Seizure-associated brain injury in term newborns with perinatal asphyxia

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Abstract—*Background:* There is controversy over whether seizures, the most common manifestation of neonatal brain injury, may themselves damage the developing brain. *Objective:* To determine if neonatal seizures are independently associated with brain injury in newborns with perinatal asphyxia. *Methods:* Ninety term neonates were studied with MRI and single-voxel ^{1}H-MRS on median day of life 6 (range 1 to 13 days). The severity of MR abnormality in the ^{1}H-MRS regions of interest was scored using a validated scale. Seizure severity was scored based on seizure frequency and duration, EEG findings, and anticonvulsant administration. Multivariable linear regression tested the independent association of seizure severity with impaired cerebral metabolism measured by lactate/choline and compromised neuronal integrity measured by *N*-acetylaspartate/choline in both regions. *Results:* Clinical seizures occurred in 33 of 90 infants (37%). Seizure severity was associated with increased lactate/choline in both the intervascular boundary zone ($p < 0.001$) and the basal nuclei ($p = 0.011$) when controlling for potential confounders of MRI abnormalities and amount of resuscitation at birth. Each increase in seizure score was independently associated with a 21% increase in lactate/choline in the intervascular boundary zone (95% CI, 5.1–38.2%) and a 15% increase in the basal nuclei (95% CI, 0.1–31.7%). Seizure severity was independently associated with diminished *N*-acetylaspartate/choline in the intervascular boundary zone ($p = 0.034$). *Conclusion:* The severity of seizures in human newborns with perinatal asphyxia is independently associated with brain injury and is not limited to structural damage detectable by MRI.

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Seizures are the most common overt manifestation of brain injury in human newborns, with an incidence of 1 to 3.5/1,000 live births.[1,2] Although seizures in the neonatal period most frequently accompany hypoxic–ischemic injury,[1] there has been considerable controversy over whether seizures, in this setting, damage the developing brain.

Neonatal animals of several species have been shown to be resistant to seizure-induced brain injury, even in the context of existing hypoxic–ischemic brain damage.[3-5] However, with improved methodologies, seizure-related neuronal apoptosis and damage have been demonstrated, albeit less than those seen in adult animals, even in the absence of systemic complications.[6,7] Neonatal seizures in the immature rat have been associated with impaired neurogenesis in contrast to that reported in adult animals.[8] Recurrent seizures in the immature brain also derange neuronal structure, connectivity, and function even when significant neuronal loss has not been reported.[9-11] While the consequences of seizures at different stages of brain development differ substantially, neonatal seizures, at least in animal models, do not appear to be benign.[7,12,13]

Unfortunately, because of interspecies variability and the differences in experimental methodologies, it is difficult to extrapolate directly from animal studies to the human newborn condition.[1] While some cohort studies have demonstrated a strong relationship between neonatal seizures in asphyxiated term infants and a poor neurologic outcome,[14,15] others have not found this relationship.[16] Thus, the deleterious effects of seizures in the human newborn, if any, remain undetermined.

Advances in MRI and ^{1}H-MRS have allowed the safe, noninvasive assessment of brain structure and metabolism in critically ill newborns. Using these advanced technologies, we sought to determine, in newborns with perinatal asphyxia, whether neonatal seizures are independently associated with brain injury.

Methods. The primary aim of this prospective cohort study of term neonates with perinatal asphyxia is to determine the MR predictors of neurodevelopmental outcome. This cohort, extensively described elsewhere,[17] is derived from screening 5,389 consecutive term neonates born in or transferred to our institution's intensive care nursery. The cohort now includes 90 infants studied by ^{1}H-MRS, all of

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whom have one of the following: (1) umbilical artery pH of <7.1, (2) umbilical artery base deficit of >10, or (3) 5-minute APGAR score of ≤5. These broad criteria for perinatal asphyxia were chosen to include newborns with the entire range of neurodevelopmental outcomes, from normal to severe impairment, but with some marker of asphyxia. Newborns were excluded if (1) gestational age was <36 weeks or (2) there were suspected or confirmed congenital malformations, congenital metabolic disease, or congenital infections. The protocol was approved by the Committee on Human Research at the University of California, San Francisco. Infants were studied only after voluntary informed consent was obtained from parents.

