

# Correlations between magnetic resonance spectroscopy alterations and cerebral ammonia and glucose metabolism in cirrhotic patients with and without hepatic encephalopathy

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**Background:** Hepatic encephalopathy is considered to be mainly caused by increased ammonia metabolism of the brain. If this hypothesis is true, cerebral glucose utilisation, which is considered to represent brain function, should be closely related to cerebral ammonia metabolism. The aim of the present study was to analyse whether cerebral ammonia and glucose metabolism in cirrhotic patients with early grades of hepatic encephalopathy are as closely related as could be expected from current hypotheses on hepatic encephalopathy.

**Methods:**  $^{13}\text{N}$ -ammonia and  $^{18}\text{F}$ -fluorodesoxyglucose positron emission tomography, magnetic resonance imaging and magnetic resonance spectroscopy (MRS) were performed in 21 cirrhotic patients with grade 0–1 hepatic encephalopathy. Quantitative values of cerebral ammonia uptake and retention rate and glucose utilisation were derived for several regions of interest and were correlated with the MRS data of the basal ganglia, white matter and frontal cortex.

**Results:** A significant correlation between plasma ammonia levels and cerebral ammonia metabolism, respectively, and MRS alterations could be shown only for white matter. In contrast, MRS alterations in all three regions studied were significantly correlated with the glucose utilisation of several brain regions. Cerebral ammonia and glucose metabolism were not correlated.

**Conclusion:** Increase of cerebral ammonia metabolism is an important but not exclusive causal factor for the development of hepatic encephalopathy.

Cerebral ammonia metabolism is considered to play a key role in the pathophysiology of hepatic encephalopathy in acute and chronic liver failure.<sup>1,2</sup> According to current hypotheses, hyperammonemia caused by liver dysfunction leads to an increased brain supply of ammonia. There the removal of ammonia totally depends on its astrocytic metabolism into glutamine.<sup>3</sup> With an increasing ammonia load the astrocytic content of glutamine also increases, leading to cell swelling if the accumulation of glutamine cannot be counterbalanced by release of the osmolyte myo-inositol.<sup>4</sup> Cell swelling is considered to be one of the major mechanisms in the development of hepatic encephalopathy. A second mechanism is the modulatory effect of ammonia on inhibitory and excitatory neurotransmission.<sup>1,5</sup>

Although there is a role for ammonia in the pathogenesis of hepatic encephalopathy, without doubt the relationship between plasma ammonia levels and the severity of cerebral dysfunction is controversial, and it has been suggested that the toxic effects of ammonia are mediated by alteration of its metabolism rather than by itself.<sup>3,5</sup>

Modern brain imaging methods allow the study of different steps of cerebral ammonia metabolism and its functional consequences in humans *in vivo*. Cerebral ammonia uptake and metabolism, for example, can be studied using  $^{13}\text{N}$ -ammonia positron emission tomography (PET), whereas the glutamine content of the brain can be evaluated by magnetic resonance spectroscopy (MRS).  $^{18}\text{F}$ -fluorodesoxyglucose (FDG)-PET measures cerebral glucose utilisation, which is dedicated to the maintenance of ion gradients after depolarisation on principle, and can be considered to represent brain function.

The aim of the present study was to look for interdependencies between regional cerebral ammonia metabolism and glucose utilisation in patients with early grades of hepatic

encephalopathy. As an increased cerebral supply with and metabolism of ammonia is considered a key factor in the pathogenesis of hepatic encephalopathy,<sup>5</sup> a significant interdependence between the different aspects of cerebral ammonia metabolism (plasma ammonia levels, ammonia uptake and retention rate, metabolic rate of ammonia and glutamate/glutamine signal intensities) and neuronal function as represented by the FDG-PET data was expected.

Parts of the results have been presented in abstract form.<sup>6</sup>

## PATIENTS AND METHODS

Twenty-one patients with biopsy-confirmed liver cirrhosis (16 men, five women) aged 32–74 ( $50.4 \pm 11.8$ ) years underwent magnetic resonance imaging (MRI) and  $^1\text{H}$  MRS of the basal ganglia, white matter and frontal gray matter. In 17 patients (12 men, five women, aged  $49.1 \pm 12.2$  years)  $^{13}\text{N}$ -ammonia PET of the brain was also performed. Eighteen of the patients (13 men, five women, aged  $49.3 \pm 11.9$  years) underwent FDG-PET of the brain.

Fourteen patients suffered from posthepatic cirrhosis, two from primary biliary cirrhosis, one from secondary biliary cirrhosis, two from cryptogenic cirrhosis, one from haemochromatosis, and one from alcoholic cirrhosis.

Applying the criteria of Pugh *et al.*,<sup>7</sup> the grade of liver dysfunction was scored Child A in four, Child B in nine and Child C in eight patients ( $^{13}\text{N}$ -ammonia PET: Child A: 4, Child B: 7, Child C: 6; FDG-PET: Child A: 4, Child B: 8, Child C: 6).

