AJNR

Detection of Cerebral β-Hydroxy Butyrate, Acetoacetate, and Lactate on Proton MR Spectroscopy in Children with Diabetic Ketoacidosis

Sandra L. Wootton-Gorges, Michael H. Buonocore, Nathan Kuppermann, James Marcin, Joseph DiCarlo, E. Kirk Neely, Patrick D. Barnes and Nicole Glaser

AJNR Am J Neuroradiol 2005, 26 (5) 1286-1291
http://www.ajnr.org/content/26/5/1286

AJNR Am J Neuroradiol 26:1286–1291, May 2005

Detection of Cerebral β-Hydroxy Butyrate, Acetoacetate, and Lactate on Proton MR Spectroscopy in Children with Diabetic Ketoacidosis

Sandra L. Wootton-Gorges, Michael H. Buonocore, Nathan Kuppermann, James Marcin, Joseph DiCarlo, E. Kirk Neely, Patrick D. Barnes, and Nicole Glaser

***BACKGROUND AND PURPOSE*: Ketone bodies provide important alternate fuel for brain metabolism, and their transport into the brain increases with prolonged fasting. During diabetic ketoacidosis (DKA), serum ketone concentrations markedly increase; however, little is known about whether ketone bodies accumulate in cerebral tissues during DKA. We used proton MR spectroscopy (MRS) to detect cerebral β-hydroxy butyrate (βOHB) and acetone/acetocaetate (AcAc) in children with DKA.**

***METHODS:* Twenty-five children underwent brain MRS: nine within 4 hours of the start of treatment for DKA; 11, at 4–8 hours; and five, at 8–12 hours. MRS was repeated after their recovery from the DKA episode at ≥72 hours after the start of treatment. MRS was evaluated for peaks corresponding to βOHB (doublet centered on 1.20 ppm) and lactate (doublet centered on 1.33 ppm). Difference spectroscopy was used to identify the AcAc peak at 2.22–2.26 ppm.**

***RESULTS:* βOHB was detected in 13 children (52%), more frequently within 4 hours (eight children, 89%) than after 4 hours (five children, 31%). AcAc was detected in 15 children (60%), more frequently at >4 hours after the start of treatment (12 patients, 75%) than in the first 4 hours (three patients, 33%). Lactate was detected in five children (18%), all within the first 8 hours of treatment.**

***CONCLUSION:* In children, βOHB and AcAc accumulate in the brain during DKA, and they can be detected on MRS. Care should be taken in interpreting MRS results in patients with DKA to avoid erroneously attributing βOHB peaks to lactate.**

Diabetic ketoacidosis (DKA) is a serious complication of diabetes mellitus type 1. Insulin deficit results in hyperglycemia, hyperosmolality, acidemia, and the production of ketones by the liver (1). These ketones can cross the blood-brain barrier and may be used as an alternative to glucose as an energy source in the brain (2, 3). However, whether these ketone bodies accumulate in cerebral tissues during DKA is unclear. Understanding the uptake and metabolism of ketones in the brain during DKA is important, as ketones may be involved in the development of the potentially life-threatening complication of cerebral edema. Recent data suggest that ketones themselves may alter permeability of the vascular endothelium and that they may be directly involved in the production of cerebral edema (4). MR spectroscopy (MRS) has provided a noninvasive means to begin to investigate this issue. The purpose of our study was use MRS to detect cerebral β-hydroxy butyrate (βOHB) and acetone/acetocaetate (AcAc) in children with DKA.

Received June 14, 2004; accepted after revision September 24.

From the Departments Radiology (S.L.W.-G., M.H.B.), Pediatrics (N.K., J.M., N.G.), and Internal Medicine, Division of Emergency Medicine (N.K.)‡, University of California, Davis School of Medicine, and the Departments of Pediatrics (J.D., E.K.N.) and Radiology (P.D.B.), Stanford University School of Medicine.

Supported by research grants from the American Diabetes Association and from the University of California, Davis Health System.

Address reprint requests to Sandra L. Wootton-Gorges, MD, Department of Radiology, UCDMC, 4860 Y Street, Suite 3100, Sacramento, CA 95817.