Neonatal condition. To quantify and summarize the presence of seizures and the overall clinical condition of our cohort in the neonatal period, three scores were calculated.

1. Seizure score: All of the newborns had a clinical seizure score assigned by one of two investigators by chart review of nursing and physician progress notes. This composite score measures seizure frequency and onset, neonatal EEG abnormalities, and number of anticonvulsant medications used (table 1). The score ranges from no documented seizure (0) to severe seizures (10). Seizures were diagnosed clinically by experienced neonatology staff as a paroxysmal alteration in motor and possibly autonomic function, including clonic, tonic, and "subtle" seizure manifestations.[1] Seizures were distinguished from "jitteriness" and other nonepileptic paroxysmal movements using standard clinical criteria.[1] The EEG most temporally proximate to the seizure was interpreted by a clinical neurophysiologist with expertise in the interpretation of neonatal EEG. As it is well recognized that neonatal seizures may not be temporally related with seizure activity on the EEG, seizures were defined by clinical criteria alone.[1] As seizures in newborns are often multifocal, lateralization of the seizures was not included in the score.
2. Resuscitation score: All of the newborns had a resuscitation score assigned by chart review. This scores the type of resuscitation required at birth from no intervention (0) to endotracheal intubation with positive pressure ventilation and medication (sodium bicarbonate with or without epinephrine) (6) (table 2). The need for cardiac massage was noted separately and was found only in scores above 5.
3. Score for Neonatal Acute Physiology—Perinatal Extension (SNAP-PE): Eighty of the newborns were prospectively scored using the SNAP-PE, a validated measure of illness severity. The SNAP-PE scores reflect the worst physiologic derangement in each organ system during the first 24 hours after admission, with increasing scores reflecting more severe derangement.[18,19]

MR data. The 90 MRI and ^{1}H-MRS exams were acquired at a median of 6 days of life (range 1 to 13 days). The same MR scanner and techniques were utilized for the entire cohort. The newborns were imaged as soon as they were stable enough to be transported safely to the MR scanner and imaging time was available. MRI of the brain in all newborns included 4 mm (1 mm gap) sagittal spin echo (500/11/2 repetition time/echo time/excitations) images, 4 mm (1 mm gap) axial spin echo (500/11/2) images, and 4 mm (2 mm gap) axial spin echo (3,000/60, 120/1) images through the entire brain. MRI abnormalities in the basal nuclei and intervascular boundary zone, the ^{1}H-MRS regions of interest, were scored using a system previously validated for the determination of neuromotor outcome.[20]

Table 1 *Neonatal seizure score*

Component	Score
Documented seizure	
No	0 (stop)
Yes	Proceed to score below
Seizure frequency	
1 seizure	0
>1 seizure	1
Status epilepticus*	2
Seizure onset	
Day of life 2 or later	0
Day of life 1	1
Neonatal EEG	
Normal	0
Abnormal background without spikes	1
Abnormal background with spikes	2
Electrographic seizures (isolated)	3
Electrographic status epilepticus*	4
Anticonvulsant therapy†	
No medications	0
<3 medications	1
>3 medications	2
Barbiturate coma	3
Composite seizure score	
Addition of points above	
Minimum score	0
Maximum score	10

* Status epilepticus is defined as continuous seizures lasting >20 minutes or multiple seizures without regaining consciousness between the seizures.

† Anticonvulsant medications included phenobarbital, phenytoin, diazepam, lorazepam, and pentobarbital.

Table 2 *Neonatal resuscitation score*

Score	Intervention
1	No intervention
2	Blow-by oxygen with or without tactile stimulation
3	Endotracheal suctioning only
4	Bag–mask positive pressure ventilation
5	Endotracheal intubation with positive pressure ventilation
6	Endotracheal intubation with positive pressure ventilation and medication (sodium bicarbonate with or without epinephrine)

