Early brain temperature elevation and anaerobic metabolism in human acute ischaemic stroke

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Early after acute ischaemic stroke, elevation of brain temperature might augment tissue metabolic rate and conversion of ischaemic but viable tissue to infarction. This might explain the observed link between pyrexia, severe stroke and poor outcome. We tested this hypothesis by measuring brain temperature and lactate concentration with multi-voxel magnetic resonance spectroscopic imaging across the acute ischaemic stroke lesion and normal brain as determined on diffusion imaging. We compared patterns of lactate concentration (reported in ‘institutional units’) and temperature elevation in diffusion lesion core, potential penumbra, ipsilateral and contralateral normal brain and with stroke severity. Amongst 40 patients with moderate to severe acute stroke imaged up to 26 h after onset, lactate concentration was highest in the ischaemic lesion core (42 versus 26 units in potential penumbra, \(P<0.05\)), whereas temperature was highest in the potential penumbra (37.7 versus 37.3\(^\circ\)C in lesion core, \(P<0.05\)). Neither sub-regional temperature nor lactate concentration correlated with stroke severity. Amongst patients scanned at later times; lactate remained elevated in the lesion core, but declined in potential penumbral and ipsilateral normal tissue at later times. We conclude that early brain temperature elevation after stroke is not directly related to lactate concentration, therefore augmented metabolism is unlikely to explain the relationship between early pyrexia, severe stroke and poor outcome. Early brain temperature elevation may result from different mechanisms to those which raise body temperature after stroke. Further studies are required to determine why early brain temperature elevation is highest in potential penumbral tissue.

Keywords: ischaemic stroke; lactate metabolism; brain temperature; MR spectroscopy; pyrexia

Abbreviations: FID = free induction decay; MRSI = magnetic resonance spectroscopic imaging; OCSP = Oxfordshire Community Stroke Project; PRESS = Point Resolved Spectroscopy; T2W = T2-weighted; UCP-2 = uncoupling protein 2
Introduction

Brain and body temperature rise after ischaemic stroke (Reith et al., 1996; Schwab et al., 1997; Castillo et al., 1998; Zaremba, 2004; Karaszewski et al., 2006). In ischaemic stroke models, artificially raised brain and body temperature were associated with ischaemic lesion enlargement (Barber et al., 2004; Krieger and Yenari, 2004; Ren et al., 2004). In stroke patients, pyrexia is associated with severe stroke and poor functional outcome (Reith et al., 1996; Castillo et al., 1998). Hypothermia reduces ischaemic lesion growth in animal models (Zhang et al., 2001; Berger et al., 2004; Iwata et al., 2005) and cooling may improve outcome in stroke patients (Schwab et al., 1998; De Georgia et al., 2004).

One potential explanation for the association between pyrexia and poor outcome is that pyrexia would increase brain temperature and, by augmenting the brain metabolic rate, could result in more rapid exhaustion of limited energy and oxygen supplies, and increased production of free radicals and other toxic substances in ischaemic tissue, thereby increasing conversion of ischaemic but viable tissue to infarction (LaManna et al., 1988; Ichord et al., 1999; Asai et al., 2000). In keeping with this concept, pyrexia after stroke was associated with worsening neurological deficit, larger infarcts and higher cerebrospinal fluid (CSF) concentrations of glutamate and glycine (Castillo et al., 1999). Cooling, started at a mean of 16 h after severe hemispheric ischaemic stroke in 12 patients, reduced glutamate, lactate and pyruvate concentrations in normal and peri-infarct brain but not in infarct core, as assessed with brain microdialysis catheter measurements (Berger et al., 2002).

In ischaemic tissue, elevated lactate concentration indicates that energy production has switched mainly to anaerobic mechanisms (Stillman and Latchaw, 1993; Schurr, 2002; Fillenz, 2005). As some lactate is produced and used as an energy substrate in the normal brain, the increased lactate in ischaemic brain reflects the net effect of overproduction, impaired usage and decreased washout. Most previous studies suggested that the ratio of lactate synthesis to utilization in ischaemic brain was positive (Stillman and Latchaw, 1993; Schurr, 2002; Fillenz, 2005) and correlated with the level of oxygen deprivation and ischaemia (Fryholm et al., 2005).

