Proton Magnetic Resonance Spectroscopy of Cerebral Lactate and Other Metabolites in Stroke Patients

Glenn D. Graham, MD, PhD; Andrew M. Blamire, PhD; Alistair M. Howseman, PhD; Douglas L. Rothman, PhD; Pierre B. Fayad, MD; Lawrence M. Brass, MD; Ognen A.C. Petroff, MD; Robert G. Shulman, PhD; and James W. Prichard, MD

Background and Purpose: Proton magnetic resonance spectroscopy can measure in vivo brain lactate and other metabolites noninvasively. We measured the biochemical changes accompanying stroke in 16 human subjects with cortical or deep cerebral infarcts within the first 3 weeks after symptom onset, and performed follow-up studies on six.

Methods: One-dimensional proton spectroscopic imaging encompassing the infarct region was performed with a 2.1-T whole-body magnet using the stimulated echo pulse sequence and an echo time of 270 msec.

Results: All but one of the cortical stroke patients had increased lactate within or near the infarct. Persistently elevated cerebral lactate was documented in five of six cases studied serially as long as 251 days after infarction. N-acetylaspartate levels were decreased in most cortical strokes. Elevated lactate, accompanied by minimal reduction in N-acetylaspartate, was recorded in two of four patients in the first week following a small subcortical infarct.

Conclusions: Long-term elevation of lactate commonly occurs after stroke. This lactate may arise from ongoing ischemia or infiltrating leukocytes, or it may be a residual of the lactate formed during the initial insult. The ability to observe stroke-elevated lactate pools at any time after lesion onset provides an approach to distinguishing among these possibilities in the future. (Stroke 1992;23:333-340)

KEY WORDS • cerebral infarction • lactates • nuclear magnetic resonance

In vivo nuclear magnetic resonance (NMR) spectroscopy can measure key metabolites in the human brain. Its noninvasiveness allows it to be used as often as is clinically useful. The hardware is similar to that used routinely for clinical magnetic resonance imaging (MRI); performance of both kinds of studies on the same instrument is practical and adds little expense. Standardized techniques have recently been developed to acquire spatially localized spectra from user-selected small volumes within the brain.1-3

Proton NMR spectroscopy has been used to study abnormalities in brain biochemistry associated with several neuropathologic processes, including brain tumors,4,5 multiple sclerosis plaques,6 and cerebral infarcts.7-12 In stroke, these early observations have detected prolonged lactate elevation and loss of N-acetylaspartate (NAA) in the affected region. One possible source of persistent lactate is production by ischemic but viable brain cells shifted toward anaerobic glycolysis. Such an “ischemic penumbra”13-15 is conceivably amenable to therapeutic interventions. We report here an initial proton NMR spectroscopy study of the temporal and spatial variation of metabolites after cerebral infarction in humans.

Subjects and Methods

All patients were selected from the neurology or medicine services of Yale–New Haven Hospital. Appropriate candidates had a new motor deficit or aphasia of <3 weeks’ duration and evidence of a corresponding recent hemispheric infarct on computed tomography or MRI examination. Subjects with radiographic evidence of hemorrhagic infarction were excluded due to the adverse effects of methemoglobin on spectral quality.16 All patients were screened for contraindications to entering a strong magnetic field. Informed consent was obtained from the patient or an immediate family member. The protocol was approved by the Human Investigation Committee of the Yale University School of Medicine and Yale–New Haven Hospital.

We used an Oxford Research Systems (Oxford, UK) 1-meter bore whole-body spectrometer operating at 2.1 T. A low-pass shielded birdcage resonator was used for...
transmission and reception of 89-MHz 'H radiofrequency signals.

A three-stage experimental protocol was carried out. First, an axial magnetic resonance imaging scan was obtained to identify the location of the infarct, and a volume encompassing this region was selected for localized spectroscopy. In follow-up examinations care was taken to select the same volume based on anatomic landmarks in the image. Second, spectroscopic parameters were optimized for the chosen volume. The third stage was acquisition of water-suppressed proton spectra. The whole procedure required the patient to be in the magnet for approximately 45 minutes.