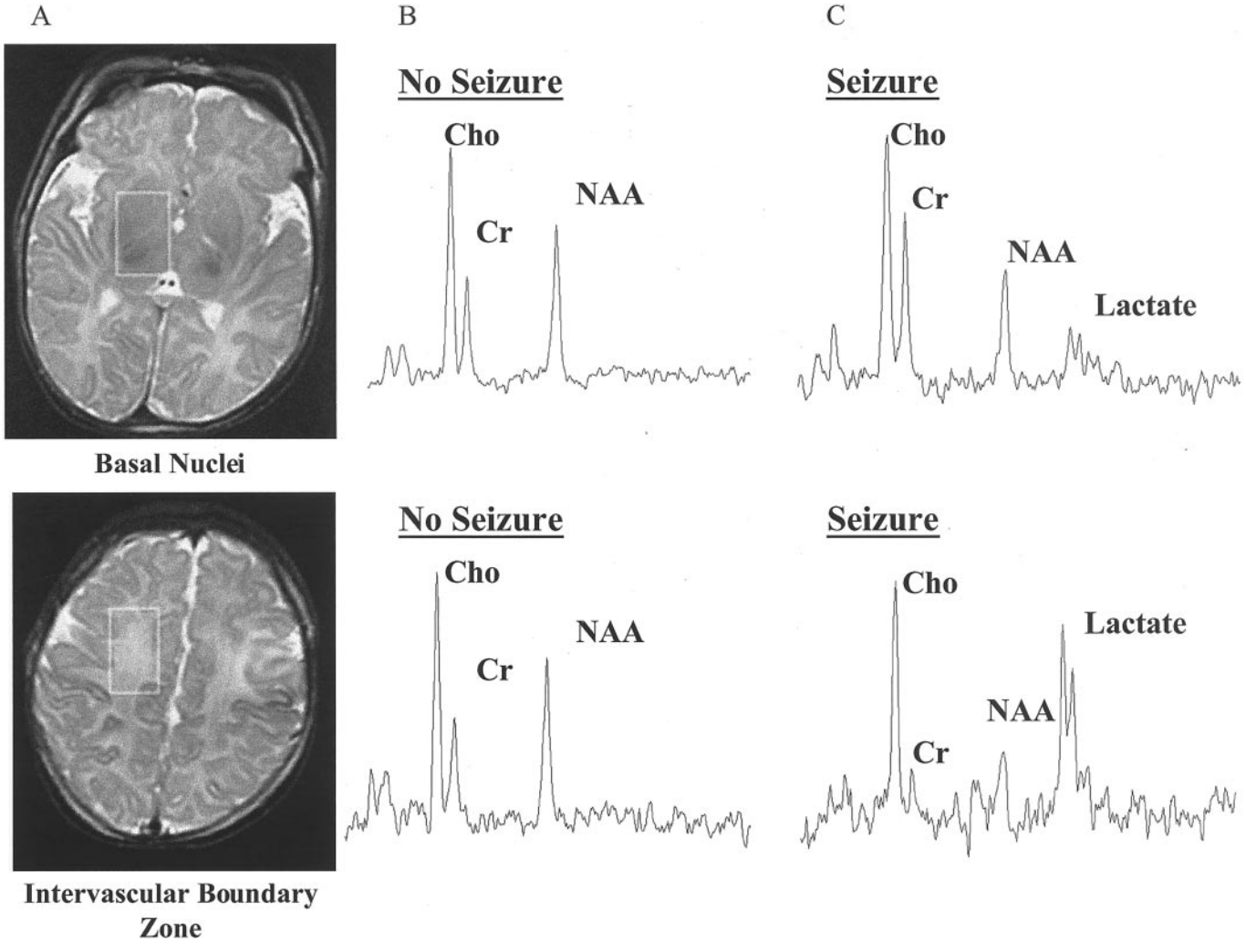

Figure 1. (A) Location of [1]H-MRS voxels demonstrated on axial T2 images (boxes). The basal nuclei voxel includes lentiform nuclei, ventrolateral thalamus, and posterior limb of the internal capsule. The intervascular boundary zone voxel includes primarily subcortical white matter in the region between the anterior cerebral artery and middle cerebral artery distributions. (B) Representative proton spectra of a newborn with no seizures (voxels as shown, point-resolved spectroscopy [PRESS] technique with repetition time 2 seconds, echo time 288 milliseconds, and 128 acquisitions). Choline (Cho), creatine (Cr), and N*-acetylaspartate (NAA) peaks are labeled. Note that the amplitude of the NAA peak is intermediate between that of the choline and creatine peaks. Note also the absence of a lactate peak. (C) Representative proton spectra of a newborn with seizures (voxels as shown, PRESS technique with repetition time 2 seconds, echo time 288 milliseconds, and 128 acquisitions). Note the presence of a lactate doublet and relative diminution of* N*-acetylaspartate compared with the spectrum in (B).*