**Abbreviations:**  $\text{CMR}_A$ , cerebral metabolic rate for ammonia;  $\text{CMR}_{\text{glc}}$ , cerebral glucose utilisation rate; FDG,  $^{18}\text{F}$ -fluorodesoxyglucose; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAA, *N*-acetyl-aspartate; PET, positron emission tomography; PSE, Present State Examination; ROI, region of interest

The grading of hepatic encephalopathy was done with regard to the results of a comprehensive neurological examination and the results of the Present State Examination (PSE) Syndrome test, a battery of five neuropsychological tests, which has been recommended for the assessment of minimal hepatic encephalopathy.<sup>8,9</sup> Those patients who presented with normal neurological examination and normal PSE-Syndrome test results were graded hepatic encephalopathy 0, those with normal results in the physical examination but pathological PSE-Syndrome test results as minimal hepatic encephalopathy, and those with psychomotor slowing but no disorientation as grade I hepatic encephalopathy. Nine patients had grade 0 hepatic encephalopathy, six minimal hepatic encephalopathy and six grade I hepatic encephalopathy (<sup>13</sup>N-ammonia PET: hepatic encephalopathy 0: 7, minimal hepatic encephalopathy: 6, hepatic encephalopathy I: 4; <sup>18</sup>FDG-PET: hepatic encephalopathy 0: 8, minimal hepatic encephalopathy: 6, hepatic encephalopathy I: 4). In addition to the PSE-Syndrome test, the cancelling d test<sup>10</sup> was performed. Clinical and psychometric examination, MRI, MRS and PET studies were performed within 24 hours. All patients gave their written informed consent. The study was, *a priori*, approved by the local ethics committee. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki.

### Magnetic resonance imaging/MRS

MRI and MRS were performed using a 1.5 T scanner (GE Medical Systems, Milwaukee, Wisconsin, USA). Transverse and coronal T1-weighted images (TR 500 ms/TE 15 ms/slice thickness 5 mm/slice gap 1.5 mm) were obtained. These images were used for the co-registration with PET studies and for the definition of regions of interest (ROI) for the MRS study. The ROI, approximately 8 cm<sup>3</sup> in size, were placed within the basal ganglia, the parietal white matter and the frontal gray matter (fig 1). Within these ROI localised proton spectra were achieved using the STEAM/VOSY technique (TR 3000 ms/TE 18 ms/TM 14 ms/128 averages) using the PROBE/SV packet (GE Medical Systems). For quantification of the metabolite ratios (*N*-acetyl-aspartate; NAA, glutamate/glutamine, myo-inositol, choline) relative to creatine, the spectra were transferred to a workstation for calculation of the peak area integrals.

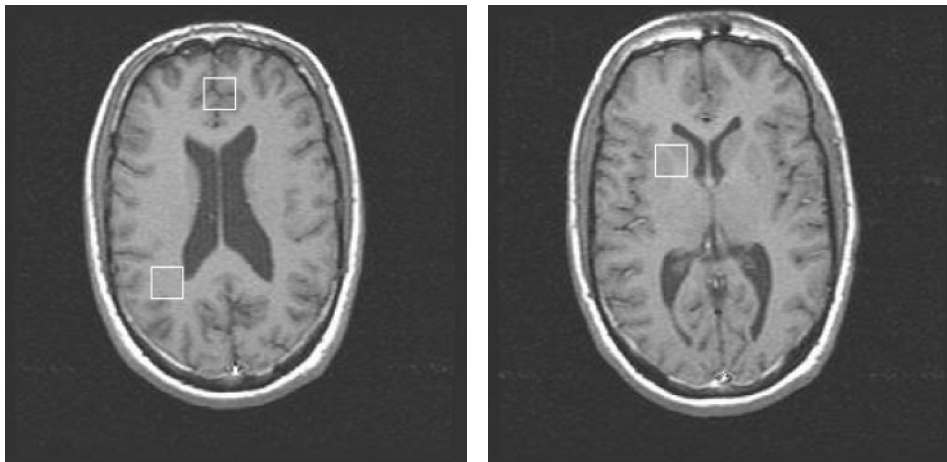
### Positron emission tomography

All patients were investigated using a Siemens 951/31 ECAT tomograph in standard head position. <sup>13</sup>N-ammonia PET and FDG-PET were performed consecutively. Thirty-one slices (plane separation 3.4 mm) were obtained simultaneously. The axial and transaxial resolution of the reconstructed image (filtered backprojection, Hann filter, cut-off frequency 0.4,