For all but the first four observations on patient A, we subdivided the localized volume into smaller discrete regions using the technique of spectroscopic imaging,1,2 which provides additional information on the spatial distribution of metabolites. A 3×6×3 cm³ or, in the studies on patients I through L, a 3×8×2 cm³ volume of tissue within the brain was selected using the stimulated echo (STEAM) technique.3 The long axis of the volume was aligned perpendicular to the sagittal plane, from ear to ear. Scalp and marrow were excluded to prevent contamination of the lactate signal by coresonant lipid.

In the first echo time (TE)/2 period, one-dimensional phase encoding divided the signal along the long axis into 10 equal voxels. The initial studies on patient A used STEAM spectroscopy obtained from a single 3×3×3-cm³ volume located within the infarct. Sixteen phase-encoding steps were used with a phase-cycle scheme of eight combinations, requiring a minimum of 128 acquisitions, corresponding to an acquisition time of 8.5 minutes. Spoiler gradients of adjustable amplitude were applied in both the TE and mixing time (TM) periods to eliminate unwanted coherences. The TE spoilers were positioned adjacent to the TM period to reduce phase variations from motion. Water suppression was achieved by a hyperbolic secant pulse18 that selectively inverted the water signal 800 msec before the start of the acquisition sequence. Additional water suppression was gained by application of a three-lobe sinc pulse during TM for selective elimination of residual water z-axis magnetization. Optimization of the J-modulated signal from lactate required a TE delay of 270 msec. The full echo was acquired, facilitating a Fourier transform in magnitude mode. Spectral bandwidth was 2,500 Hz, and sine wave apodization was applied in both dimensions.

Areas of metabolite resonances were determined by curve tracing on a digitizing tablet and scaled to the area of the NAA resonance in the hemisphere opposite the stroke to obtain relative metabolite signals. With the assumption that brain regions distant from the stroke had similar and constant NAA concentrations, this allowed comparison of data across patients and between examinations of the same patient. The lactate and NAA peak areas from the single-volume spectra of patient A were multiplied by correction factors derived from the metabolite distribution on poststroke day 203 to permit comparison to the spectroscopic imaging volumes.

Results

Spectra from a normal 56-year-old volunteer are shown in Figure 1. Peaks corresponding to NAA and other N-acetylated compounds at 2.03 ppm, creatine at 3.02 ppm, and trimethylamines at 3.20 ppm are readily apparent and are fairly constant in amplitude throughout the selected volume of interest. The use of long TEs greatly reduced signal from lipids and other metabolites with short T₂ times. Under these conditions, normal lactate concentrations (<~1 mM) are below the threshold of detection.

A representative set of spectra obtained from patient C, one of the 12 patients with cortical strokes, is shown in Figure 2, along with an MRI scan of the lesion. Figure 3A shows lactate profiles obtained from five examinations of patient C. Each graph displays relative lactate signal as a function of spectroscopic imaging voxel position. Lactate decreased after the first month but was still elevated 251 days after the stroke. The NAA spatial distribution (Figure 3B) was essentially reciprocal to the lactate.

Overall, 13 of the 16 patients had increased lactate in or near the infarct identified on MRI, as shown in Table 1. The average initial relative lactate for the 12 patients with cortical strokes was significantly greater than zero (1.26±0.36, mean±SEM, p<0.005). Patient H, the only patient with a cortical infarct who never demonstrated lactate elevation, was examined 8 days after a small embolic occlusion of a right middle cerebral artery branch, at which time he had normal metabolite signals in this region and minimal neurological deficit.

Figure 4 shows the temporal behavior of lactate for the six patients examined more than once. All but one (patient F) continued to have measurable lactate by NMR spectroscopy, lasting to poststroke day 251 in our longest follow-up. Changes in lactate varied over the initial month, but levels fell thereafter. The three patients with the largest lactate elevations had larger infarcts and were left with greater neurological deficits than the patients with smaller lactate elevations. The voxel of highest lactate often moved over time, usually by <1.5 cm. The total

![Figure 1](http://stroke.ahajournals.org/)

Nuclear magnetic resonance spectroscopic image of a normal 56-year-old man. Repetition time=4 seconds, echo time=270 msec, mixing time=70 msec. TMA, trimethylamines; NAA, N-acetylaspartate.
decay of lactate may therefore be smaller than suggested by looking at single volumes alone.