We previously found that brain temperature was elevated in ischaemic brain soon after stroke, prior to any increase in body temperature, and was significantly higher in probable penumbral tissue than in infarct core or normal brain (Karaszewski et al., 2006). The temperature in the contralateral normal cerebral hemisphere (which may be more closely related to body temperature) only became elevated close to 24 h after stroke. Furthermore, in contrast to the known association between pyrexia and poor outcome, we found no clear association between elevated brain lesion temperature within the first 24 h and clinical outcome (Karaszewski et al., 2006), suggesting that early perilesional brain temperature elevation might precede pyrexia and might reflect a different mechanism to that which causes pyrexia after stroke.

We hypothesized that if the early rise in brain temperature increased the metabolic rate in ischaemic tissue, and was thus the link between elevated body temperature, infarct growth, severe stroke and poor outcome, then lactate concentration should be highest in the same areas of the brain as those with the highest temperature. Therefore, using magnetic resonance spectroscopic imaging (MRSI), we determined the distribution of lactate concentration (reported in MRSI-relevant ‘institutional units’) and temperature across normal and ischaemic brain, in acute stroke patients and tested for any association with stroke severity.

Methods

Patient recruitment

We prospectively recruited patients with symptoms of moderate to severe acute cortical ischaemic stroke without contraindications to MRI. A stroke physician conducted a detailed medical history and examination, measured baseline stroke severity using the National Institutes of Health Stroke Scale (NIHSS) and determined the Oxfordshire Community Stroke Project (OCSP) classification of the stroke subtype (Bamford et al., 1991). The time of onset was taken as the time when the symptoms were first observed or, if the patient awoke with a stroke, then the time last known to be well. Patients underwent MRI as soon as possible after stroke, but within a maximum of 26 h. The study was approved by the local Research Ethics Committee, and informed consent was obtained from the patient or assent from their relative.

MRI and MRSI technique

All scans were carried out using a GE Signa Echospeed LX 1.5T (General Electric, Milwaukee, WI, USA) MR scanner with self-shielding gradients (22 mT/m maximum) and ‘birdcage’ quadrature head coil. We performed axial T2-weighted (T2W) fast spin echo imaging, axial diffusion tensor imaging (DTI, with field-of-view (FOV) 240 × 240 mm, 15 axial slices of thickness 5 mm, slice gap 1 mm, acquisition matrix 128 × 128, echo time 97.4 ms, repetition time 10 s and diffusion sensitizing gradients with scalar b-values of 1000 s/mm2 applied in six non-collinear directions), and multi-voxel Point Resolved Spectroscopy (PRESS)-localized proton MRSI with the voxel grid centered on the slice showing the maximum ischaemic lesion extent on diffusion imaging. The MRSI voxel grid was carefully placed within brain to include as much of the visible ischaemic lesion, ipsilateral and contralateral normal brain as possible and to avoid contamination of the spectra with lipid signal from bone marrow or subcutaneous fat (Fig. 1). The imaging parameters for MRSI were: FOV 320 × 320 mm, slice thickness 10 mm, acquisition matrix 24 × 24, echo time 145 ms and repetition time 1000 ms. We used the echo time of 145 ms as previous work showed it to be more reproducible than MRS detection of metabolites at short echo times (Marshall et al., 2002). We used the scanner’s standard three-pulse chemical shift selective (CHESS) water suppression and shimming, optimized on the slice of interest. There is sufficient residual water signal in each voxel for frequency (and hence temperature) estimation. Spectroscopic data were Fourier transformed for display and visual quality control purposes, but were modelled in the time domain by five Gaussian components (corresponding to choline, creatine, N-acetyl aspartate containing compounds and lactate) using the Advanced Method for Accurate Robust and Efficient Spectral Fitting (AMARES) algorithm within the Magnetic Resonance User Interface (MRUI) package (http://www.mrui.uab.es/mrui).
The chemical shifts (i.e. frequency) of the fitted metabolite peaks were reported to a precision of 0.001 ppm. Spectra were automatically discarded if fitted line widths were less than 1 Hz or greater than 10 Hz, if the metabolite peaks were more than 0.1 ppm offset from their expected values, if the voxels lay on the edges of the PRESS excitation region or came from voxels containing CSF. In addition, all spectra were inspected visually, and discarded if judged to be of poor quality, e.g. having a badly elevated baseline or containing spurious peaks. For each phase encoding, 512 complex data points were acquired with a sampling interval of 1 ms. Body temperature was not monitored during scanning as all sequences operated well within the specific absorption rate limits. The image acquisition took 20 min, not including patient settling time. Regular quality assurance checks were performed (including a weekly spectroscopy check) with appropriate phantoms to ensure stability of the scanner.