Methods

Patient Population

Patients were eligible for participation if they were younger than 18 years, if they had a diagnosis of diabetes mellitus type 1, and if they had DKA (defined as serum glucose >300 mg/dL, venous pH < 7.25 and/or serum bicarbonate level <15 mEq/l, and a positive result for urine ketones or serum ketones of >3 mmol/L).

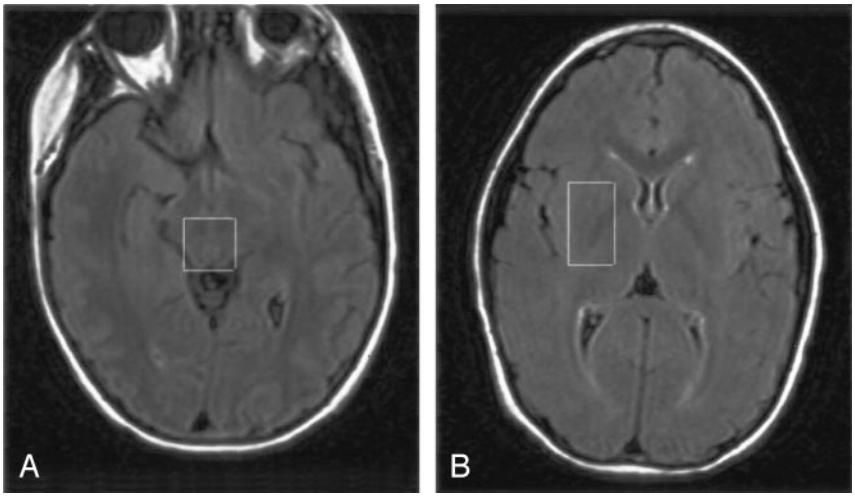

FIG 1. Position of voxels for spectroscopy in the periaqueductal region (*A*) and in the basal ganglia (*B*).

Treatment Protocol

The institutional review boards of the two participating institutions approved the study. After obtaining written informed consent from parents or guardians, we treated enrolled patients according to a standardized DKA protocol. All patients received an initial 10–20-mL/kg infusion of 0.9% saline, depending on the assessed degree of hypovolemia. Patients with persistently poor perfusion or hemodynamic instability after this infusion were given additional infusions of 0.9% saline until normal perfusion and hemodynamic stability were established. Subsequent intravenous fluids were administered as 0.67% saline (112 mEq sodium/L) with the rate calculated to replace an estimated fluid deficit of 70 mL/kg over 48 hours. Insulin was administered by means of continuous intravenous infusion at an initial rate of 0.1 U/kg/hour. Patients were not treated with bicarbonate. Potassium replacement was initiated with 20 mEq potassium chloride and 20 mEq potassium phosphate per liter of intravenous fluid and adjusted to maintain normal serum potassium concentrations. Glucose was added to the intravenous fluids when the patient's serum glucose concentration was less than 300 mg/dL. Serum electrolyte concentrations, venous pH, and partial pressure of CO_2 were measured at presentation and monitored during treatment.

Imaging Procedures

Patients enrolled in the study underwent brain MR imaging with proton MRS at two points: 2–12 hours after the start of treatment for DKA and after their recovery from the episode of DKA (at ≥72 hours after the start of treatment for DKA). Images were obtained by using a standard quadrature head coil and a 1.5-T imaging system (Signa; GE Medical Systems, Milwaukee, WI). During the each examination, all images were obtained at the same section locations with identical fields of view so that the anatomic and spectroscopic images were spatially registered. Three-plane localizing imaging was performed, followed by a T2-weighed fluid-attenuated inversion recovery (FLAIR) sequence (TR/TE/TI = 10,000/147/2200, 4.2-mm-thick sections).

Single-voxel spectroscopy was performed by using the Probe-P sequence (probeSI.psd; GE Medical Systems), with TR/TE 1500/144. One 8-cm^3 voxel was selected from the FLAIR images; it contained periaqueductal gray matter (18 patients) or basal ganglia (seven patients). Automatic shimming on the voxel was performed and routinely produced a line width of 4 Hz or less, water suppression of 99%, and a flip angle of 135° ± 30°. Figure 1 shows examples of voxel placement. The periaqueductal gray matter and basal ganglia were chosen for spectroscopy on the basis of the known regional pattern of brain injury seen in children with DKA and cerebral edema (5, 6).