In most patients, the initial NAA resonance was significantly decreased in the stroke region compared with the contralateral hemisphere (0.52±0.10 versus 1.00, p<0.001). Patients with the greatest elevations in lactate generally exhibited the most marked lesion-associated depressions of NAA (p<0.01) and had the largest lesions on imaging studies. In some of the subjects studied on multiple occasions, NAA continued to decline after the initial examination.

Changes in the magnitude of the trimethylamine relative signal in the area of the stroke occurred in several subjects. Three patients (D, E, and I) exhibited notable increases in this resonance. In three other patients (A, C, and J), both trimethylamine and creatine signals were decreased in the infarct zone. These changes bore no consistent relationship to changes in lactate and NAA.

Results of spectroscopic examinations of four patients with purely subcortical strokes are given in the lower part of Table 1. An increased lactate signal unaccompanied by a decrease in NAA was found in patient M 6 days after an infarct. The MRI and spectroscopic imaging data are illustrated in Figure 5. The lactate signal accurately corresponds to the position of the stroke, estimated to be <3 cm³ in volume. A second patient (N) exhibited a slight increase in lactate and
A. Lactate Profiles

B. NAA Profiles

FIGURE 3. Graphs showing relative lactate (panel A) and N-acetylaspartate (NAA) (panel B) of patient C versus position within spectroscopic image volume. Metabolites are reported as a ratio to NAA signal in region contralateral to infarct. Each line represents single experiment.

decrease in NAA in the two volumes corresponding to the position of her infarct 5 days after symptom onset. Two additional patients (O and P), both with smaller-volume subcortical infarcts than in patients M and N, were studied on poststroke days 3 and 10, respectively, and showed no detectable elevation of lactate.

Discussion

Chemically specific, noninvasive observation of lactate elevation associated with human stroke is a unique capability of NMR spectroscopy. This study demonstrates persistence of elevated lactate and decreased NAA for months after a large cortical infarct. Smaller and less-prolonged changes accompanied strokes of smaller volume. Our observations are consistent with initial proton NMR spectroscopy data obtained by other workers.7,8,12

Several etiologies, either alone or in concert, may account for the persistence of the lactate pool. Lactate formed in the initial period of ischemia could remain sequestered in necrotic tissue and leave the region of injury only very slowly by passive diffusion after cell lysis. Lactate could also arise from a shift toward anaerobic glycolysis in potentially viable cells that continue to metabolize glucose under locally hypoxic conditions. Or it might be produced by inflammatory and phagocytic cells infiltrating the brain parenchyma after a stroke. Both polymorphonuclear leukocytes and peritoneal macrophages metabolize glucose primarily to lactate even under normoxic conditions.18-21

Some preliminary inferences can be drawn about the origin of lactate present long after cerebral infarction. Estimates of lactate washout after brain ischemia22 and of blood–brain barrier permeability to lactate23 suggest far more rapid clearance of initially formed lactate than we have observed. Lactate in cerebrospinal fluid is increased for days after stroke,24,25 and significant differences in cerebral venous versus arterial lactate have been reported in human subjects26,27 and in animals following cerebral ischemia.28 These results imply that clearance of lactate from killed tissue occurs within days after ischemic injury and that persistent lactate is due to a dynamic balance between synthesis and clearance.

Recently, the metabolic activity of the lactate pool associated with a 32-day-old stroke was assessed by monitoring the appearance of 3-13C-lactate in the affected brain tissues by NMR spectroscopy after the infusion of 1-13C-glucose.29 Label incorporation into brain lactate reached the level predicted by the serum glucose 13C isotopic fraction, showing that, within experimental error, all of the brain lactate arose from glycolysis of serum glucose. Labeling of blood lactate reached only half the brain level. This direct evidence supports the hypothesis that elevated lactate present weeks after a stroke is the product of ongoing lactate synthesis in the brain.