Image processing

Bulk patient motion and eddy current-induced artifacts were removed from the DTI data using a three dimensional (3D) computational image alignment program to register the component echo-planar imaging volumes to the T2W volumes acquired with the DTI protocol. Maps of the average DTI signal were obtained from the six DTI images acquired for each slice. Spectroscopic images were interpolated to a $32 \times 32$ matrix, yielding 1000 mm$^3$ voxels and all processing was carried out on a voxel-by-voxel basis (Figs. 1 and 2). Subsequent processing consisted of zero-order phase correction and frequency reference using the residual water signal (effectively bringing water to a chemical shift of 4.70 ppm) followed by removal of the residual signal using the Hankel-Lanczos Singular Value Decomposition method (van den Boogaart et al., 1994). Each MRSI data set took ~9 minutes to acquire, and the data are effectively ‘averaged’ over this period. Since frequency analysis was carried out on each individual spectrum in parts-per-million, the absolute scanner frequency (i.e. Bo) was not relevant.

Lactate concentration estimation

Lactate was identified by its characteristic appearance at echo time = 145 ms (namely, an inverted doublet with peak separation of 7 Hz corresponding to the $J$-coupling). Metabolite quantification took into account coil loading (using the scanner’s radio-frequency
transmitter gain) and receiver gain thus enabling inter-subject comparison of individual metabolite levels. Furthermore, all patients were positioned in nearly the same place in the head coil; it was found previously that coil uniformity is very good across an axial slice near the centre (data not published). Our metabolite quantification was in ‘institutional units’ (Fig. 2). It is worth noting that using relative concentrations of lactate to choline, creatine or N-acetyl aspartate would not be appropriate as an alternative method for comparisons between patients as all three of these metabolites change in stroke (Mun˜ oz Maniega et al. 2008). We did not correct for T1 and T2 effects since measuring T1 and T2 would make the scan sessions unacceptably long for severely ill patients.

Brain temperature estimation

Cerebral temperature for each remaining voxel was calculated from the relative chemical shifts of water and N-acetyl aspartate (Fig. 2). Temperature-dependent changes in hydrogen bonding cause the water chemical shift to vary linearly with temperature at 0.01 ppm per °C (Cady et al., 1995; Germain et al., 2001), whilst the chemical shift of N-acetyl aspartate is independent of temperature. Both chemical shifts are essentially independent of pH (Martin et al., 1993). The use of MRUI for spectral quantification is advantageous because the frequency resolution of MRUI is very high as quantification is carried out in the time domain, effectively looking for small phase changes in the signals over the entire duration of the free induction decay (FID). In other words, frequency resolution is determined by the Cramer-Rao bounds (Marshall et al., 2006) and not by the apparent digital resolution of the spectra, giving better determination of any chemical shifts.