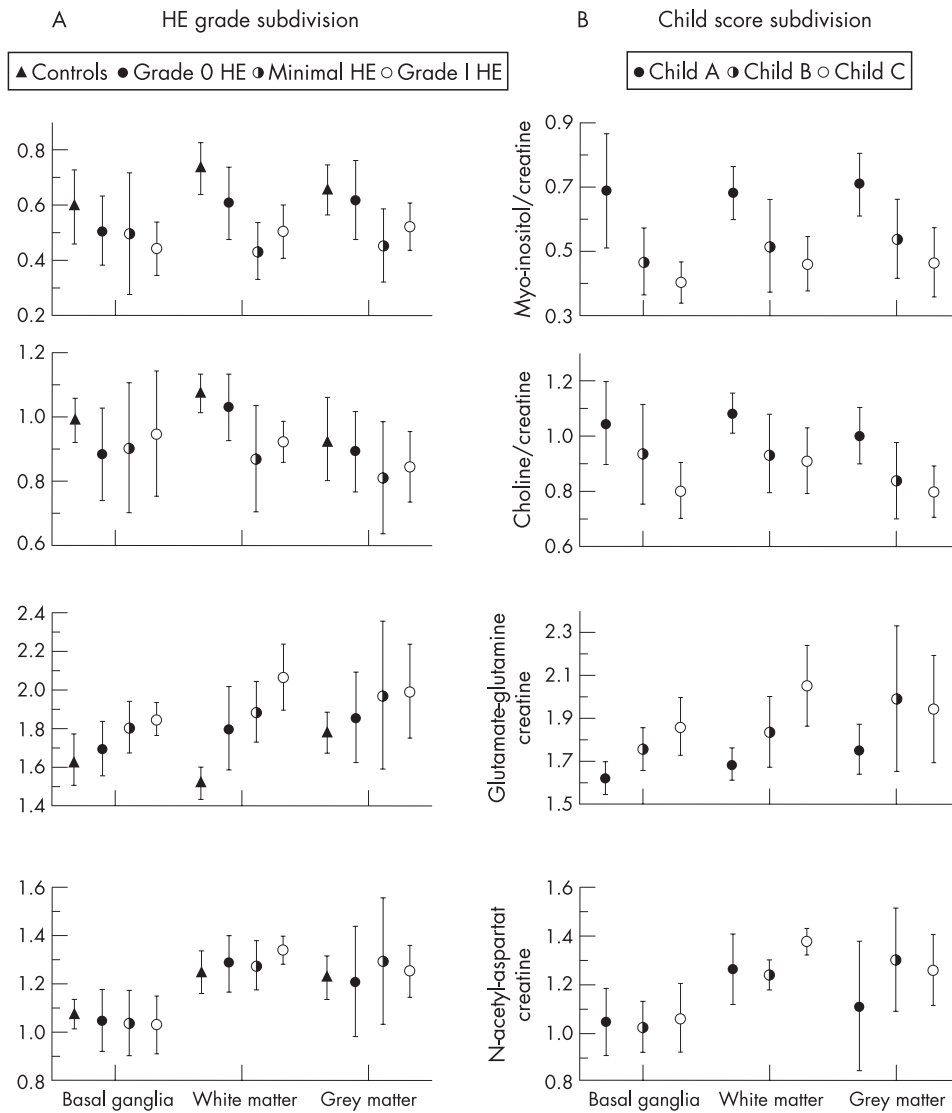
128 × 128 matrix, pixel size 1.9 mm) was approximately 8 mm full-width half maximum. Attenuation correction was performed with a measured transmission scan (10 minutes). After a slow bolus intravenous injection of 740 MBq <sup>13</sup>N-ammonia, the data were recorded dynamically (12 × 10 s, 5 × 30 s, 2 × 120 s, 1 × 900 s frames; = 23.5 minutes). Arterial blood samples were taken simultaneously. After reconstruction (including correction of the PET data for decay and attenuation) parametric images were created. Like Keiding *et al.*<sup>11</sup> we applied an irreversible two tissue compartment model with three transport rate constants for kinetic analysis (the term "irreversible" refers to the fact that the tracer is, at least partly, trapped in tissue, namely the second compartment of the model).<sup>12</sup> The model was originally developed for the quantification of ammonia studies of the heart<sup>13</sup> and is comparable to the model used to study cerebral glucose utilisation by FDG-PET. It is in accord with the findings of Lockwood and colleagues<sup>14,15</sup> on ammonia metabolism in humans. For the generation of parametric images we used a modified linearised approach<sup>16</sup> with automatic correction of input function delay and dispersion.<sup>17</sup> In addition, correction for ammonia metabolism was performed in accordance with the data of Rosenspire *et al.*<sup>18</sup> Images were generated for the rate constants K1, K2, K3, Km and the fractional blood volume.<sup>19</sup> For details of the method see Ahl *et al.*<sup>12</sup>

The cerebral metabolic rate for ammonia (CMR<sub>A</sub>) was not calculated in this study as arterial plasma ammonia levels were not available for all patients. According to recent data of Ong *et al.*,<sup>20</sup> however, there is a close correlation between venous and arterial plasma ammonia levels with a correlation coefficient of 0.86639 (*p* < 0.0001). Ong *et al.*<sup>20</sup> were kind enough to provide us with the linear regression equation that describes the regression between venous and arterial plasma ammonia levels in a group of 53 patients with grades 0 and 1 hepatic encephalopathy: arterial plasma ammonia level 18.74 + 0.892 × venous plasma ammonia level. On the basis of this equation we estimated the arterial plasma ammonia levels in our patients using their venous ammonia levels and used these data for an estimation of the metabolic rate for ammonia (estimated CMR<sub>A</sub>).

Sixty minutes after the injection of <sup>13</sup>N-ammonia, 370 MBq FDG were administered intravenously after a second transmission scan had been performed. The tissue activity concentration was measured dynamically over a period of 50 minutes (6 × 20 s, 3 × 60 s, 2 × 150 s, 2 × 300 s, 3 × 600 s frames; ... 50 minutes). To obtain the tracer input function, 20 arterialised blood samples of 1 ml each were taken according to the dynamic measurement protocol at midframe time. Quantification of glucose metabolism was performed using the method of Patlak *et al.*<sup>19</sup>



**Figure 1** Location of the magnetic resonance spectroscopy voxels used in this study.



**Figure 2** Magnetic resonance spectroscopy results of the patients subdivided with regard to their grade of hepatic encephalopathy (HE; on the left) and with regard to their grade of liver dysfunction. The metabolite ratios of normal controls are added to this figure to give an impression of the extent of the metabolic alterations in the different patient groups compared with normal controls.