Use of pharmacologic sedation during the imaging procedures was avoided whenever possible. However, when sedation was necessary, sodium pentobarbital (≤2 mg/kg) or midazolam (≤0.1 mg/kg) was used.

Peaks of cerebral metabolites were identified according to their chemical shifts, as follows (7): *N*-acetylaspartate (NAA, 2.02 ppm), creatine and phosphocreatine (Cr, 3.0 ppm), free choline compounds (Cho, 3.20 ppm), acetone (2.22 ppm), lactate (doublet with peaks at 1.28 and 1.38 ppm), and βOHB (doublet with peaks at 1.15 and 1.25 ppm). With a TE of 144 ms, lactate and βOHB were defined as inverted peaks, and lipids were suppressed.

To identify lactate and distinguish it from βOHB, we used a phantom model. Three 500 mL water-filled MR spectroscopy test phantoms were constructed (Huntington Medical Research Institute, Pasadena, CA) based on published methods and metabolite concentrations (8). NAA, Cr hydrate (Cr), and Cho chloride (Cho) concentrations were 12.5, 10.0, and 2.0 mmol/L, respectively. The phantoms were identical in composition with the exception that the first phantom contained βOHB but no L-lactate, the second phantom contained L-lactate but no βOHB, and the third phantom contained both βOHB and L-lactate. To obtain larger βOHB and lactate peaks, the concentrations of both these metabolites were increased to 15 mmol/L, three times that described in a previous report (9).

Single-voxel MRS was performed in each phantom by using a point-resolved spectroscopic (PRESS) sequence with timing parameters identical to those used in patients (TR/TE = 1500/144). PRESS spectra with TEs of 192 and 288 ms were also obtained to confirm the inversion properties of the J-coupled doublets, and a stimulated echo-acquisition mode (STEAM) sequence with TE = 35 ms was performed to confirm the presence of the full set of metabolites. The phantoms were scanned at room temperature, and the spectra were shifted according to the established dependence of chemical shift of these peaks on temperature. The phantom PRESS spectra clearly showed NAA, CR, Cho peaks, in addition to the βOHB and or L-lactate peaks.

The main peaks of NAA, Cr, and Cho were used to register the phantom spectra with the *in vivo* spectra from the patients. A best fit between these peaks was manually determined on the phantom and the *in vivo* spectra to allow for both shifting and horizontal scaling of the *in vivo* spectra. The doublet peak thought to corresponding to βOHB and or L-lactate on the patient spectra could then be confirmed on the basis of the alignment of the peaks with the doublet or triplet in the phantom spectra.

For each subject, we calculated the difference spectrum, defined as the subtraction of the spectrum obtained during

Clinical characteristics of the 25 study participants at presentation

Characteristic	Value
Age (y)	12.0 ± 2.7
Sex (%)	
Female	52
Male	48
New-onset diabetes (%)	44
Serum sodium level (mmol/L)	132 ± 4
Serum potassium level (mmol/L)	4.7 ± 0.9
Serum urea nitrogen level (mg/dL)	21 ± 9
Serum creatinine level (mg/dL)	1.3 ± 0.4
Serum glucose level (mg/dL)	647 ± 216
Venous pH	7.12 ± 0.06
Partial pressure of CO_2	22 ± 9

Note.—Data are the mean ± standard deviation unless otherwise specified.

DKA treatment from the spectrum obtained after recovery. Each spectrum was first converted to a continuous function by fitting third-order interpolating functions between each measured value of the spectrum. Each spectrum was then adjusted by subtracting a baseline value computed as the mean value in the frequency range of 0.6–1.0 ppm for the spectrum obtained during DKA treatment, and the range 0.6–1.6 ppm for the spectrum obtained after recovery. To prepare the spectra for subtraction, an overall scale factor and a frequency shift was applied to the spectrum obtained after recovery. This scale factor and frequency shift were determined by means of least-squares fit of the admission spectrum to the scaled and shifted spectrum obtained after recovery. This fit allowed us to match the main spectrum peaks of NAA, Cr, and Cho and thereby produce the smallest appearance in the difference spectrum.