Lactate may serve as a marker of chronic cellular ischemia; however, we cannot at present distinguish lactate formed by neural tissue from that due to macrophage metabolism. Animal models of prolonged cerebral ischemia30 and infarction31 document an immediate increase in cerebral lactate before a significant infiltration of brain tissue by leukocytes could occur.32,33 In some of our patients, lactate persisted beyond the time that phagocytosis of necrotic cells is mostly complete.34 These facts suggest that sources of brain lactate other than leukocytes must be considered in the acute phase, and perhaps also in the late chronic phase.
Observe the first 48 to 72 hours after acute cerebral infarction, before significant leukocyte infiltration, will detect lactate elevations principally in dead or impaired neural tissue.

The NAA signal was markedly decreased in large cortical lesions with high lactate, but usually was little changed in subcortical strokes, regardless of size. As the in vivo peak at 2.02 ppm likely arises from other N-acetylated molecules in addition to NAA, at least some of this decrease may be due to the loss of other hypoxia-sensitive compounds. N-acetylaspartate is known to be concentrated in neurons and may be relatively less affected in white than in gray matter lesions. However, smaller cortical infarcts usually also had more modest declines in lesion-associated NAA. Given the voxel size employed, partial volume effects with considerable signal contribution from normal brain tissue surrounding the lesion are likely. Also, a small portion of the signal ascribed to a given voxel by phase encoding arises from neighboring regions, tending to further limit spatial resolution and increase the apparent distribution of the relatively large proton NAA signal. Use of smaller spectroscopic imaging volumes to increase the proportion of signal from pathological tissue and reduce partial volume effects may detect more subtle changes in NAA.

In four of our patients, we observed increased levels of trimethylamines following stroke. When it rose, the trimethylamine peak tended to increase over the first few weeks after the infarct. The principal contributors to the in vivo trimethylamine signal are choline-containing compounds. In animal models, increased formation of trimethylamines following stroke is observed.

NAA, N-acetylaspartate; TMA, trimethylamines; M, male; F, female; L, left; R, right; MCA, middle cerebral artery; ACA, anterior cerebral artery; PCA, posterior cerebral artery; IC, internal capsule; ant, anterior; post, posterior.

*Voxel chosen had the largest lactate accumulation at time of initial examination.

### Table 1. Clinical Data and Normalized Metabolite Signal Intensities, Reported as a Fraction of the N-Acetylaspartate in Uninfarcted Brain, From a Single Spectroscopic Imaging Voxel for Each Patient*

<table>
<thead>
<tr>
<th>Patient/age (yr)/sex</th>
<th>Location</th>
<th>Poststroke day</th>
<th>Fraction of Contralateral NAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lactate</td>
</tr>
<tr>
<td><strong>Cortical infarcts</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/60/M</td>
<td>R temporoparietal MCA</td>
<td>19</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>117</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>203</td>
<td>0.7</td>
</tr>
<tr>
<td>B/65/M</td>
<td>Proximal L and distal R ACA</td>
<td>8</td>
<td>4.5</td>
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<td></td>
<td></td>
<td>45</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65</td>
<td>1.1</td>
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<tr>
<td></td>
<td></td>
<td>101</td>
<td>0.7</td>
</tr>
<tr>
<td>C/72/F</td>
<td>L parietal MCA</td>
<td>8</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
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<td>27</td>
<td>2.8</td>
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<td></td>
<td></td>
<td>153</td>
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<td></td>
<td></td>
<td>251</td>
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<td>D/48/M</td>
<td>L parietal MCA</td>
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<td></td>
<td></td>
<td>20</td>
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<tr>
<td></td>
<td></td>
<td>25</td>
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<tr>
<td>E/77/F</td>
<td>L lenticulostriate and frontal</td>
<td>2</td>
<td>0.7</td>
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<tr>
<td></td>
<td></td>
<td>9</td>
<td>0.6</td>
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<tr>
<td></td>
<td></td>
<td>30</td>
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<td></td>
<td></td>
<td>150</td>
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<td>F/45/M</td>
<td>R ant parietal</td>
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<td></td>
<td></td>
<td>18</td>
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<tr>
<td>G/68/F</td>
<td>L post parietal</td>
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<td>H/63/M</td>
<td>Lateral R MCA branch</td>
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<tr>
<td>I/56/M</td>
<td>L ant and post MCA</td>
<td>15</td>
<td>1.0</td>
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<tr>
<td>J/82/M</td>
<td>L post temporoparietal</td>
<td>6</td>
<td>0.6</td>
</tr>
<tr>
<td>K/71/F</td>
<td>R temporo-occipital PCA</td>
<td>8</td>
<td>1.3</td>
</tr>
<tr>
<td>L/63/M</td>
<td>L post parietal</td>
<td>6</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Small subcortical infarcts</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/51/F</td>
<td>L corona radiata</td>
<td>6</td>
<td>0.6</td>
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<tr>
<td>N/60/F</td>
<td>L head of caudate</td>
<td>5</td>
<td>0.4</td>
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<tr>
<td>O/47/M</td>
<td>L post limb IC</td>
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<td>None</td>
</tr>
<tr>
<td>P/56/M</td>
<td>Small R IC</td>
<td>3</td>
<td>None</td>
</tr>
</tbody>
</table>