With use of first-echo T2 abnormalities, since these abnormalities correlate most closely with neurodevelopmental outcome, MRI abnormalities were scored in the basal nuclei as follows: (0) normal or isolated focal cortical infarct, (1) abnormal signal in thalamus, (2) abnormal signal in thalamus and lentiform nucleus, (3) abnormal signal in thalamus, lentiform nucleus, and perirolandic cortex, and (4) more extensive involvement.[20] Findings in the intervascular boundary zone were scored as follows: (0) normal, (1) single focal infarction, (2) abnormal signal in anterior or posterior watershed white matter, (3) abnormal signal in anterior or posterior watershed cortex and white matter, (4) abnormal signal in both anterior and posterior watershed zones, and (5) more extensive cortical involvement.

[1]H-MRS may be used to detect elevation in lactate, indicating disturbance in cerebral oxidative metabolism, and diminution in *N*-acetylaspartate, indicating neuronal damage, dysfunction, or death.[21-24] Proton spectra were acquired from two 5-cm^3 voxels: one centered on the basal nuclei, including the lentiform nucleus and thalamus (90 newborns), and one centered on the frontal intervascular boundary zone (89 newborns) (figure 1).[17] The voxels used were the same in all subjects with the hemisphere for the voxels chosen to best fit with the imaging planes; the side of voxel placement was not determined by the lateralization of seizures or EEG findings. [1]H-MRS was performed using the point-resolved spectroscopy (PRESS) technique to acquire long echo time spectra with the PROBE (PROton Brain Exam; General Electric Medical Systems, Milwaukee, WI) sequence in <5 minutes with a repetition time of 2 seconds, an echo time of 288 milliseconds, and a total of 128 acquisitions. The [1]H-MRS timings and voxel

Table 3 *Neonatal and MR predictor variables in newborns without and with seizures*

Variable	No seizure, mean (SD) n = 57	Seizure, mean (SD) n = 33	*p* Value
Seizure score	0 (0)	4.7 (2.0)	—
Resuscitation score	4.35 (0.79)	5.00 (0.70)	0.0002
Cardiopulmonary resuscitation at birth, n (%)	8 (14)	7 (21)	0.4
SNAP-PE	26.1 (8.4), n = 51	28.9 (9.2), n = 29	0.2
Umbilical cord artery pH	7.06 (0.13), n = 41	7.07 (0.17), n = 25	0.9
Age at MRI, d*	7 (2–13)	5 (1–12)	0.09
Basal nuclei MRI score*	0 (0–2)	2 (0–4)	<0.0001
Intervascular boundary zone MRI score*	2 (0–4)	5 (0–5)	0.009
Basal nuclei lactate/choline	0.10 (0.08)	0.36 (0.54)	0.0006
Intervascular boundary zone lactate/choline	0.14 (0.11)	0.50 (0.64)	0.0001
Basal nuclei NAA/choline	0.72 (0.11)	0.63 (0.15)	0.003
Intervascular boundary zone NAA/choline	0.74 (0.14)	0.64 (0.17)	0.005

* Median (range).

SNAP-PE = Score for Neonatal Acute Physiology—Perinatal Extension; NAA = *N*-acetylaspartate.

localization were chosen to facilitate the detection of lactate and *N*-acetylaspartate while minimizing extracranial adipose tissue contamination of the spectra. After acquisition, the ^{1}H-MRS data were transferred off-line and analyzed on a SPARC workstation (Sun Microsystems, Mountain View, CA) using spectral quantitation software developed in our laboratory. The ^{1}H-MRS data were Fourier transformed and baseline fitted. The peak areas were integrated for the choline, creatine, *N*-acetylaspartate, and lactate resonances, and peak area ratios were calculated. All spectra were analyzed by both a pediatric neuroradiologist and a basic MR scientist with extensive expertise in ^{1}H-MRS.