For both glucose and ammonia metabolism, ROI were defined after co-registration of the PET and MRI studies of each patient. ROI were defined on the respective MRI studies using a stereotactical anatomical atlas.<sup>21</sup> Defined ROI were the thalamus, caudate nucleus, lenticular nucleus, hippocampus, cerebellum, frontomedial and frontolateral cortex, motor cortex, sensory cortex, parieto-occipital cortex and parietal white matter. Absolute values for KI and Km for ammonia PET and the cerebral metabolic rate of glucose were extracted as a maximum of the defined ROI. In addition, each regional value was individually normalised to global data.

### Statistical analysis

For each parameter the closed test procedure was used to compare the different patient groups. Thereby, the global null hypothesis was tested using analysis of variance, and pairwise comparison was based on the *t*-test. Dependencies between MRI, MRS and PET parameters were analysed by pairwise Pearson correlations. Multiplicity within each MRS and MRI parameter with respect to the PET parameters was controlled for by the Bonferroni–Holm procedure. Furthermore, for each PET parameter as a dependent variable a stepwise multiple regression analysis with backward elimination was performed on MRI and MRS parameters by applying an exclusion limit of  $p > 0.05$ . The results are described as standardised regression

coefficients and the proportion of explained variance,  $\beta$  and  $R^2$ . All tests were performed two-sided on the level  $\alpha = 0.05$ .

### RESULTS

The key findings of our study relate to the correlation between different parameters of cerebral ammonia metabolism and cerebral glucose metabolism in cirrhotic patients with mild grades of hepatic encephalopathy. As cerebral MRS alterations, especially the increase in the glutamate–glutamine/creatinine signal intensity ratio and the decrease in the myo-inositol/creatinine ratio, are considered to represent the metabolic endpoint of increased ammonia metabolism in cirrhotic patients we used the MRS results as a reference parameter to detect possible interrelationships between cerebral ammonia and glucose metabolism.

The MRS alterations found in our patient group resembled those published by other groups in the past (fig 2, table 1),<sup>4 21–26</sup> whereas the NAA/creatinine ratio remained constant, the myo-inositol/creatinine and choline/creatinine signal ratio decreased and the glutamate–glutamine/creatinine ratio increased with increasing grade of encephalopathy (table 1). However, except for the myo-inositol/creatinine and the choline/creatinine ratio in the white matter the differences between the three patient groups (hepatic encephalopathy 0, minimal hepatic encephalopathy, hepatic encephalopathy I) were not significant.

**Table 1** Magnetic resonance spectroscopy data of the patient group subdivided according to the grade of encephalopathy and the grade of liver dysfunction, respectively

	Myo-inositol/creatinine			Choline/creatinine			Glutamate-glutamine/creatinine			NAA/creatinine		
	BG	WM	GM	BG	WM	GM	BG	WM	GM	BG	WM	GM
HE 0	0.51 ± 0.13	0.61 ± 0.13	0.62 ± 0.14	0.89 ± 0.14	1.03 ± 0.10	0.89 ± 0.13	1.70 ± 0.14	1.80 ± 0.22	1.86 ± 0.23	1.05 ± 0.13	1.29 ± 0.12	1.21 ± 0.23
Minimal HE	0.50 ± 0.22	0.43 ± 0.10	0.45 ± 0.13	0.90 ± 0.20	0.87 ± 0.16	0.81 ± 0.17	1.81 ± 0.14	1.89 ± 0.16	1.97 ± 0.39	1.04 ± 0.14	1.28 ± 0.10	1.30 ± 0.26
HE I	0.44 ± 0.10	0.50 ± 0.10	0.52 ± 0.09	0.95 ± 0.20	0.92 ± 0.06	0.85 ± 0.11	1.85 ± 0.09	2.07 ± 0.17	2.00 ± 0.25	1.03 ± 0.12	1.34 ± 0.06	1.25 ± 0.11
ANOVA	p=0.71 R <sup>2</sup> 0.037	p=0.032 R <sup>2</sup> 0.333	p=0.064 R <sup>2</sup> 0.264	p=0.791 R <sup>2</sup> 0.026	p=0.046 R <sup>2</sup> 0.304	p=0.529 R <sup>2</sup> 0.068	p=0.070 R <sup>2</sup> 0.256	p=0.075 R <sup>2</sup> 0.262	p=0.598 R <sup>2</sup> 0.055	p=0.965 R <sup>2</sup> 0.004	p=0.535 R <sup>2</sup> 0.071	p=0.746 R <sup>2</sup> 0.032
Child A	0.69 ± 0.18	0.68 ± 0.08	0.71 ± 0.10	1.05 ± 0.15	1.09 ± 0.07	1.00 ± 0.10	1.62 ± 0.08	1.67 ± 0.08	1.76 ± 0.12	1.04 ± 0.14	1.26 ± 0.15	1.11 ± 0.27
Child B	0.47 ± 0.11	0.52 ± 0.14	0.54 ± 0.12	0.94 ± 0.18	0.94 ± 0.14	0.84 ± 0.14	1.76 ± 0.10	1.84 ± 0.08	1.99 ± 0.34	1.02 ± 0.10	1.24 ± 0.06	1.30 ± 0.22
Child C	0.41 ± 0.06	0.46 ± 0.08	0.47 ± 0.11	0.81 ± 0.10	0.91 ± 0.12	0.80 ± 0.09	1.87 ± 0.14	2.05 ± 0.19	1.95 ± 0.25	1.06 ± 0.14	1.38 ± 0.06	1.26 ± 0.15
ANOVA	p=0.002 R <sup>2</sup> 0.512	p=0.016 R <sup>2</sup> 0.384	p=0.009 R <sup>2</sup> 0.411	p=0.041 R <sup>2</sup> 0.299	p=0.081 R <sup>2</sup> 0.256	p=0.032 R <sup>2</sup> 0.318	p=0.008 R <sup>2</sup> 0.416	p=0.005 R <sup>2</sup> 0.465	p=0.377 R <sup>2</sup> 0.103	p=0.808 R <sup>2</sup> 0.023	p=0.009 R <sup>2</sup> 0.423	p=0.305 R <sup>2</sup> 0.124