Validity of MRS temperature measurement

This technique has been validated previously (Corbett et al., 1995; Corbett et al., 1997; Karaszewski et al., 2006; Marshall et al., 2006; Harris et al., 2008). Brain MRS thermometry readings correlated highly (r = 0.93) with temperatures measured by the fluoroptic method in pigs during body cooling and heating (Corbett et al., 1997). We previously validated the MRSI thermometry technique with phantoms of known temperature in normal volunteers (to test within and between session repeatability) (Marshall et al., 2006) and during head cooling in normal subjects (Harris et al., 2008). We found very close correlation between temperature measured spectroscopically and by thermometer in a phantom (Marshall et al., 2006). We scanned four healthy volunteers, four consecutive times within one MRI session: the mean brain temperature across all scan times was 36.5°C; within each volunteer and each scan time, and there was no statistically significant difference between the hemispheres (difference 0.14°C, P = 0.302). Moreover, across the four sequential MRS scan acquisitions within each volunteer, we detected a statistically significant reduction in brain temperature of 0.09°C per scan (P = 0.0001), possibly due to the cooling effect of the air current in the bore of the magnet or relative cerebral inactivity during the scan (Marshall et al., 2006). Head cooling in healthy volunteers caused a statistically significant drop in brain temperature of 0.45°C (P = 0.01) as measured by MR spectroscopy and was 0.3°C lower in voxels located near the brain surface compared with those located centrally (Harris et al., 2008) but this difference was not statistically significant (P = 0.15). This contrasts with numerical modelling which suggests that the brain surface temperature relative to the brain core may only differ in the outer 1 cm and by a maximum of 1°C (Nelson and Nunneley, 1998). This was confirmed using direct

Figure 2 Example of spectral data as acquired (left) and the corresponding fitted model (right). Voxel 433 of this patient was within the PAL+ region.
temperature probes inserted during neurosurgery where lower temperatures were only found in the outer 1 cm of brain tissue in normothermic patients, but this could reflect direct surface cooling by exposure during surgery (Stone et al., 1997) and would explain the difference to our results in volunteers through the intact cranium (Harris et al., 2008).

**Tissue regional classification**

MRSI and DTI data were co-registered using an affine transformation. The MRSI voxel grid was superimposed on the DTI data, and the voxels were coded according to the DTI visual appearance, blind to all other information. We used an operational tissue classification based on the DTI visual appearance (Fig. 1), as described previously (Karaszewski et al., 2006). This distinguished definitely abnormal DTI voxels (DAL, probable core); possibly abnormal DTI voxels (PAL); a rim of normal appearing tissue one voxel thick immediately outside the possibly abnormal tissue (PAL+); and ipsi- and contra-lateral normal voxels (INL and CNL respectively). In this operational classification, ‘possibly abnormal’ and ‘possibly abnormal plus’ tissues were considered to correspond to ‘potential penumbra’ (Karaszewski et al., 2006).

**Statistical analysis**

We calculated mean temperatures and lactate concentrations in definitely and possibly abnormal, possibly abnormal+ and normal voxels. We examined lactate concentrations, tissue temperatures and National Institutes of Health Stroke Scale (NIHSS) for associations within the whole group and in those imaged within 12 h and after 12 h. Some data were not normally distributed (W Shapiro-Wilk test) so we used non-parametric tests where appropriate. We tested for correlations between lactate and temperature, and between temperatures or lactate concentrations and delay from stroke to imaging using Pearson correlation co-efficients, and between temperatures or lactate concentrations and NIHSS scores using Spearman correlation co-efficients. We used t-tests to compare lactate concentrations/temperature readings in different brain sub-regions in patients scanned before versus after 12 h of stroke. We performed all comparisons ‘within patient’ or ‘within region and within patient’ thereby avoiding potential confounding by fluctuations in scanner performance or other factors that might influence apparent between-patient and between-tissue region differences. For maximum transparency, the statistically insignificant results are shown in the tables in addition to the significant ones. The P-values were not adjusted to allow for multiple testing since the statistical methods for their correction may be inappropriate (Perneger, 1998).

**Results**

We recruited 40 patients aged 58–95, with NIHSS of 1–29 (for full demographic details see Karaszewski et al., 2006). Brain lactate concentrations were not obtained for one patient due to technical reasons. Twenty-six patients were scanned within the first 12 h of stroke and fourteen patients between 12 and 26 h after stroke.

**Lactate concentration and temperature in brain sub-regions**

Mean lactate concentration (i.u.) was highest in the definitely abnormal tissue on DTI and lowest in contralateral normal voxels (DAL = 42.1, SD = 24.3; PAL = 26.0, SD = 16.3; PAL+ = 14.5, SD = 10.6; INL = 10.4, SD = 8.8; CNL = 8.7, SD = 4.6; P < 0.05; Fig. 3). Temperature was highest in potential penumbral tissues (37.66 °C), then in definitely abnormal tissue (37.3 °C), and then contralateral (37.25 °C) and ipsilateral...
(37.16°C) normal voxels respectively (Fig. 3). Thus the highest lactate levels and highest temperatures occurred in different regions of the ischaemic brain. There was no association between lactate and temperature in any of the individual sub-regions.