Results

Twenty-five children with DKA were enrolled (Table). At enrollment, 11 children complained of headache, and two had altered mental status (Glasgow Coma Scale [GCS] scores of less than 15). One of the two had a GCS score of 13, which returned to normal (GCS score of 15) 18 hours after the start of therapy for DKA. The other child was severely obtunded, with an initial GCS score of 6. Her mental status improved after 5 hours of treatment for DKA and returned to normal after 12 hours. Seven additional children had deterioration of mental status during treatment. Six had mild mental status decline (minimum GCS scores of 14). The seventh child had a decline in the GCS score to 11 at hour 7, but this returned to normal by hour 10. None of these children were treated for cerebral edema because their inversion-recovery images did not show overt evidence of cerebral swelling (i.e., reduced ventricular size, unapparent basilar cisterns, or intensity abnormalities). All patients fully recovered without neurologic deficits. Four children received pharmacologic sedation for initial imaging, and three children received sedation for follow-up imaging.

Nine children (36%) were initially imaged within 4 hours after beginning treatment for DKA. Eleven (44%) were initially imaged 4–8 hours after beginning treatment, and five (20%) were initially imaged 8–12 hours after beginning treatment. βOHB was

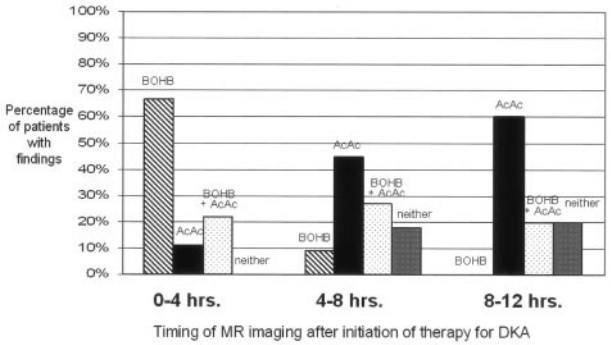

FIG 2. Patients with βOHB, AcAc, both, or neither on initial imaging during DKA therapy.

detected in 13 children (54%): eight (89%) of nine patients in the 0–4-hour group, four (36%) of 11 patients in the 4–8-hour group, and in one (20%) of five in the 8–12-hour group (Figs 2–4).

AcAc was detected during difference spectroscopy in 15 patients (54%) (Figs 2, 4, 5). AcAc was seen in three (33%) patients in the 0–4-hour group, in eight (73%) in the 4–8-hour group, and in four (80%) in the 8–12-hour group.

In five patients, lactate was detected during their initial studies. Four of these patients had both βOHB and lactate (Fig 6), and one had lactate alone. All of these patients had been imaged within the first 8 hours of therapy. Serum lactate concentrations were measured in four of five children with detectable lactate on MRS. Serum lactate concentrations were elevated in two of these four. Among the patients without detectable lactate on MRS, serum lactate concentrations were measured in 13 and were found to be elevated in seven. Therefore, the frequency of elevated serum lactate levels was similar in those patients with and in those without detectable lactate on MRS.

Of nine children who had an abnormal mental status (GCS score <15) at presentation or during treatment, six (67%) had βOHB detectable on MRS, and seven (78%) had AcAc. In comparison, βOHB and AcAc were detected less frequently in 16 children with normal mental status at presentation and during treatment; βOHB was detected in seven (44%), and AcAc in six (37%). The difference in the frequency of detecting βOHB was not significant ($P = .25$); however, the difference in the frequency of detecting AcAc showed a trend toward significance ($P = .06$). Lactate was detected in four (44%) of nine children with abnormal mental status compared with one (6%) of 16 with normal mental status ($P = .04$).

Lactate, βOHB and AcAc were not detected in any of the spectra obtained during follow-up studies at 72 hours or longer after treatment for DKA.

Discussion

We documented the accumulation of ketone bodies in the brain in children with DKA and demonstrated that the time course of changes in cerebral ketone-