ANOVA, Analysis of variance; BG, basal ganglia; GM, gray matter (frontal cortex); HE, hepatic encephalopathy; NAA, N-acetyl-aspartate; WM, white matter.

A comparison of the MRS results after partitioning of the patient group according to the Child score (table 1) showed a significant decrease of the myo-inositol/creatinine signal ratio with an increasing grade of liver dysfunction for all regions studied. The choline/creatinine signal intensity significantly decreased with increasing Child grade in the basal ganglia and the gray matter, whereas the level of significance was failed in the white matter. A significant increase in the glutamate-glutamine/creatinine ratio with an increasing Child score could be observed in the basal ganglia and white matter, but not in the frontal cortex. The NAA/creatinine ratio remained constant with the increasing Child score in the basal ganglia and gray matter, but was significantly increased, especially in Child C compared with Child B patients, in the white matter.<sup>27-31</sup>

Overall, MRS differences between the groups were more pronounced when the patients were divided into Child classes than into hepatic encephalopathy classes (table 1).

### MRS versus psychometric measures

A regression analysis considering the PSE-Syndrome test results and the MRS data showed a significant negative correlation between the tests' composite score (the so-called psychometric hepatic encephalopathy score) and the glutamate-glutamine/creatinine ratio of the basal ganglia ( $p = 0.038$ ,  $r = -0.462$ ). With regard to the single tests of the battery, a significant negative correlation could be observed between the score of the digit symbol test and the glutamate-glutamine/creatinine ratio of the basal ganglia ( $p = 0.02$ ,  $r = -0.503$ ). The

cancelling d test results were positively correlated with the myo-inositol/creatinine and choline/creatinine ratio of the white matter (myo-inositol/creatinine  $p = 0.007$ ,  $r = 0.617$ ; choline/creatinine  $p = 0.016$ ,  $r = 0.554$ ) and the frontal cortex (myo-inositol/creatinine  $p = 0.02$ ,  $r = 0.526$ ; choline/creatinine  $p = 0.03$ ,  $r = 0.502$ ).

### MRS versus ammonia metabolism

A correlation analysis between venous plasma ammonia levels and MRS data ( $n = 17$ ) revealed significant results only for the white matter. With increasing plasma ammonia levels the glutamate-glutamine/creatinine ratio ( $p = 0.03$ ,  $r = 0.535$ ) increased, whereas the myo-inositol/creatinine ratio ( $p = 0.02$ ,  $r = -0.557$ ) and the choline/creatinine ratio ( $p = 0.02$ ,  $r = -0.568$ ) decreased.

A significant correlation was also found between the initial ammonia uptake rate (K1) in the caudate nucleus and the myo-inositol/creatinine and choline/creatinine ratio in the basal ganglia voxel (myo-inositol/creatinine  $p = 0.042$ ,  $r = -0.498$ ; choline/creatinine  $p = 0.023$ ,  $r = -0.548$ ).

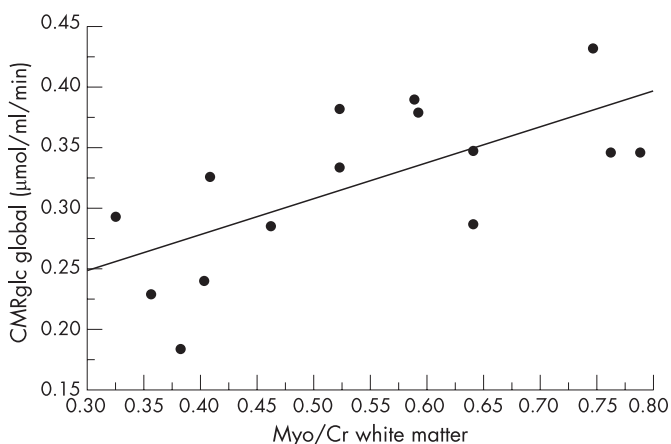
There was no correlation between the ammonia retention rate (Km) in any region and the MRS results.