**Brain lactate, temperature and stroke severity**

There was no association between lactate or temperature and NIHSS in any individual regions (Table 1).

**Brain lactate and temperature and time from stroke onset**

In patients scanned >12 h after stroke, lactate concentration was lower in possibly abnormal and ipsilateral normal tissue than in patients scanned within 12 h indicating that lactate concentrations in these regions fell with increasing time after stroke (Table 2). However there was no change over time in possibly or definitely abnormal or contralateral normal voxels (Table 2), i.e. lactate stayed elevated in possibly and definitely abnormal tissue and remained low in contralateral normal tissue. Brain temperature did not change with time, except that temperature of contralateral tissue was higher in those scanned at later than at earlier times (Table 2).

**Table 1 Correlations between lactate concentration and temperatures in individual brain regions and baseline stroke severity (NIHSS)**

<table>
<thead>
<tr>
<th>Brain sub region</th>
<th>DAL</th>
<th>PAL</th>
<th>PAL+</th>
<th>INL</th>
<th>CNL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate correlation with stroke severity by sub-region</td>
<td>Spearman r-value</td>
<td>−0.02</td>
<td>0.3</td>
<td>0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>P-value</td>
<td>0.93</td>
<td>0.07</td>
<td>0.77</td>
<td>0.54</td>
<td>0.12</td>
</tr>
<tr>
<td>Temperature correlation with stroke severity by sub-region</td>
<td>Spearman r-value</td>
<td>0.13</td>
<td>0.22</td>
<td>−0.09</td>
<td>0.18</td>
</tr>
<tr>
<td>P-value</td>
<td>0.49</td>
<td>0.18</td>
<td>0.62</td>
<td>0.29</td>
<td>0.29</td>
</tr>
</tbody>
</table>

DAL = definitely abnormal tissue; PAL = possible abnormal tissue; PAL+ = tissue one voxel thick immediately outside the definitely or possible abnormal tissue; INL = ipsilateral normal brain; CNL = contralateral normal brain.

**Table 2 Association between time to scanning, mean lactate concentrations and temperatures in brain sub-regions**

<table>
<thead>
<tr>
<th>Brain sub region</th>
<th>DAL</th>
<th>PAL</th>
<th>PAL+</th>
<th>INL</th>
<th>CNL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate concentration</td>
<td>Pearson r-value (correlation)</td>
<td>−0.17</td>
<td>−0.35</td>
<td>−0.52</td>
<td>−0.45</td>
</tr>
<tr>
<td>P-value (correlation)</td>
<td>0.41</td>
<td>0.076</td>
<td>0.006</td>
<td>0.02</td>
<td>0.23</td>
</tr>
<tr>
<td>Mean&lt;12 h of stroke</td>
<td>43.35</td>
<td>29.09</td>
<td>17.21</td>
<td>12.66</td>
<td>7.9</td>
</tr>
<tr>
<td>Mean&gt;12 h of stroke</td>
<td>40.43</td>
<td>20.01</td>
<td>9.13</td>
<td>5.95</td>
<td>10.18</td>
</tr>
<tr>
<td>P-value (t-test)</td>
<td>0.75</td>
<td>0.10</td>
<td>0.029</td>
<td>0.029</td>
<td>0.15</td>
</tr>
</tbody>
</table>

| Brain Temperature | Pearson r-value (correlation) | −0.07 | −0.03 | −0.002 | −0.18 | 0.39 |
| P-value (correlation) | 0.74 | 0.88 | 0.99 | 0.37 | 0.046 |
| Mean<12 h of stroke | 37.47 | 37.67 | 37.46 | 37.25 | 37.07 |
| Mean>12 h of stroke | 37.42 | 37.63 | 37.68 | 37.09 | 37.5 |
| P-value (t-test) | 0.91 | 0.9 | 0.56 | 0.54 | 0.03 |

Significant values are indicated in boldface.

