Background and Purpose—It is generally considered that tissue that appears abnormal on T2 MRI is already infarcted and that any penumbra lies outside the T2-visible lesion. We investigated the distribution of infarcted tissue using proton spectroscopic MRI.

Methods—In patients with symptoms of acute hemispheric ischemic stroke, imaged within a maximum of 3 days of stroke, we explored the distribution of $N$-acetylaspartate (NAA), a marker of intact neurons, within and around the abnormal (hyperintense) areas on T2-weighted MR images, using proton spectroscopic MRI.

Results—In 11 patients, imaged 24 to 72 hours after stroke onset, there was little evidence of damaged neurons (reduced NAA) beyond the margins of hyperintensity on the T2 image. However, within the abnormal T2 area, there were statistically significant differences in the amount of NAA (ie, the proportion of intact neurons) between areas that were obviously abnormal on T2 (very hyperintense) and those that were only slightly abnormal (slightly hyperintense).

Conclusions—The extent and degree of hyperintensity of the T2-visible lesion directly reflect the amount of neuronal damage; lack of a T2-visible lesion would suggest predominantly intact neurons at the time of imaging. We hypothesize that once tissue damage has reached a critical (probably irreversible) level, the T2 image quickly becomes abnormal without any significant time lag between the pathological staging of the infarct and its visualization on T2. Further testing in a larger study with information on blood flow levels would be required to confirm this. (Stroke. 2000;31:3008-3014.)

Key Words: cerebral infarction ■ magnetic resonance imaging ■ pathology ■ spectroscopy
not show any visible lesion on T2 until such time as the neurons have been ischemic for so long that they die in sufficient numbers to produce a T2-visible lesion. Restoration of blood flow before the neurons had died might mean that the infarct would never become visible on T2. When an infarct first becomes visible on T2 imaging, it is only slightly hyperintense (pale white) compared with normal brain. Close observation of the T2-visible lesion often demonstrates that the degree of hyperintensity is not uniform within the boundaries of the visible lesion but rather is patchy. In humans it progresses to maximal hyperintensity (very white) over the first 3 to 5 days, concomitant with the time of maximum infarct swelling, although the time may be quicker in rats.

In the present study we used proton MR spectroscopic imaging to determine the relationship between the likely degree of permanent neuronal damage (ie, cerebral infarction) and the visible lesion on T2. Intact neurons contain the metabolite N-acetylaspartate (NAA), whose methyl protons can readily be detected by 1H MR spectroscopic imaging. The amount of NAA detectable with 1H MR spectroscopic imaging rapidly falls as neurons die in a cat stroke model. In stroke patients (mostly studied days after the stroke), the spectroscopic signal from NAA is reduced in infarcted brain compared with healthy brain. Thus far, the majority of MR spectroscopic studies in humans have used single-voxel spectroscopy, in which a region of interest (of typical volume 8 cm^3) is placed over the area of brain thought to be infarcted (as seen on T2-weighted imaging) and in the contralateral region for comparison. Therefore, this gives little information about the distribution of metabolites across the whole infarct, into the surrounding presumed-penumbral and then presumed-normal brain. 1H MR spectroscopic imaging is potentially more useful because it gives metabolic information from a much larger region of the brain with a spatial precision comparable to single-voxel methods. Previous 1H MR spectroscopic imaging studies of human stroke have found reduced NAA in the core of the infarct demonstrated on T2 imaging, similar to the results of single-voxel spectroscopy studies.

Systematic analysis of all the voxels in a spectroscopic image (to get a fuller metabolic picture of an ischemic brain) has not been undertaken in previous human in vivo stroke studies. There have been experimental studies (in rat models of stroke) in which the spatial and temporal variations in NAA have been compared with changes in diffusion images, although unfortunately the small size of the rat brain precludes high spatial resolution regional analysis of small localized brain regions even in a dedicated animal magnet.

We wanted to know first whether there was evidence of any infarcted but not yet T2-visible tissue beyond the boundary of the T2-visible lesion, ie, significantly reduced NAA in areas where the T2 signal was normal. We also wanted to determine whether there was evidence of variable tissue damage corresponding with different hyperintensities within the T2-visible lesion. If the T2-visible lesion appearance lags behind the point of actual tissue damage, there should be little NAA in the center of the T2-visible lesion (because the neurons would have died already), and the NAA might be reduced beyond the margins of the T2-visible lesion in damaged areas that had not yet become visible on T2. If, however, there is no significant time lag between the point of tissue damage and the appearance of the lesion on T2, then the NAA level should correspond with the degree of T2 hyperintensity within the T2-visible lesion but be relatively normal beyond the T2 lesion boundary. Therefore, the present study was performed as a further detailed analysis of spectroscopic image data obtained as part of a larger study in patients with recent stroke.