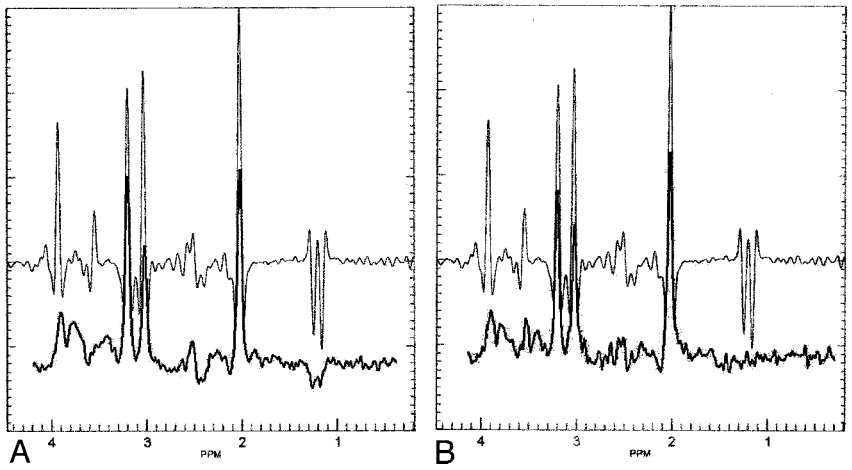

FIG 3. Findings in a 3-year-old girl with new-onset DKA.

A, Initial spectra were obtained 2 hours after the start of therapy for DKA (*black*) in the patient and a phantom containing only βOHB (*gray*). βOHB is seen as a doublet centered at 1.2 ppm.

B, βOHB is absent in the patient's spectrum (*black*) obtained after her recovery from DKA.

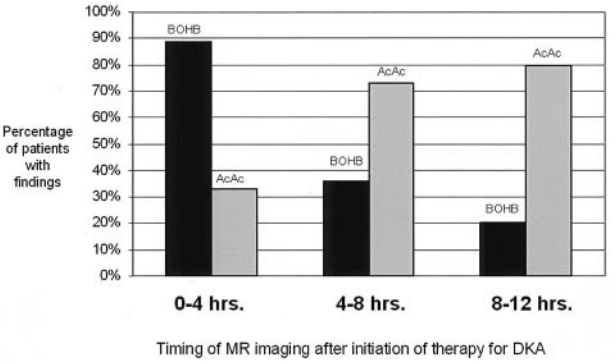

FIG 4. Patients with βOHB and/or AcAc on initial imaging by the timing of the study after the start of therapy.

body concentrations paralleled the typical pattern of changes in serum ketone-body concentrations observed during DKA treatment. We believe we are the first to document the presence of βOHB in cerebral tissues in patients with DKA and the first to document the presence of AcAc in a pediatric study.

In children, insulin-dependent diabetes is a complex metabolic disorder, and cerebral edema is a serious and potentially life-threatening complication of DKA. Metabolic alterations in DKA include insulin deficiency, hyperglycemia, hyperosmolality, acidemia, and the accumulation of ketone bodies produced by the liver (1). Acidemia can increase levels of the ketone bodies AcAc and βOHB by as much as 30 times, and the accumulation of lactate may augment this acidemia. Although serum levels of ketone bodies are known to be elevated, no human study has demonstrated βOHB accumulation in the brain during DKA, to our knowledge. One study has demonstrated the presence of AcAc in cerebral tissues in a small number of adults with diabetes (9).

In the normal state, glucose is the predominant fuel used by the brain. Previous studies have demonstrated that the brain can use ketone bodies as an alternative energy source for glucose during starvation, fasting, or strenuous exercise (2, 3, 10). However, acute infusion of βOHB in the nonfasting state does not increase brain βOHB levels (11, 12). With prolonged fasting, upregulation of monocarboxylic acid transporters in the blood-brain barrier induces the entry of ketones into the brain (11). Ketone bodies enter the tricarboxylic acid cycle after being converted to acetyl coenzyme A. Metabolism of ketones leads to an inhibition of glycolysis and a decrease in glucose utilization by the brain (13, 14).

Previous studies of proton MRS in adults with diabetes mellitus have shown elevated AcAc levels in patients with DKA. Using difference spectroscopy, Kreis and Ross (9) found a peak at 2.22 ppm in two of nine adults with DKA. This peak, which disappeared after the patients' recovery from DKA, was attributed to acetoacetate or acetone. These investigators did not detect βOHB in any of their patients. Acetone has also been identified during proton spectroscopy in the occipital lobes of children treated with a ketogenic diet for epilepsy control (15).