**Discussion**

Lactate elevation and temperature elevation occur in different regions of the acute ischaemic stroke lesion. Lactate was highest in the lesion core and temperature was highest in potential penumbral regions. There was no association of either lactate or temperature with initial stroke severity as measured by NIHSS. We did not find any correlations between brain temperature in individual sub-regions and functional outcome in contrast to the well established relationship between pyrexia, severe stroke and clinical outcome (Reith et al., 1996; Castillo et al., 1998). Lactate concentrations and tissue temperature elevation also showed different temporal profiles. In patients scanned within the first 12 h, lactate concentration was significantly higher in potential penumbral tissue and in ipsilateral normal tissue than in patients scanned after 12 h, suggesting that patients being scanned later had less potential penumbral tissue. We previously showed that patients scanned within the first few hours of stroke had significantly higher temperatures in ischaemic lesion core than in contralateral brain, whereas in those scanned later there was no difference in temperature between these brain regions because the temperature in contralateral normal voxels rose with time (Karaszewski et al., 2006). Temperature in the normal brain contralateral to the ischaemic lesion is most likely to reflect body core temperature. Contralateral brain temperature elevation 12–26 h after stroke is temporally consistent with patterns of pyrexia observed in other studies. Considering the ischaemic hemisphere, these observations suggest that increased lactate concentration is unlikely to be consequent upon rises in local brain temperature, at least within the first 26 h. This, in turn, suggests that the early rise in brain temperature is not simply an early systemic inflammatory response to ischaemia—if it were then the most ischaemic areas i.e. those where lactate concentration was highest—reflecting a positive lactate production/utilization ratio in the ischaemic brain (Stillman and Latchaw, 1993; Schurr, 2002; Fillenz, 2005), should have shown the highest temperatures.

The study has limitations including the relatively small sample size, and the difficulty in drawing conclusions about temporal changes from individual patients imaged at different times. It is...
possible that patients with larger strokes presented earlier than those with small lesions, were scanned earlier and could influence the analysis of the time-dependent changes. Alternatively, MRI timing may have been delayed in the most severely ill patients due to the longer time needed for stabilization of their clinical condition, but there was no evidence of this. Serial imaging of the same patients every few hours soon after acute stroke would interfere with their acute stroke care. We limited the potential bias due to differences in stroke severity between patients arriving early and late by focusing on within patient analyses. However there are likely to have been differences between patients scanned early and late, emphasizing the need for larger studies. We performed quality controls for MRS by regular checking against phantoms of known metabolite concentration. Our metabolite measurements were in arbitrary units, but corrected for scanner gain settings to enable comparisons between patients, and therefore referred to as ‘institutional units’. Because of the unknown relaxation times (T1 and T2) in pathological tissues, we were unable to ‘normalize’ the measurements and interpret them in terms of absolute millimolar metabolite concentrations. Unfortunately, detailed measurement of T1 and T2 would considerably prolong imaging times and not be possible in acutely ill stroke patients. A limitation of the spectroscopic imaging technique is the difficulty of achieving a satisfactory shim over the whole slice, especially on scanners that are not fitted with high-order shimming coils. Several spectra from frontal regions had to be discarded because of susceptibility effects from the nearby sinuses. The radio-frequency excitation is non-uniform near the edges of the PRESS localized MRS volume, leading to several poor quality spectra from cortical regions of interest. We discarded all poor quality spectra as part of a rigorous quality control process. In *in vitro* studies, we have found that modest variations in shimming (linewidths ranging from 1.4 Hz at the centre of a phantom to 1.7 Hz at the edges) and water suppression factor (range from 85 to 105) do not lead to corresponding variations in estimated temperatures (data submitted for publication). Although the situation is more complex *in vivo*, MRSI appears to be capable of regional temperature estimation. Future studies should examine the degree to which metabolite concentrations can be evaluated precisely in the situation where limited signal-to-noise ratio is limited and to quantify the effect of other variables that could affect temperature estimation. Inevitably, patients with small lesions will have contributed more normal brain voxels than patients with large lesions, the influence of which we tried to minimize using within patient analyses. The variable lesions with different numbers of voxels contributing to each tissue type are an inevitable factor in acute stroke studies and emphasize the need for larger confirmatory studies.