Considering the estimated metabolic rate of ammonia we found a significant positive correlation between the eCMR<sub>A</sub> and the myo-inositol/creatinine ( $p = 0.0102$ ,  $r = 0.660$ ) and the choline/creatinine ( $p = 0.0015$ ,  $r = 0.763$ ) ratio for the white matter, but not for any other region studied.

### MRS versus cerebral glucose utilisation

The cerebral glucose utilisation showed significant correlations with the MRS results, both globally as well as with regard to several ROI (table 2). Using a stepwise multiple regression analysis that included the global cerebral glucose utilisation rate (CMR<sub>glc</sub>) as a dependent and the relative signal intensities of the four metabolites NAA, choline, myo-inositol and glutamate-glutamine in the three regions studied as independent variables, we found a significant correlation between the MRS data and the CMR<sub>glc</sub> ( $p = 0.0058$ ) that was predominantly based on a correlation between CMR<sub>glc</sub> and the myo-inositol/creatinine signal ratio in the white matter voxel (fig. 3).

Considering the normalised regional glucose utilisation rates, we found that the thalamic glucose utilisation ( $R = 0.575$ ;  $p = 0.0157$ ) and the glucose utilisation in the hippocampus ( $R = 0.730$ ;  $p = 0.0009$ ) increased with an increasing glutamate-glutamine/creatinine ratio in the basal ganglia voxel, whereas the glucose utilisation in the lenticular nucleus ( $R = -0.547$ ;  $p = 0.0230$ ) and the motor cortex ( $R = -0.716$ ;  $p = 0.0012$ ) decreased (fig 4). In addition, we found a significant increase in the glucose utilisation rate of the frontomedial cortex with decreasing myo-inositol/creatinine



**Figure 3** The global cerebral metabolism rate of glucose decreases with increasing magnetic spectroscopy alterations. This figure shows the regression line between the myo-inositol/creatinine (Myo/Cr) ratio in the white matter and the global cerebral glucose utilisation rate (CMR<sub>glc</sub>).



( $R = -0.564$ ;  $p = 0.0183$ ) and choline/creatine ( $R = -0.641$ ;  $p = 0.0056$ ) ratios in the basal ganglia.

The glucose utilisation of the motor cortex also decreased with decreasing myo-inositol/creatine and choline/creatine ratios and with increasing glutamate–glutamine/creatine intensity in the white matter, and with decreasing myo-inositol/creatine and choline/creatine ratios in the gray matter voxel.

In the frontomedial cortex the glucose utilisation increased with the increasing glutamate–glutamine/creatine ratio in the gray matter, whereas it increased in the frontolateral cortex with decreasing myo-inositol/creatine ratios in the white and gray matter voxels and with decreasing choline/creatine ratios in the white matter voxel.

The myo-inositol/creatine ratio in the white matter was also significantly negatively correlated with the glucose utilisation of the hippocampus, cerebellum and thalamus. That of the gray matter was significantly negatively correlated with the glucose utilisation of the cerebellum and the thalamus.

After  $\alpha$ -correction, the number of regions according to Bonferroni–Holm significance could be assumed for the following correlations: (1) myo-inositol/creatine ratio in all regions studied versus the glucose utilisation of the motor cortex; (2) glutamate–glutamine/creatine ratio of the basal ganglia and the white matter voxel versus glucose utilisation rate of the motor cortex; (3) glutamate–glutamine/creatine ratio of the basal ganglia versus glucose utilisation rate of the hippocampus; and (4) choline/creatine ratio of the white matter versus glucose utilisation of the motor cortex (table 2). There is thus a significant correlation between the MRS changes in the brain in cirrhotic individuals and the glucose utilisation especially of the motor cortex. The glucose utilisation in this area decreased with an increasing glutamate–glutamine/creatine ratio in different regions of the brain and also with decreasing myo-inositol/creatine and choline/creatine ratios.

A stepwise multiple regression analysis confirmed the findings of the linear correlation analysis. Again we found

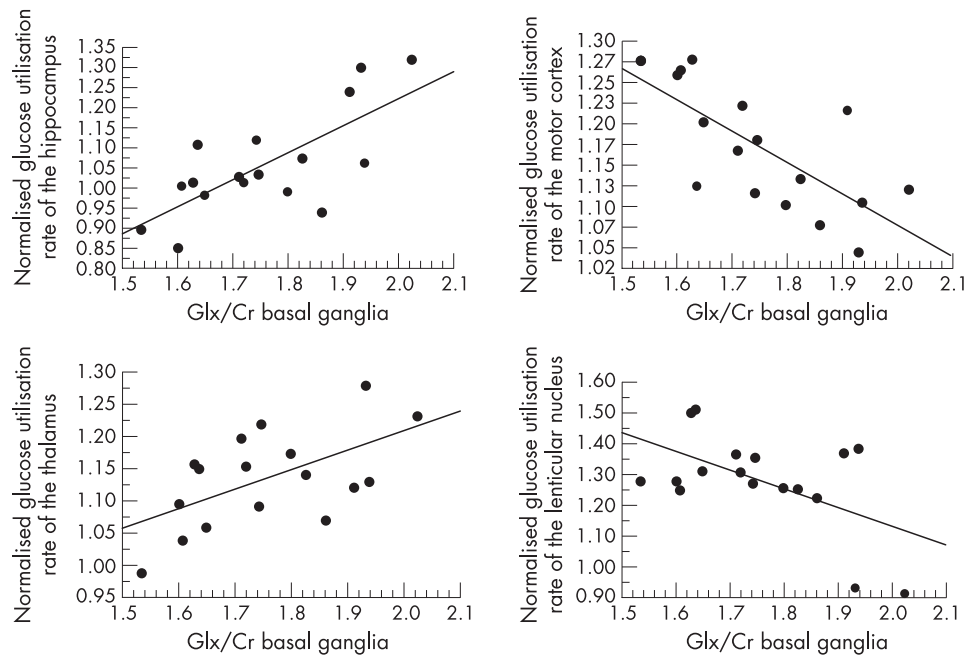
**Table 2** The table shows the correlation coefficients ( $r$ ) between magnetic resonance spectroscopy data and glucose utilisation of several brain regions