Lapidot and Haber (14) found elevated plasma and brain levels of βOHB in diabetic, hyperketonemic young adult rabbits. βOHB was observed in some of the ^{13}C spectra in brain tissue. However, though an infusion of βOHB into normal nondiabetic rabbits significantly increased plasma levels, it did not increase cerebral βOHB levels. βOHB has also been documented on 3-T proton MRS in the basal ganglia of two children with persistent hyperinsulinemic hypoglycemia of infancy who received oral βOHB supplementation as treatment for hypoglycemia (16). However, to our knowledge, we are the first to document the presence of βOHB in the brain in children with DKA. Our finding of accumulated βOHB likely results from two factors: First, we imaged patients with DKA earlier in the course of treatment than did previous investigators. Because detection of cerebral βOHB was greatest during the first 4 hours of therapy, earlier imaging allowed us to detect βOHB more readily. Second, healthy children have overnight fasting values of ketone bodies higher than those of adults, and they appear to use βOHB in the brain to a greater extent than do adults (17).

The serum ratio of βOHB to acetoacetate is known to rise during DKA from a normal ratio of 1:1 to as high as 10:1. During treatment, this ratio returns to normal as βOHB is metabolized to acetoacetate. Our

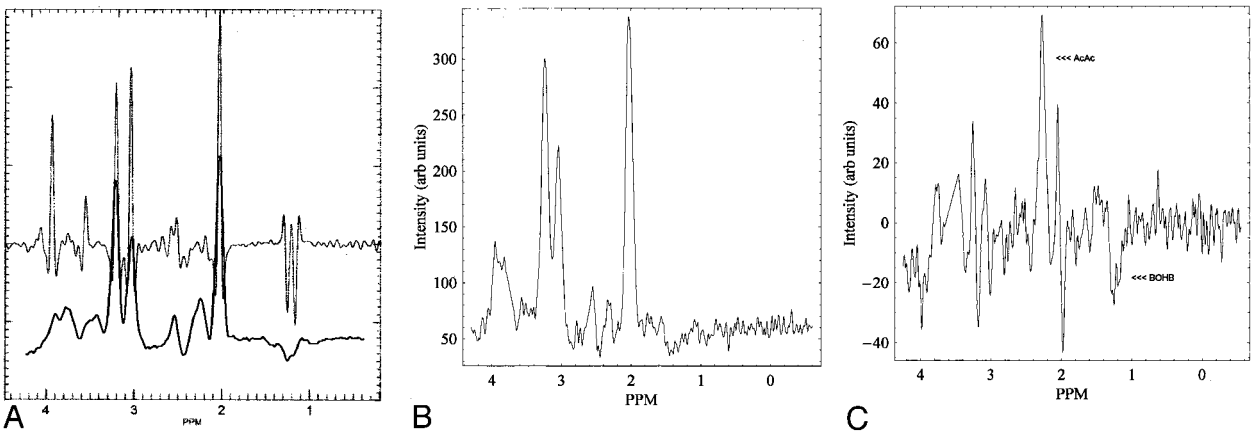

FIG 5. Findings in a 14-year-old male adolescent; *arb* indicates arbitrary.
A, Initial spectrum (*black*) during DKA shows AcAc (peak at 2.22 ppm) and βOHB. Spectrum from the phantom containing βOHB is *gray*.
B, On the spectrum obtained after the patient's recovery from DKA, AcAc and βOHB are absent.
C, Difference spectrum between *A* and *B*.