In the meantime, MRS appears to be a valid method for measuring brain temperature in patients non-invasively using the principle that water frequency shift relative to N-acetyl aspartate is temperature-dependent (Cady *et al.*, 1995; Corbett *et al.*, 1997; Marshall *et al.*, 2006). Experimental studies in test phantoms (Corbett *et al.*, 1995; Ishihara *et al.*, 1995) and experimental models (Corbett *et al.*, 1995; Ishihara *et al.*, 1995; Corbett *et al.*, 1999; Kuroda *et al.*, 2003; Trubel *et al.*, 2003; McDannold *et al.*, 2004) show very close correlation between temperature measured by MRS and implanted probes (*r*=0.93 in Corbett *et al.*, 1997). In 4 normal volunteers, we showed that the technique is capable of detecting small differences in brain temperature as small as 0.09°C (Karaszewski *et al.*, 2006; Marshall *et al.*, 2006). Each volunteer was scanned four times and within each volunteer and each scan time there was no statistically significant difference in MR measured brain temperatures between the hemispheres (difference 0.14°C, *P*=0.30) and the mean temperature across all scan times was 36.5°C. However, across the four sequential MRS scan acquisitions within each volunteer, we were able to detect a statistically significant reduction in temperature of 0.09°C per scan (*P*=0.0001), presumably due to cooling by the air current in the bore of the magnet (or relative cerebral inactivity during the scan). Convective device head cooling in the scanner in healthy volunteers caused a statistically significant drop in brain temperature of 0.45°C (*P*=0.01) as measured by MRS, of similar magnitude within the ‘core’, ‘intermediate’, and ‘surface’ located voxels (Harris *et al.*, 2008). These data did not support the hypothesis that there is a major surface-to-core temperature gradient in the brain (Nelson and Nunneyeley, 1998) in the intact skull.

Previous studies of brain temperature and its relation to different metabolites were limited by reliance on invasive measurement techniques. Brain tissue temperatures were measured using temperature probes implanted either directly in brain tissue (Stone *et al.*, 1997; LaManna *et al.*, 1998; Nemoto *et al.*, 2005) or suspended in CSF (e.g. Meylaerts *et al.*, 2000). Although both methods might be useful in some circumstances, they can only sample brain temperatures from discrete regions. Inserting any probe into the brain exposes the brain surface to cooler room air, is likely to change any physical heat exchange conditions and thus alter temperature readings. Invasive probes may cause microlesions and a local inflammatory response, thus possibly changing the tissue temperature. The MRS thermometry is not confounded by any of these artifacts. Although further standardization, including in *in vivo* models is essential to quantify the effect of different known and unknown factors, the available data suggest that the technique provides reliable and valid temperature measurements within the brain. Other potential methods for non-invasive thermometry, such as infrared thermal imaging, are useless for deep brain thermometry due to their limited depth penetration.

Previous studies indicate that the highest lactate concentrations should be in the most ischaemic tissues (Stillman and Latchaw, 1993; Schurr, 2002; Frykholm *et al.*, 2005; Fillenz, 2005). Lactate is synthesized constantly at low levels in healthy brain and is the principal product mainly of anaerobic and, as suggested recently, possibly also aerobic neuronal metabolism (Schurr and Payne, 2007). It might be utilized by mitochondria as a substrate for oxidative energetic pathways followed by oxidation to pyruvate (Bergersen, 2007; Schurr and Payne, 2007). Additionally, some CSF lactate is likely to derive from brain cells although there are no actual studies to support this thesis. Deprivation of tissue oxygen causes increased lactate production, but simultaneously might impair its rate of cellular utilization (Frykholm *et al.*, 2005; Hillered *et al.*, 2005; Macrè *et al.*, 2006; Bergersen, 2007). Nevertheless, the lactate synthesis/utilization ratio is
thought to be positive in ischaemic nervous tissue and therefore useful to estimate the level of ischaemia in acute ischaemic stroke. Our data concur with this interpretation as the lactate concentration was the highest in the core of the ischaemic lesion and the lowest in the brain remote from the ischaemic area. A previous study (Parsons et al., 2000) suggested that ischaemic core lactate/choline ratio measured by single voxel MRS was associated with poor functional outcome. In our sample, core lactate concentration was not associated with stroke severity or clinical outcome. This difference might be because we did not use the ratio of lactate to choline because choline (or other potential reference-metabolites such as creatine or N-acetyl aspartate) is normal and therefore an unreliable denominator in ischaemic tissue (Muñoz Maniega et al., 2008), or our core lactate concentration was taken as a mean of all ischaemic core voxels rather than from a single large lesion-centered voxel (Parsons et al., 2000), or both factors combined.