Data analysis. Statistical analysis was performed using Stata (Stata Corp., College Station, TX). The resuscitation score, SNAP-PE, the day of life the MRI was performed, the MRI scores, and the ^{1}H-MRS metabolite ratios were compared in the groups with and without seizures using *t*-tests for normally distributed data, Mann–Whitney *U* tests for skewed data, and Fisher's exact test for categorical variables.

The outcome variables examined were lactate/choline and *N*-acetylaspartate/choline in both the basal nuclei and the intervascular boundary zone. The predictor variable of interest was the seizure score. Linear regression was used to test the unadjusted association of lactate/choline and *N*-acetylaspartate/choline with seizure score. Multivariable linear regression was used to test the association of lactate/choline and *N*-acetylaspartate/choline in both regions of interest with seizure score while controlling for potential confounders. To measure the effect of seizure severity independent of coexisting brain injury, we considered the resuscitation score, the SNAP-PE score, and the MRI score in the model. The resuscitation score was chosen as a measure of perinatal depression as the amount of resuscitation at birth is a powerful predictor of outcome following perinatal asphyxia.[25] SNAP-PE was considered as an estimate of the overall systemic injury.[18,19] Adjusting for the severity of regional MRI-detectable brain injury is necessary as more than two thirds of newborns with seizures have MRI-detectable lesions, most frequently secondary to hypoxic–ischemic injury.[26]

To directly determine the percentage change in lactate/choline for each increase in seizure score, the multivariate linear regression was repeated with a log-transformed lactate/choline outcome variable.[27] As several subjects had zero lactate/choline, the transformation used was log(lactate/choline + 0.001).

Results. Thirty-three of the 90 newborns (37%) had clinical seizures. Of the 33 newborns with seizures, 30 (91%) had at least one EEG in the neonatal period. Each of the newborns that did not have an EEG had a single seizure on day of life 2; two of these three newborns were treated with fewer than three anticonvulsant medications.

The lactate/choline and *N*-acetylaspartate/choline values for the basal nuclei and intervascular boundary zone differed (all $p < 0.005$) in the groups with seizures compared with those without seizures (table 3). The resuscitation scores and MRI scores differed significantly in the groups with seizures compared with those without seizures (see table 3). The SNAP-PE scores, umbilical cord artery pH, and the day of life on which the MRI was done did not differ significantly in newborns with seizures and those without (see table 3).

In the unadjusted analyses, increasing seizure score was associated with increased lactate/choline in the intervascular boundary zone (coefficient 0.07; 95% CI, 0.04–0.1; $p < 0.0001$) and basal nuclei (coefficient 0.06; 95% CI, 0.04–0.09; $p < 0.0001$). With increasing seizure score, *N*-acetylaspartate/choline decreased more prominently in the intervascular boundary zone (coefficient −0.02; 95% CI, −0.03–0.005; $p = 0.006$) than the basal nuclei (coefficient −0.01; 95% CI, −0.02–0.003; $p = 0.01$).

In the multivariable linear regression models, seizure severity was associated with increased lactate/choline in both the intervascular boundary zone ($p < 0.001$) and the basal nuclei ($p = 0.01$), when controlling for the severity of MRI abnormalities and amount of resuscitation at birth (table 4). When controlling for these potential confounders,

Table 4 *Multivariable linear regression coefficients for ^{1}H-MRS metabolite ratios in both regions of interest, expressed as coefficient (95% CI)*

Predictor variable	Basal nuclei lactate/choline	*p* Value	Intervascular boundary zone lactate/choline	*p* Value	Basal nuclei NAA/choline	*p* Value	Intervascular boundary zone NAA/choline	*p* Value
Seizure score	0.04 (0.009 to 0.06)	0.01	0.06 (0.03 to 0.09)	<0.0001	−0.003 (−0.014 to 0.008)	0.6	−0.01 (−0.03 to −0.001)	0.03
Resuscitation score	0.003 (−0.08 to 0.09)	0.9	0.03 (−0.07 to 0.14)	0.5	−0.04 (−0.07 to −0.005)	0.03	−0.007 (−0.05 to 0.03)	0.7
MRI score in region of interest	0.1 (0.05 to 0.16)	<0.0001	0.07 (0.03 to 0.11)	0.001	−0.03 (−0.05 to −0.005)	0.02	−0.017 (−0.03 to −0.001)	0.04