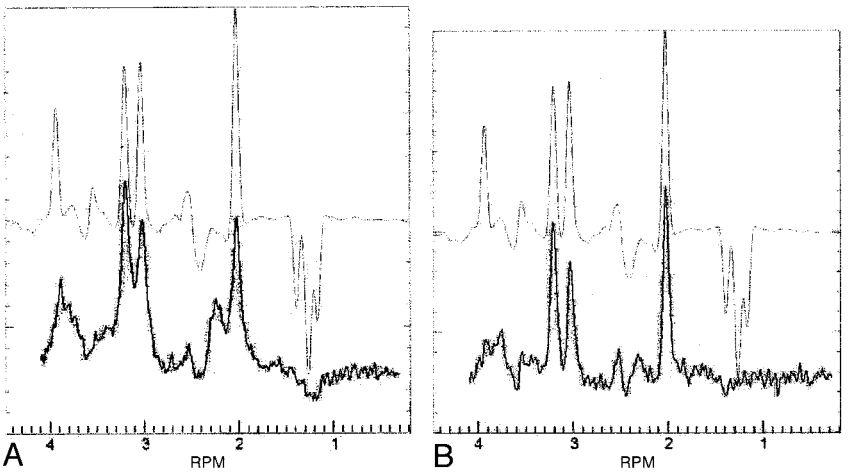

FIG 6. Findings in an 11-year-old girl with DKA.
A, Initial spectrum (*black*) during treatment for DKA and spectrum from phantom containing both lactate and βOHB (*gray*). Patient's spectrum shows lactate and βOHB as a triplet with peaks from 1.15 to 1.38 ppm. AcAc is noted at 2.22–2.26 ppm.
B, After recovery, no lactate or βOHB is detected on the patient's spectrum (*black*).

study demonstrated that brain concentrations of ketone bodies parallel those typically seen in the serum, with βOHB present early in DKA treatment and with AcAc appearing later.

Although none of our patients had overt cerebral edema, previous studies have demonstrated that most children with DKA have evidence of subtle, subclinical cerebral edema (18–20). Studies in children without overt manifestations of cerebral edema nonetheless may contribute useful information regarding the pathophysiology of edema formation. The current data provide important insights into the alterations in cerebral metabolic state that occur during DKA. Ketone accumulation in the brain may be important because ketones have been found to have direct effects on the brain microvascular endothelium (4), and they may alter permeability of the blood brain barrier. Elevated brain glucose and ketone levels may also cause oxidative tissue damage, resulting in disruption of membrane structure. This may result from both production of reactive oxygen species, as well as from a compromised free radical scavenger system (21, 22). This combination of microvascular effects and membrane disruption could possibly contribute to the development of cerebral edema.

In our study, lactate was present on MRS in several patients. Lactate was detected with significantly greater frequency in children with mental status abnormalities during DKA treatment than in those with normal mental status. Detection of lactate may indicate anaerobic cerebral metabolism; these findings therefore support the possibility that cerebral hypoperfusion occurs during DKA and that is plays a role in the pathogenesis of cerebral edema. For ethical reasons, treatment for DKA cannot be withheld to conduct MRS; therefore, the cerebral metabolic state during untreated DKA could not be evaluated. However, because lactate was detectable in some patients several hours after the start of treatment for DKA, lactate might have been present in more patients or in greater concentrations if MRS could have been performed before start of treatment.

These data have practical implications for the use of MRS in evaluating patients with diabetes. Lactate, βOHB, and AcAc may all be detected during brain proton MRS in children with DKA. In particular,

lactate and βOHB arise in overlapping spectral locations and must be differentiated from one another. Lactate is an indicator of anaerobic cerebral metabolism. In other studies of patients with brain injury, detection of lactate on MRS was correlated with poorer neurologic outcomes (23, 24). Because of the overlapping spectral locations of lactate and βOHB, βOHB might be easily mistaken for lactate and interpreted as representing cerebral ischemia, with a poor prognosis. Our findings should alert radiologists performing MRS to the frequent detection of βOHB in children with DKA, so that MR spectra in these patients can be interpreted accurately.

Acknowledgments

We gratefully acknowledge the assistance of John Ryan, Greg Davis, and Manju Dhaliwal in conducting the MR studies.

