentrations were calculated for normal and abnormal tissues in each patient at each time point. A general linear model repeated measures regression analysis was used to compare changes over time in the concentrations of choline and creatine in normal and abnormal tissue. All statistical analyses were performed in SPSS 13.

Results
Fifty-one patients (mean ±SD: 75±15 years; National Institutes of Health Stroke Scale 11±8) were recruited. The scanning times were 11±7 hours for the admission scan and 5±1, 12±2, 31±2, and 95±6 days, respectively, for the subsequent scans. Follow-up scanning was not possible in all cases with 23 patients scanned up to 3 months.

Figure 2 shows the change with time of mean choline and creatine concentrations in abnormal and normal tissue regions for all patients at all time points. The general linear model regression analysis did not show any overall difference in the
temporal evolution of choline concentration between abnormal and normal tissue over the 3 months of study, possibly due to sigmoid shape of the recovery curve of abnormal voxels values after 2 weeks (Table; Figure 2A). A further analysis including only data from the first 3 scans showed that the concentration of choline in abnormal tissue was in fact significantly lower than normal over the first 2 weeks after stroke (general linear model regression $P=0.034$). The general linear model regression analysis showed that creatine concentration was significantly reduced in abnormal tissue after the first scan up to 3 months after stroke ($P=0.011$).

**Figure 1.** A, Classification grid overlaid on the averaged diffusion-weighted image; the classification in each voxel is indicated by a color cross: Yellow, normal; blue, abnormal; gray, cerebrospinal fluid or background. B, MR spectroscopic image of choline levels of the same subject; each 10×10×10 mm$^3$ voxel is color-coded according to concentration of choline measured. Color bar shows choline concentration in institutional units.

**Discussion**

Early studies reported no significant changes in choline concentrations in the ischemic lesion, which prompted the use of choline as a denominator to assess changes in other metabolites in stroke. However, other studies found either an increase or a decrease in choline levels in stroke lesions. Discrepancies might be related to individual variability, the small sample sizes of 10 patients or less in some studies, or to the use of different patients scanned at different time points rather than the same patients scanned at similar times. The current study is the largest to date following the same group.

**Figure 2.** Temporal evolution of mean (A) choline and (B) creatine concentrations in institutional units. The error bars indicate SD. Note: graphs include data from all patients imaged at each time point.
Table. Summary of Tests of Between-Subject Effects for General Linear Model Repeated Measurements Analysis of Choline and Creatine Time Evolution*

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*Bold type indicates significant differences.

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Disclosures

None.

References

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