Subjects and Methods

Materials and Patients

Patients with symptoms of an extensive hemispheric stroke (ie, at least 2 of the following 3 deficits: hemiparesis, hemianopia, deficit in higher cognitive function), with no contraindications to MR, were included if they could be imaged within 3 days of onset of their symptoms. The patients were examined by a stroke physician, and their clinical data were prospectively entered into our hospital stroke registry. Patients underwent MRI as soon as possible after hospital admission with the use of a 1.5-T Siemens SP63 Magnetom clinical MR scanner. T1 midline sagittal localizer and T2 and proton density (PD) axial brain imaging were performed before spectroscopy. Patients with hemorrhagic lesions were not imaged further, but those with either an obvious or possible infarct on the T2/PD-weighted imaging underwent MR spectroscopic imaging. Ethical approval was granted by the Lothian Area Ethics of Medical Research Committee. The T2 sequence was a spin-echo axial acquisition of the whole brain, with the following parameters: repetition time, 3500 ms; echo time, 93 ms; slice thickness, 5 mm; field of view, 240; and number of excitations, 1.

Spectroscopic Imaging

The acquisition and data processing methods for spectroscopic imaging have been described in detail previously. The prescan protocols included shimming, calculation of the optimum 1H nuclear MR chemical shift selective suppression (CHESS) water suppression voltage, and positioning of the point-resolved spectroscopy (PRESS) localized inner volume excitation volume of interest. Water-suppressed and water reference data sets were then acquired with 16×16 phase encodings over a 240-mm field of view, giving cubic voxels of 15 mm (Figure 1). The echo time was 135 ms, and the repetition time between successive phase encoding steps was 1600 ms. The data were then processed offline with software written in C^4 to give single-slice images of proton metabolites within the PRESS inner volume placed in the brain. The data processing steps were briefly as follows: reading of the raw files, voxel shifting of the spectroscopic imaging grid, 2-dimensional spatial fast Fourier transformation, phase correction with the water reference free induction decay, 4-Hz line broadening, Hankel-Lanczos singular value decomposition water removal, spectral fast Fourier transformation, and peak area calculation for all voxels. In addition, the 16×16 spectroscopic image grid was zero-filled to 32×32 (Figure 2). No further smoothing of the spectroscopic imaging grid was performed because excessive smoothing may introduce an erroneous picture of the actual metabolite distribution. Finally, the spectroscopic images were normalized for head coil (B1) inhomogeneity using phantom data, and the radio frequency coil loading was accounted for using the amplitude of the 90° radio frequency pulse voltage. The resulting images represent the relative concentration of the metabolite in system-dependent units, which allow intrapatient and interpatient comparisons to be made.

Image Analysis

The MR images from patients were closely examined to ensure that most of the T2-visible lesion was visible on both adjacent T2 slices. The spectroscopic slice was 15 mm thick, whereas the T2 slice was...
interest facility of the ANALYZE (Mayo Clinic) image processing package. Fulfillment of this condition of 75% overlap on adjacent slices should have minimized inaccuracies introduced by partial volume effects.30

The distributions of NAA levels from the spectroscopic grid in and around the T2-visible lesion were determined as follows. Each spectroscopic voxel was divided into quarters (subvoxels) by the process of zero-filling (see Spectroscopic Imaging). Subvoxels containing cerebrospinal fluid alone were omitted, as any NAA signal would have been, because of partial volume effects. These subvoxels were then coded blind to spectroscopic data according to 2 separate methods. The 2 coding methods were applied blind to the NAA values, and the clinical data on 2 different days were separated by a period of several weeks by the same neuroradiologist. The levels of NAA in the coded voxels were then read directly from the raw spectroscopic image.

In the first coding method, each subvoxel was coded N, P, or A according to whether it was located in an area of definitely normal brain on the T2 image (N), in an area of possible T2 signal hyperintensity (P), or in an area of definitely abnormal T2 hyperintense signal (A) (Figure 3, top panel). The N, P, A coding scheme was used to determine whether the intensity of the T2 signal abnormality (ie, very hyperintense or only slightly hyperintense) related to the degree of neuronal death as determined by spatially corresponding NAA measurements. If there was a significant time lag between neuronal death and the appearance of the lesion on T2, then the NAA values in voxels coded P should be very similar to those coded A, whereas if T2 changes closely mirrored neuronal death without any time lag, then there should be a significant difference between the amount of NAA in P and A.