PET glucose utilisation	MRS results									Multiple regression after backward elimination
	Basal ganglia			White matter			Gray matter			
	Glx/Cr	Myo/Cr	Cho/Cr	Glx/Cr	Myo/Cr	Cho/Cr	Glx/Cr	Myo/Cr	Cho/Cr	
Motor cortex										
$r$	-0.716	0.787	0.560	-0.832	0.826	0.737	-0.283	0.807	0.530	$p < 0.0001$ $R^2 0.963$
$p$	0.0012*	0.0002*	0.0193	0.0001*	<0.0001*	0.0011*	0.2715	<0.0001*	0.0285	
$\beta$		0.424		-0.535	0.251					
Sensory cortex										
$r$	-0.017	0.285	-0.083	-0.069	0.123	0.047	-0.369	0.329	-0.215	n.s.
$p$	0.9494	0.2668	0.7511	0.7984	0.6512	0.8629	0.1451	0.1969	0.4078	
Parieto-occipital cortex										
$r$	-0.076	0.013	0.165	0.023	0.006	-0.026	0.226	0.265	-0.075	n.s.
$p$	0.7721	0.9605	0.5276	0.9340	0.9828	0.9237	0.3841	0.3047	0.7758	
Frontolateral cortex										
$r$	-0.086	-0.466	-0.380	0.235	-0.531	-0.606	0.379	-0.639	-0.344	$p = 0.0032$ $R^2 0.475$
$p$	0.7427	0.0594	0.1327	0.3818	0.0342	0.0128	0.1335	0.0058	0.1768	
$\beta$								-0.689		
Frontomedial cortex										
$r$	0.182	-0.564	-0.641	0.252	-0.408	-0.424	0.528	-0.422	-0.424	$p = 0.0089$ $R^2 0.516$
$p$	0.4853	0.0183	0.0056	0.3465	0.1169	0.1015	0.0294	0.0915	0.0899	
$\beta$			-0.506				0.409			
Hippocampus										
$r$	0.730	-0.425	-0.326	0.503	-0.499	-0.474	0.005	-0.405	-0.250	$p = 0.0024$ $R^2 0.494$
$p$	0.0009*	0.0890	0.2098	0.0557	0.0490	0.0635	0.9838	0.1072	0.3335	
$\beta$										
Cerebellum										
$r$	0.621	-0.148	-0.150	0.454	-0.558	-0.421	0.164	-0.594	0.147	$p = 0.0026$ $R^2 0.696$
$p$	0.0177	=0.6138	0.6080	0.1195	0.0474	0.1521	0.5746	0.0251	0.6148	
$\beta$								-0.994	0.628	
Caudate nucleus										
$r$	-0.106	0.157	0.121	-0.056	0.164	0.334	0.031	0.087	-0.217	n.s.
$p$	0.6847	0.5478	0.6447	0.8370	0.5440	0.2064	0.9071	0.7406	0.4022	
Lenticular nucleus										
$r$	-0.547	0.391	0.387	-0.336	0.376	0.514	0.059	0.284	0.154	$p = 0.0104$ $R^2 0.385$
$p$	0.0230	0.1208	0.1250	0.2036	0.1510	0.0417	0.8212	0.2686	0.5543	
$\beta$	-0.620									
Thalamus										
$r$	0.575	-0.378	-0.420	0.341	-0.602	-0.330	0.036	-0.641	-0.328	$p = 0.0076$ $R^2 0.409$
$p$	0.0157	0.1352	0.0932	0.1956	0.0136	0.2113	0.8914	0.0056	0.1984	
$\beta$								-0.640		

Cho, Choline; Cr, creatine; Glx, glutamate–glutamine; MRS, magnetic resonance spectroscopy; Myo, myo-inositol; PET, positron emission tomography.

\* $p$  Values that remain significant after Bonferroni–Holm correction.

Added are the results of a stepwise multiple regression analysis  $R^2$ , significance of variables after backward elimination and the standardised regression coefficient  $\beta$ .