**Observations within the first 48 to 72 hours after acute cerebral infarction, before significant leukocyte infiltration, will detect lactate elevations principally in dead or impaired neural tissue.**

The NAA signal was markedly decreased in large cortical lesions with high lactate, but usually was little changed in subcortical strokes, regardless of size. As the in vivo peak at 2.02 ppm likely arises from other N-acetylated molecules in addition to NAA, at least some of this decrease may be due to the loss of other hypoxia-sensitive compounds. N-acetylaspartate is known to be concentrated in neurons and thus may be relatively less affected in white than in gray matter lesions. However, smaller cortical infarcts usually also had more modest declines in lesion-associated NAA. Given the voxel size employed, partial volume effects with considerable signal contribution from normal brain tissue surrounding the lesion are likely. Also, a small portion of the signal ascribed to a given voxel by phase encoding arises from neighboring regions, tending to further limit spatial resolution and increase the apparent distribution of the relatively large proton NAA signal. Use of smaller spectroscopic imaging volumes to increase the proportion of signal from pathological tissue and reduce partial volume effects may detect more subtle changes in NAA.

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Enhanced choline production has been found to be limited to brain tissue fractions containing membranes. A recent in vitro experiment found that choline levels were not decreased by treatment with an acetylcholinesterase inhibitor during ischemia, implying that phospholipid membrane breakdown and not acetylcholine metabolism was responsible for the rise in choline. These studies suggest that increases in trimethylamine signal may arise from cell membrane breakdown. However, as the magnitude of the trimethylamine resonance varied greatly from patient to patient, better understanding of this phenomenon will require more data.

Magnetic resonance spectroscopy may prove to be of value in the management of individual stroke patients. In our study, patients with large and persistent elevations in cerebral lactate had significant neurological impairment and moderate-to-profound residual disability, whereas those with lesser changes had a more benign clinical course. Correlation of early metabolite profiles with lesion volume and the patient's clinical status may establish NMR spectroscopy as a useful predictor of clinical outcome. For instance, a hemiparetic patient with normal NAA and little lactate 24 hours after onset of a cortical stroke may be shown in future studies to have a reliably better prognosis than one whose hemiparesis is associated with a large lactate...
signal and substantial loss of NAA. In the first 4–8 hours after infarction, labeling of stroke-elevated lactate from 1-13C-glucose can provide immediate assessment of the proportion of cerebral tissue still metabolically active and thus potentially salvageable by aggressive therapeutic interventions. These direct assessments of tissue metabolic state may well be the earliest reliable indicators of future course after stroke. Together with structural and flow measurements, they can make possible a new level of precision in tailoring therapy to the individual stroke patient.

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