References

1. Sperling MA. **Diabetic ketoacidosis.** *Pediatr Clin North Am* 1984;31:591–610
2. Balasse EO, Fery F. **Ketone body production and disposal: effects of fasting, diabetes and exercise.** *Diabetes Metab Rev* 1989;5: 247–270
3. Robinson AM, Williamson DH. **Physiological role of ketone bodies as substrates and signals in mammalian tissues.** *Physiol Rev* 1989;60:143–187
4. Isales CM, Min L, Hoffman WH. **Acetoacetate and β-hydroxybutyrate differentially regulate endothelin-1 and vascular endothelial growth factor in mouse brain microvascular endothelial cells.** *J Diabetes Complications* 1999;13:91–97
5. Roe TF, Crawford TO, Huff KR, Costin G, Kaufman FR, Nelson MD Jr. **Brain infarction in children with diabetic ketoacidosis.** *J Diab Comp* 1996;10:100–108
6. Shrier DA, Shibata DK, Wang HZ, Numaguchi Y, Powers JM. **Central brain herniation secondary to juvenile diabetic ketoacidosis.** *Am J Neuroradiol* 1999;20:1885–1888
7. Moore GJ. **Proton magnetic resonance spectroscopy in pediatric neuroradiology.** *Pediatr Radiol* 1998;28:805–814
8. General Electric Company. *Operator Manual for Signa Horizon LX and Signa MR/I Systems: Multinuclear Spectroscopy Features.* Release 8.3,.Direction 2229281–100. Revision E. June 1999. Milwaukee: GE Medical Systems; 1999;6:6
9. Kreis R, Ross BD. **Cerebral metabolic disturbances in patients with subacute and chronic diabetes mellitus: detection with proton MR spectroscopy.** *Radiology* 1992;184:123–130
10. Owen OE, Morgan AP, Kemp HG, Sullivan JM, Herrera MG, Cahill GF. **Brain metabolism during fasting.** *J Clin Invest* 1967;46:1589–1595
11. Pan JW, Telang FW, Lee JH, DeGraaf RA, Rothman DL, Stein DT, Hetherington HP. **Measurement of β-hydroxybutyrate in acute hyperketonemia in human brain.** *J Neurochem* 2001;79:539–544
12. Blomqvist G, Alvarsson M, Grill V, et al. **Effect of acute hyperketonemia on the cerebral uptake of ketone bodies in nondiabetic subjects and IDDM patients.** *Am J Physiol Endocrinol Metab* 2002;283:E20–E28
13. Lapidot A, Haber S. **Effect of endogenous β-hydroxybutyrate on brain glucose metabolism in fetuses of diabetic rabbits, studied by ^{13}C magnetic resonance spectroscopy.** *Brain Res Dev Brain Res* 2002;135:87–99
14. Lapidot A, Haber S. **Effect of endogenous β-Hydroxybutyrate on glucose metabolism in the diabetic rabbit brain: a ^{13}C-magnetic resonance spectroscopy study of [U-^{13}C] glucose metabolites.** *J Neurosci Res* 2001;64:207–216
15. Seymour KJ, Bluml S, Sutherling J, Sutherling W, Ross BD. **Identification of cerebral acetone by ^{1}H-MRS in patients with epilepsy controlled by ketogenic diet.** *MAGMA* 1999;8:33–42
16. Plecko B, Stoeckler-Ipsironglu S, Schober E, et al. **Oral β-hydroxybutyrate supplementation in two patients with hyperinsulinemic hypoglycemia: monitoring of β-hydroxybutyrate levels in blood and cerebrospinal fluid, and in the brain by in vivo magnetic resonance spectroscopy.** *Pediatr Res* 2002;52:301–306
17. Settergren G, Lindblad BS, Persson B. **Cerebral blood flow and exchange of oxygen, glucose, ketone bodies, lactate, pyruvate and amino acids in anesthetized children.** *Acta Paediatr Scand* 1980;69:457–465
18. **Risk factors for cerebral edema in children with diabetic ketoacidosis: the Pediatric Emergency Medicine Collaborative Research Committee of the American Academy of Pediatrics.** *N Engl J Med* 2001;344:264–9
19. Krane EJ, Rockoff MA, Wallman JK, Wolfsdorf JI. **Subclinical brain swelling in children during treatment of diabetic ketoacidosis.** *N Engl J Med* 1985;312:1147–1151
20. Hoffman WH, Steinhart CM, Gammal TE, Steele S, Cuadrado AR, Morse PK. **Cranial CT in children and adolescents with diabetic ketoacidosis.** *Am J Neuroradiol* 1988;9:733–739
21. Baynes JW. **Role of oxidative stress in development of complications in diabetes.** *Diabetes* 1991;40:405–412
22. Bhardwaj SK, Sharma P, Kaur G. **Alterations in free radical scavenger system profile of type I diabetic rat brain.** *Mol Chem Neuropathol* 1998;35:187–202
23. Auld KL, Ashwal S, Holshouser BA, Tomasi LG, Perkin RM, Ross BD, Hinshaw DB. **Proton magnetic resonance spectroscopy in children with acute central nervous system injury.** *Pediatr Neurol* 1995;12:323–334
24. Holshouser BA, Ashwal S, Shu S, Hinshaw DB. **Proton MR spectroscopy in children with acute brain injury: comparison of short and long echo time acquisitions.** *J Magn Reson Imaging* 2000;11: 9–19