**Figure 4** With increasing magnetic resonance spectroscopy (MRS) alterations in the brain of cirrhotic patients we found alterations in the cerebral glucose utilisation rate that were oppositely directed. Whereas the glucose utilisation rate of the hippocampus and thalamus increased with increasing MRS alterations, it decreased in the motor cortex and lenticular nucleus, for example. Glx/Cr, Glutamate–glutamine/creatinine ratio.##

significant correlations between MRS alterations and the glucose utilisation of several brain regions, with an increase in the glucose utilisation in the thalamus, cerebellum, frontomedial and frontolateral cortex and hippocampus, as well as a decrease in glucose utilisation in the motor cortex and lenticular nucleus (table 2). There was no hint that the MRS alterations in one of the three regions studied were more related to the glucose utilisation of selected brain regions than the other.

#### Cerebral ammonia metabolism versus cerebral glucose utilisation

There was no correlation between the  $^{13}\text{N}$ -ammonia and FDG-PET data.

#### DISCUSSION

Cerebral MRS alterations in cirrhotic individuals are considered to represent alterations of astrocytic metabolism; as a result of an increase in brain ammonia levels the astrocytic glutamine content increases, followed by a decrease in myo-inositol, an astrocytic osmolyte that counterbalances the osmotic consequence of an increased glutamine synthesis.<sup>4</sup> A close correlation between plasma ammonia levels, cerebral ammonia metabolism and MRS alterations could thus be expected. If ammonia detoxification to glutamine is the key factor in the pathogenesis of hepatic encephalopathy, then the parameters of ammonia metabolism should also be correlated with cerebral glucose utilisation.

Actually, the present study showed a clear correlation between plasma ammonia levels and the estimated cerebral metabolic rate of ammonia and MRS alterations in the white matter. This fits with a recent observation of Balata *et al.*,<sup>32</sup> who were able to show that even acute induced hyperammonemia leads to the characteristic MRS alterations in cirrhotic patients. We did not, however, find a significant correlation between plasma ammonia levels or the estimated cerebral metabolic rate of ammonia and the MRS data in the basal ganglia and gray matter. In addition, we were not able to show a close correlation between the actual cerebral ammonia uptake and retention rates and MRS alterations. Only the ammonia uptake rate in the caudate nucleus was significantly correlated with the myo-inositol/creatinine and choline/creatinine alterations in the

basal ganglia. In none of the regions studied, however, was a correlation between the ammonia retention rate and the MRS results observed. These results suggest diverse conclusions: (1) the MRS changes seem not to be much related to the actual amount of ammonia that is metabolised by the brain in cirrhotic individuals; or (2) the correlation analysis failed to show a significant interdependence because of the remarkable variances of both MRS results and  $^{13}\text{N}$ -ammonia PET results, which could be overpowered only in the white matter voxel, a region that, at least in MRS, is less prone to artefacts than the basal ganglia or frontal cortex.

In contrast to  $^{13}\text{N}$ -ammonia PET results, the FDG-PET results were closely correlated with the patients' MRS data. The data can be summarised as follows: with increasing MRS alterations, the glucose utilisation of the lenticular nucleus and several cortical regions, especially the motor cortex, decreased whereas the glucose utilisation of the frontomedial and frontolateral cortex, thalamus, hippocampus and cerebellum increased or remained unchanged. When dealing with normalised glucose utilisation rates it has to be taken into consideration that in subjects with MRS abnormalities global glucose utilisation decreases. In these patients a decrease in the normalised utilisation rate within a special region can be interpreted as a real decrease, whereas an increase in the normalised rate may reflect either a real increase or only stability of regional glucose utilisation.

The alterations in cerebral glucose utilisation in cirrhotic individuals observed in this study fit well with those described in former studies,<sup>33–35</sup> except for two findings: the reduction in glucose metabolism within the motor cortex, a region that has not been studied in detail before, and the finding of stable or increased glucose utilisation rates within the frontolateral cortex. The latter could be because the ROI defined in the present study differs from the frontal regions where glucose utilisation was shown to be decreased in hepatic encephalopathy by SPM analysis. In conclusion, we found a correlation between plasma ammonia levels and the estimated cerebral metabolic rate of ammonia and the extent of the characteristic MRS changes in the brain of cirrhotic individuals, especially for the white matter voxels. We did not find any correlation between the parameters of cerebral ammonia metabolism and global or regional cerebral glucose utilisation as measured by

PET. We were, however, able to show a strong correlation between alterations in the glutamate/glutamine, choline and myo-inositol content of the brain (related to creatine) and cerebral glucose utilisation.

How can the difference between ammonia and glucose metabolism be explained in their relationship to MRS data? Again methodological restrictions have to be considered. Correlation analysis can not be used to prove or exclude a causal relationship between different parts of brain metabolism, on principle. In addition, MRS and PET results show a remarkable variance, which might obscure the presence and extent of interdependencies between cerebral ammonia and glucose metabolism. It may also be assumed, however, that actual cerebral ammonia metabolism, as represented by the  $eCMR_A$  for example, is just one influencing variable with regard to both the concentration of the metabolites measured by MRS and cerebral glucose utilisation in cirrhotic individuals with hepatic encephalopathy. In particular, the concentration of the osmolytes myo-inositol and choline is regulated by many factors beyond the intracellular glutamine concentration.<sup>36</sup> It was the myo-inositol/creatine ratio that was especially significantly correlated with cerebral glucose utilisation. Our data may thus be considered as an additional indication that cerebral dysfunction in cirrhotic patients depends on many more metabolic alterations than just ammonia detoxification via the synthesis of glutamine.<sup>5, 36</sup>

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