How do we interpret our findings? We hypothesized that elevated brain temperature might promote conversion of ischaemic but viable tissue to infarction by augmenting metabolic reactions and promoting rapid exhaustion of residual energy. However, the tissue with the highest temperature elevation was not the most ischaemic as assessed by lactate concentration. This questions which other factors might promote the ischaemic brain temperature increase. An important one is local tissue blood flow (Parry-Jones et al., 2008), discussed in our previous work (Karaszewski et al., 2006). Reduced brain perfusion could act in several ways. Reduced blood flow could impair heat exchange resulting in higher ischaemic lesion temperature where cells were still actively metabolizing. Alternatively, the same scenario might result in lower ischaemic than normal brain temperature if body and brain temperature were elevated due to pyrexia due to failure to transfer heat into the ischaemic lesion, whether or not the ischaemic lesion cells were still actively metabolizing. Blood flow is rarely completely absent from the ischaemic lesion so the relative contribution of blood flow to temperature distribution probably lies between the two scenarios. We previously found that patients with higher blood flow levels in the ischaemic tissues had lower tissue temperatures (Karaszewski et al., 2006) consistent with recent experimental data showing marginally lower ischaemic lesion temperatures following reperfusion (Parry-Jones et al., 2008). However, this may not account for the difference in temperatures observed between definitely abnormal and probable penumbral tissues which would have fallen within the whole area of reduced perfusion (by mean transit time). Similarly, if we assume that local inflammatory response, which is likely to be more intense in the more ischaemic tissues, is linked to the tissue temperature elevation, than the temperature should be the highest within the lesion core rather than in the healthy-looking tissue surrounding the core, but it is not.

The early rise in ischaemic lesion temperature may result from different processes to those which lead to pyrexia following stroke. One explanation for the observed brain temperature distribution, and the lack of association between brain temperature and stroke severity or clinical outcome, might be the existence of a local process that is at first neuroprotective and secondly, causes regional heat generation. An example is up-regulation of the gene for uncoupling protein 2 (UCP-2), an inner mitochondrial membrane molecule, which regulates ATP synthesis by uncoupling oxidation from phosphorylation, thus dissipating energy as local heat (Horvath et al., 2003). UCP-2 is up-regulated in human ischaemic brain (Nakase et al., 2007) and following transient ischaemic attack in animal models, increasing resistance to neuronal damage with subsequent repeated ischaemia (Mattiaison et al., 2003). Its overexpression is associated with decreased brain damage following experimental acute stroke and better neurological outcome (Mattiaison et al., 2003; Kim-Han and Dugan, 2005). UCP-2 up-regulation may therefore constitute a natural neuroprotective mechanism, possibly part of the ischaemic preconditioning process, and simultaneously be responsible for the early rise in lesion brain temperature after stroke.

Many other factors could influence local heat production in the brain. These might be related to some common stroke-coexisting pathologies such as impaired insulin action affecting many cellular metabolic pathways in diabetes and metabolic syndrome, or to some individual reactions e.g. the strength of immune or stress response to ischaemia, in addition to variation in time of sampling after stroke. We were unable to explore all these factors in the present analysis. Our data suggest that within the first 24h there is unlikely to be a simple relationship between higher temperature, increased energy metabolism, worse stroke and hence worse outcome. Instead, early brain temperature elevation after stroke and pyrexia probably represent responses to different stimuli occurring after stroke. The pathophysiological links between metabolic reactions taking place in ‘tissue at risk’, their thermal effects, disease severity and their ability or otherwise to limit deterioration, need further investigation with detailed measurements of brain and body temperature to profile the sequence of temperature changes with other factors like regulation of selected genes, infection, systemic inflammatory response, and local blood flow patterns in and around the infarct.

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