NAA = *N*-acetylaspartate.

each increase in seizure score was associated with a 19% increase in lactate/choline in the intervascular boundary zone (95% CI, 5.0–32.3%) and a 14% increase in the basal nuclei (95% CI 0.1–27.6%) (figure 2). Seizure severity was independently associated with diminished *N*-acetylaspartate/choline in the intervascular boundary zone ($p = 0.034$) but not the basal nuclei ($p = 0.61$), when controlling for the same potential confounders. *N*-acetylaspartate/choline in the intervascular boundary zone decreased 2.4% (95% CI, 0.2–4.6%) with each increase in seizure score.

SNAP-PE scores were not included in the final multivariable model as values were missing for 10 newborns that were not scored prospectively in the neonatal period. Addition of SNAP-PE in the model did not substantially alter the results.

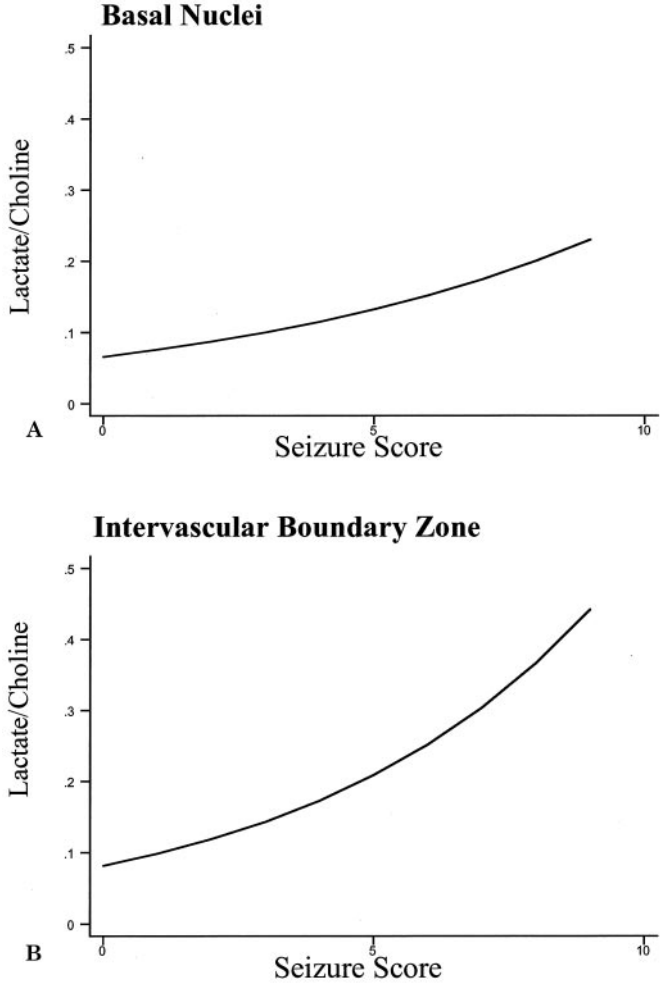

Figure 2. (A) Line graph of the average basal nuclei lactate/choline ratio, adjusted for the resuscitation score and the basal nuclei MRI score by the value of the seizure score. (B) Line graph of the average intervascular boundary zone lactate/choline ratio, adjusted for the resuscitation score and the intervascular boundary zone MRI score by the value of the seizure score.

Discussion. This study shows that the severity of seizures in human newborns with perinatal asphyxia is independently associated with brain injury. This supports the hypothesis, articulated based on animal models, that neonatal seizures are not the benign manifestation of existing brain injury.[7,13] Analogous to the relative paucity of histopathologic brain damage following seizures in immature animals, seizure-associated brain injury in the human newborn is not limited to structural damage detectable by MRI.

The elevation of lactate with increasing seizure severity is consistent with animal models in which neonatal seizures have been associated with transiently impaired cerebral energy metabolism, as measured by diminished storage forms of high-energy phosphate compounds and glucose, and elevation of the relative concentration of lactate.[1,28-30] Deranged cerebral energy metabolism has been observed in five human newborns with perinatal asphyxia, using P-MRS temporally proximate to seizures.[31] Although P-MRS does not measure lactate directly, the absence of acidosis in these five newborns raises the possibility that lactate was not produced. In contrast, we demonstrate a sustained elevation of relative lactate concentrations beyond the ictal period, indicating a persistent impairment in brain energy metabolism independent of the amount of resuscitation required at birth and structural brain damage detected by MRI.