In the second coding method, the subvoxels were coded core, inner rim, outer rim, or normal according to their position in the area of T2 abnormality. Subvoxels in the central area of the T2-weighted lesion were classified as follows: core (C) = the main lesion area; inner rim (I) = a rim 1 subvoxel thick delineating the inner margin of the edge of the visible T2 lesion; abnormal on T2 filled to at least 50% to 100% with abnormal-appearing tissue; outer rim (O) = a rim 1 subvoxel thick on the outer edge of the T2-visible lesion that appeared normal on T2 or was only filled to <50% with visibly abnormal tissue; and normal (N) = subvoxels containing 100% normal-appearing tissue (remote from the T2 lesion) (Figure 3, bottom panel). In this scheme, the core of the presumed infarct was taken to be any subvoxel overlying brain with any degree of T2 hyperintensity except for the rim of subvoxels around the edge of the T2 hyperintense area. The square shape of the subvoxels necessitated the use of a definition for inner rim of subvoxels filled to between 50% and 100% with T2 hyperintensity and for outer rim of subvoxels filled to <50% with T2 hyperintensity to account for the visible boundary of the lesion often cutting obliquely across its edge. The C, I, O, N coding scheme was used to determine whether the infarcted tissue actually extended beyond the edges of the T2-visible lesion, ie, whether T2 normal brain was in fact infarcted—as inferred from low NAA levels—beyond the edge of the visible lesion.

Statistical Analysis
The mean NAA levels for subvoxels in each coding classification were calculated from all of the spectroscopic imaging surrounding the T2-visible lesion for each of the patients studied, ie, to give a mean value for all voxels coded N, P, or A or C, I, O, or N in each patient.

NAA levels measured from all the voxels (as categorized according to the 2 methods outlined above) were compared after log transformation between voxel categories, with the use of a 2-way ANOVA model to control for patient-specific and system-dependent effects.30 Post hoc pairwise comparisons were performed with the Bonferroni correction to allow for multiple testing. All statistical analyses were performed with SPSS for Windows 7.5.1 (SPSS Inc).

Results
The T2 images of 11 patients (of a total of 17 patients who had spectroscopic imaging) showed an area of hyperintensity

Figure 1. Example of T2 image 72 hours after stroke. This patient had a large infarct in the basal ganglia and overlying cortex of the left cerebral hemisphere. Note that within the area of T2 hyperintensity, some areas are whiter, ie, more hyperintense than others. Superimposed is the 16×16 grid of spectroscopic voxels and the PRESS localized inner volume of interest.

Figure 2. The 32×32 NAA spectroscopic image matrix corresponding to the T2 image in Figure 1. Note the correlation between depressed NAA levels (dark area) and the T2-visible infarct.
consistent with a recent infarct that was large enough to overlap by 75% the 2 images immediately adjacent to the slice on which the spectroscopic grid had been centered. The patients were imaged at a mean time of 43 hours after stroke onset (range, 24 to 72 hours).

Analysis of Subvoxels Categorized as Normal, Possibly Abnormal, or Abnormal According to T2 Appearance

There were statistically significant differences between the 3 categories of voxels (Table 1 and Figure 4, top panel). Both the overall ANOVA result ($F_{2,902} = 53.8, P < 0.001$) and all pairwise comparisons ($P < 0.001$) were highly statistically significant. There were clear differences in the NAA values between voxels coded as normal (highest NAA values), possibly abnormal, and abnormal (lowest NAA values). Thus, a marked increase in T2 signal indicated that there had been significantly greater neuronal loss compared with areas where the T2 signal was less hyperintense although still definitely abnormal and indicated that even in this possibly abnormal area, there was significant neuronal loss compared with normal brain.

Analysis of Subvoxels Categorized as Infarct Core, Inner Rim on the Edge of the Lesion, Outer Rim on the Edge of the Lesion, or Normal Brain Outside the Lesion According to T2 Appearance

There were statistically significant differences in the NAA values between the categories ($F_{3,1328} = 75.7, P < 0.001$) (Figure 4, bottom panel and Table 2). After Bonferroni adjustment of the standard errors, there were statistically significant differences between core subvoxels and all others individually ($P < 0.001$ in each case), between the inner rim subvoxels and both the outer rim and normal subvoxels ($P < 0.001$ and $P = 0.04$, respectively), but not between the outer rim and the normal subvoxels ($P = 0.06$). This indicates that there was unlikely to have been any significant neuronal loss outside the edge of the T2-visible lesion.

