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 ($P=0.011$).

**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

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**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 $10 \times 10 \times 10 \text{mm}^3$ voxel is color-coded according to concentration of choline measured. Color bar shows choline concentration in institutional units.

**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.
of patients with stroke longitudinally using proton MR spectroscopic imaging at fixed time points to reduce this variability. We found that choline levels were significantly reduced in stroke compared with healthy tissue during the first 2 weeks. Thus, using choline measured within the lesion as a denominator for metabolic ratios would overestimate other metabolites’ concentrations. This would introduce ambiguity to the conclusions drawn from MR spectroscopic studies of acute stroke using these ratios, because reported values would be subjected to changes in 2 metabolites instead of one. For example, it was previously reported that the ratio of lactate/choline in acute stroke is a better predictor of clinical outcome than the ratio NAA/choline.2 This could result from the decrease of choline in the lesion, which makes the NAA/choline ratio less sensitive to reductions in NAA, whereas it enhances the increase of lactate in lactate/choline ratios.

Creatine has been used less often than choline to calculate metabolite ratios in stroke.1 In events such as ischemia, creatine cannot be used as an internal reference because its concentration varies with the anaerobic tissue conditions and, like in the current study, other authors have consistently found a general decrease in the concentration of creatine in the ischemic lesion.7,8

Although metabolite ratios are still used because of the simplicity of their calculation, methods for quantifying individual metabolites are now well established. In vivo measurement of metabolite concentrations can be achieved, for example, by calibrating the measured spectral resonance area with the known concentration of a reference solution and using the appropriate corrections for volume normalization, coil loading, and differential $T_2$ attenuation.9

Future MR spectroscopic studies of ischemic stroke should be aware of temporal changes in choline and creatine within the lesion, particularly choline. If ratios of other metabolites to choline or creatine are to be used during the analysis, the potential ambiguity introduced into the ratios by the variation of these metabolites should be carefully considered.

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### Disclosures
None.

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