Further supporting the independent association of seizures with impaired brain energy metabolism, elevation of lactate/choline with increasing seizure severity was substantially more prominent in the subcortical intervascular boundary zone compared with the basal nuclei. This is in sharp contrast to the spatial distribution of lactate seen in term newborns with perinatal asphyxia alone, where elevation of

lactate is most prominent in the basal nuclei compared with the intervascular boundary zones.[17,32] Thus, the basal nuclei may be affected more commonly in perinatal asphyxia while seizures influence the metabolism of the cortex and subcortical white matter (of the intervascular boundary zone) more prominently. An alternative explanation would be that neonates with greater cortical damage, as manifest by higher intervascular boundary zone lactate levels, are more likely to develop seizures.

In our cohort, the severity of neonatal seizures was independently associated with diminished neuronal integrity or function in the intervascular boundary zone, the area with the greater increase in lactate/choline. While it is possible that the early neonatal scans may have missed some structural injury in the cortex, our observation is consistent with experimental models in which neonatal seizures derange neuronal function and connectivity in the absence of significant structural neuronal loss.[9-11] Neonatal seizures have been associated with impaired mossy fiber sprouting even in the absence of cell loss[10] and, in immature rat, impaired neurogenesis in the hippocampus.[8] While in adult epilepsy, decreases in *N*-acetylaspartate are thought to reflect neuronal loss or dysfunction,[33-35] the modest decreases in *N*-acetylaspartate seen here may also reflect the early changes of impaired neurogenesis or impaired fiber sprouting. In the absence of a cortical voxel, we cannot exclude primary axonal injury. More likely, however, the finding of diminished *N*-acetylaspartate/choline in the intervascular boundary zone may represent either primary cortical neuronal loss with secondary axonal degeneration, impaired neuronal maturation in the cortex, or aberrant axonal sprouting. The ^{1}H-MRS techniques being used for the duration of this study did not permit sampling of a cortical voxel because of the proximity of the cortex to the potentially contaminating subcutaneous fat of the scalp; thus, we were unable to directly observe changes in the intervascular boundary zone cortex.

Only neonates with clinical seizures documented by experienced neonatal staff using standard clinical criteria were included in this cohort. As neonatal seizures are not uniformly related with seizure activity on the EEG, not all newborns had electrographically documented seizures.[1] Given this limitation, our cohort may have included newborns without electrographic seizures; this limits our observations to those newborns with clinically manifest seizures with or without electrographic seizures on EEG. Our voxel location was not determined by the lateralization of seizures, which is often unreliable and not of the same significance in the newborn as in the adult. Chance assignment of ^{1}H-MRS voxels would be expected to bias the finding of an association of seizure score and ^{1}H-MRS metabolite ratios toward the null hypothesis of no association between these variables. In addition, as the newborns in our cohort were all studied out of the ictal period, at a median of 6 days of age, the single ^{1}H-MRS study did not allow determination of the duration of impaired cerebral metabolism following seizures. Despite these limitations, our findings demonstrate that, for a given severity of structural brain injury and amount of neonatal resuscitation, worsening seizure severity is associated with increased brain lactate even beyond the ictal period.

The impairment of energy metabolism and neuronal integrity observed in our cohort is a potential mechanism by which seizures may cause or exacerbate brain injury in newborns with perinatal asphyxia. Consistent with the experience in adult lesional partial epilepsy, where ^{1}H-MRS demonstrates neuronal dysfunction or injury in areas that are normal on MRI, it appears that seizure severity in term newborns with perinatal asphyxia is associated with ongoing cerebral metabolic dysfunction and neuronal injury beyond that visualized on structural MRI.[36-38] ^{1}H-MRS can be used to demonstrate this dysfunction beyond that visualized with conventional MRI. While abnormalities of lactate and *N*-acetylaspartate in newborns with perinatal asphyxia are associated with abnormal neurodevelopmental outcome,[17,39-41] the long-term significance of this seizure-associated injury will require continued prospective studies.

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