Discussion

In this small group of patients with large cerebral infarcts, we have demonstrated that the degree of signal abnormality (hyperintensity) on T2 imaging is directly proportional to the severity of neuronal loss as determined by the NAA level: the greater the hyperintensity in a T2 image, the greater is the neuronal damage as measured by depleted NAA levels in that region of the image plane. Furthermore, there was little evidence of significant neuronal loss beyond the margins of the T2-visible lesion. The use of the alternative classification of core, inner rim, outer rim, normal allowed us to explore the possibility that there was neuronal damage beyond the edges of the T2-visible lesion that had not yet become visible on T2. This second classification demonstrated that although there

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**TABLE 1. Mean NAA Concentrations in System-Dependent Units From All 11 Patients for Each of the 3 Categories of Spectroscopic Voxel Determined on the Basis of Position of Spectroscopic Voxel in Relation to Appearance of Brain Parenchyma on T2 MR Image Underlying Spectroscopy Grid**

<table>
<thead>
<tr>
<th>Voxel</th>
<th>Mean Log (NAA)</th>
<th>Mean NAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal</td>
<td>5.788</td>
<td>449</td>
</tr>
<tr>
<td>Possibly abnormal</td>
<td>6.213</td>
<td>675</td>
</tr>
<tr>
<td>Normal</td>
<td>6.487</td>
<td>826</td>
</tr>
</tbody>
</table>

Significant differences between each category ($P < 0.001$ in each case).
was a stepwise increase in the NAA level from the core of the infarct across the edge of the T2-visible infarct to definitely normal brain, the only statistically significant increases were from core to inner edge and inner edge to outer edge, but not outer edge to normal brain. This indicates that the edge of the T2-visible lesion is likely to be the true boundary of the infarct, there being little evidence of much neuronal loss (reduced NAA) beyond that boundary in the present group of patients. This does not completely exclude the possibility of there being some penumbral tissue beyond the T2-visible lesion, particularly since we were not able to image many of these patients very early after stroke, but indicates that if there is any, it is likely to be very minor.

These findings suggest that the appearance on T2 reflects the actual “real time” stage of the infarct during its evolution, rather than the slowly developing appearance of tissue that died many hours previously. While the traditional concept of ischemic stroke is of a central dead area and a surrounding penumbra of viable tissue, this simple geographic model may not be correct or useful. The ischemic area is probably patchy, ie, more damaged areas mingled with less damaged areas, as mirrored by the patchiness of the hyperintensity of T2. The longer the ischemia persists, the fewer viable neurons and the more dead neurons are present and the more visible the lesion becomes on T2. There is thus a balance depending on depth and duration of impaired cerebral blood flow1; perhaps, therefore, if there is no visible infarct on T2 at the time of imaging, the majority of the neurons are still alive.

In the present group, imaged >12 hours after the stroke, it is possible that the infarct had become completely established and that any penumbral tissue had either died or survived. The lack of lactate in most patients might support this hypothesis. We were unable to image patients that quickly, but others have found evidence of incompletely damaged neurons up to several days after the infarct and therefore, by implication, penumbral tissue.2,31,32

What are the sources of error or bias in the present study? The problem of time has been mentioned previously. The partial volume effect of inclusion of tissue of a different composition from that suggested by the T2 image in-plane with the spectroscopic grid30 could affect the measured NAA values. However, by studying large T2 lesions, which were as extensive on the images on either side of the image on which the spectroscopic grid was placed as on that in-plane image itself, these partial volume effects were minimized. Excessive smoothing of metabolite images might lead to an NAA voxel of abnormally high or low intensity surrounded by voxels of uniform intensity, giving the impression of a core region surrounded by a region of intermediate intensity. We have tried to avoid this source of error by avoiding excessive smoothing of the spectroscopic images. The quantitative normalization procedures24 described in Subjects and Methods removed any machine system–dependent effects. One factor not accounted for in our analysis was metabolite relaxation time changes. As the T2 of water changes in ischemia, so do the T2s of the proton metabolites.11 However,

### Table 2: Mean NAA Concentrations in System-Dependent Units From All 11 Patients for Each of the 4 Categories Determined on the Basis of Position of Spectroscopic Voxel in Relation to Core, Inner Rim, and Outer Rim of T2-Visible Infarct and Normal Brain on T2 MR Image Underlying Spectroscopy Grid

<table>
<thead>
<tr>
<th>Voxel</th>
<th>Mean Log (NAA)</th>
<th>Mean NAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core of infarct</td>
<td>5.508</td>
<td>453</td>
</tr>
<tr>
<td>Inner rim</td>
<td>6.163</td>
<td>633</td>
</tr>
<tr>
<td>Outer rim</td>
<td>6.335</td>
<td>774</td>
</tr>
<tr>
<td>Normal</td>
<td>6.550</td>
<td>868</td>
</tr>
</tbody>
</table>

Significant difference between core of lesion and all other locations (P<0.001 in each case) and between inner rim and all others (P<0.001 for normal, P=0.04 for outer rim). No significant difference between outer rim and normal voxels (P=0.06).

Figure 4. Top, Distribution of the mean NAA levels in the 3 categories of voxel (normal, possibly abnormal, definitely abnormal) in and around a T2-visible lesion. The results for each patient are represented by a continuous line. The means were computed from all voxels of the spectroscopic image surrounding the lesion for each (n=11) patient studied (see Table 1 for mean NAA values for the whole patient group). Bottom, Distribution of the mean NAA levels in the 4 categories of voxel (core, inner rim, outer rim, normal) in and around a T2-visible lesion. The results for each patient are represented by a continuous line. The means were computed from all voxels of the spectroscopic image surrounding the lesion for each (n=11) patient studied (see Table 2 for mean NAA values for the whole patient group).
multiple repetition time and echo time acquisitions, required to measure metabolite T1s and T2s, would have required prohibitively long scan times, which would be unethical in such ill patients, and therefore quantitative measurements of T1 and T2 change were not possible. Since our patients were all imaged well before the time of maximal water shift into the infarct and associated swelling (5 days), and given the magnitude of difference in NAA that we have observed across the infarcts, we think it unlikely that water content of the infarct alone would have had a major effect on the measured NAA values.

Visualization of infarction on CT depends on the time lapse from stroke to scan and the severity of the stroke.

Some patients (usually those with the most severe strokes) have visible infarction on CT even when scanned within the first 90 minutes. It is likely that increasing stroke severity and time lapse increase visible infarcts on T2 MRI as well, although this has not specifically been examined. In our previous spectroscopy work (mostly with single voxel), increasing stroke severity was associated with larger infarcts on T2 MRI and lower NAA; the lower the infarct NAA, the greater the likelihood of a poor clinical outcome. While the conclusions of the present study are based on T2 MR images, they may also apply to visible infarction on CT and would explain why patients with a clinically severe stroke and a definite visible infarct appear to be less likely to benefit from thrombolytic treatment. Other imaging techniques, more sensitive to early infarction, such as diffusion MRI, may simply be detecting tissue damage at higher blood flow levels at an earlier stage in the ischemic cascade. We were unable to perform diffusion imaging in the present study because the non–echo-planar technology imaging time was far too long for these patients to tolerate. However, we have started such a study on our new machine. Our findings in the present study are supported by studies in a rat model of middle cerebral artery occlusion in which (after a brief fall) T2 values in the infarct core rose linearly with time after arterial occlusion. We did not measure T2 values in the present study because that would also have considerably prolonged imaging time. T2 measurement would be feasible on newer, faster MR machines and might improve infarct recognition and characterization of the degree of ischemic damage. Our present results are also supported by a study in stroke patients in which those with middle cerebral artery occlusion had significantly lower NAA values in the infarct than those with either a patent middle cerebral artery or only minor branch occlusion at the time of imaging.

In summary, the results of this study support the notion that the amount or severity of irreversible neuronal loss is directly reflected in the appearance of the brain on T2 MRI at that particular moment. Furthermore, the less obvious the lesion on T2, the more likely it is that there are viable neurons within it for a given stroke severity. This result has important implications for acute ischemic stroke treatment because it means that if there is little visible infarction on T2 MRI (or possibly CT), but the patient clinically has a moderate to severe stroke, then there has been little neuronal death thus far and there is likely to be a substantial proportion of viable but functionally shut-down neurons. Patients with obvious visible infarction are likely to have mostly dead neurons, and those with faintly visible infarcts are somewhere in between. Therefore, our results suggest that stroke physicians should worry less that they might not be seeing extensively and irreversibly damaged brain that is not yet visible on T2 at the time when the patient was scanned. Rather, it would appear that there is unlikely to be any time lag between the time of neuron death and visualization; if no lesion is visible, but the patient has symptoms of a moderate to severe stroke, then the majority of neurons have probably not yet died. Clearly this finding needs to be confirmed in a larger group of patients imaged earlier after ischemic stroke and ideally with some measure of the level of blood flow.

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N-Acetylaspartate Distribution in Proton Spectroscopic Images of Ischemic Stroke: Relationship to Infarct Appearance on T2-Weighted Magnetic Resonance Imaging

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