



# Neurological Consequences of Diabetic Ketoacidosis at Initial Presentation of Type 1 Diabetes in a Prospective Cohort Study of Children

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## OBJECTIVE

To investigate the impact of new-onset diabetic ketoacidosis (DKA) during childhood on brain morphology and function.

## RESEARCH DESIGN AND METHODS

Patients aged 6–18 years with and without DKA at diagnosis were studied at four time points: <48 h, 5 days, 28 days, and 6 months postdiagnosis. Patients underwent magnetic resonance imaging (MRI) and spectroscopy with cognitive assessment at each time point. Relationships between clinical characteristics at presentation and MRI and neurologic outcomes were examined using multiple linear regression, repeated-measures, and ANCOVA analyses.

## RESULTS

Thirty-six DKA and 59 non-DKA patients were recruited between 2004 and 2009. With DKA, cerebral white matter showed the greatest alterations with increased total white matter volume and higher mean diffusivity in the frontal, temporal, and parietal white matter. Total white matter volume decreased over the first 6 months. For gray matter in DKA patients, total volume was lower at baseline and increased over 6 months. Lower levels of *N*-acetylaspartate were noted at baseline in the frontal gray matter and basal ganglia. Mental state scores were lower at baseline and at 5 days. Of note, although changes in total and regional brain volumes over the first 5 days resolved, they were associated with poorer delayed memory recall and poorer sustained and divided attention at 6 months. Age at time of presentation and pH level were predictors of neuroimaging and functional outcomes.

## CONCLUSIONS

DKA at type 1 diabetes diagnosis results in morphologic and functional brain changes. These changes are associated with adverse neurocognitive outcomes in the medium term.

The incidence of childhood-onset type 1 diabetes varies from 0.1 to 57.6 per 100,000 and is increasing worldwide (1). Long-term cognitive consequences of type 1 diabetes and associated fluctuations in glycemia during childhood and adolescence are well documented (2). Strategies to prevent or ameliorate these adverse outcomes require an

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understanding of the nature and timing of the neurological insults associated with diabetic dysglycemia.

The most severe acute diabetes-related central nervous system complication in type 1 diabetes is cerebral edema associated with diabetic ketoacidosis (DKA) (2), with 10–25% of affected children experiencing chronic central nervous system morbidity (3). Although the frequency of DKA at diagnosis is relatively high (15–70% depending on age and geographic region), fulminate clinical cerebral edema in this context is relatively rare, with an incidence rate of 0.5–0.9% (4); hence, documented brain injury is also rare. The more frequent, milder alterations in brain function and chemistry in newly diagnosed DKA have been assumed to be transitory with normalization after metabolic stabilization (5–8), but this assumption has not been formally tested. We hypothesized that just as there exists a continuum of clinical and subclinical cerebral edema (9), there also is a continuum of brain injury in DKA and that brain injury outside the context of florid cerebral edema is more common than previously recognized.

The purpose of this study in children with new-onset DKA was twofold. The first aim was to document acute alterations in magnetic resonance imaging (MRI) measures of cerebral structure and cognitive function and correlate these with clinical parameters. The second aim was to study the evolution of these alterations in brain structure and cognitive functioning over the first 6 months.

## RESEARCH DESIGN AND METHODS

We conducted a prospective, longitudinal cohort study at the Royal Children's Hospital (RCH), Melbourne, Australia, between June 2004 and October 2009 in children aged 6–18 years with a new diagnosis of type 1 diabetes. The diabetes clinic at RCH provides services to ~75% of children with diabetes in Melbourne, a large (population of 4.17 million), sociodemographically, and ethnically diverse region. The clinic population comprised 1,650 children and adolescents aged up to 19 years, with 2–3 children being diagnosed each week. Study participants were recruited sequentially as they presented to RCH at diagnosis. Participants underwent MRI, magnetic resonance spectroscopy

(MRS), and cognitive evaluation at the following four time points: Baseline was within 48 h, day 5 between 5 and 7 days, day 28 between 26–30 days, and 6 months between 25 and 27 weeks after presentation. Exclusion criteria were pre-existing neurological abnormality, evidence of established brain abnormality on MRI, clinical signs of cerebral edema (headache with obtundation [impaired sensorium] or deterioration in Glasgow Coma Scale score, bradycardia with hypertension), presence of metal-wired orthodontic braces, and inability to undergo MRI without general anesthesia. Of 205 eligible children, 95 were enrolled in the study (36 with DKA [defined as serum pH <7.30] and 59 without DKA). Clinical status of participants (DKA vs. non-DKA) was classified unambiguously according to parameters measured biochemically in a National Association of Testing Authorities, Australia-accredited laboratory. Siemens RAPIDLab 1260 with a measurement of uncertainty of  $\pm 0.02$  was used to measure pH level. All participants were treated per standard hospital protocol ([http://www.rch.org.au/clinicalguide/guideline\\_index/Diabetes\\_Mellitus](http://www.rch.org.au/clinicalguide/guideline_index/Diabetes_Mellitus)). The study was approved by the RCH Human Research Ethics Committee, and written informed consent was obtained from participants and their guardians.

## Measures

### Clinical and Demographic Information

Clinical information collected at diagnosis were age, sex, weight, height, blood pressure, pulse rate, and serum biochemical measures (pH, CO<sub>2</sub>, HCO<sub>3</sub>, base excess, glucose, osmolality, sodium, urea). Percent dehydration was calculated as  $[(\text{discharge weight} - \text{admission weight}) / \text{discharge weight}] \times 100$ . Discharge weight was measured between 3 and 5 days after admission. Socioeconomic status (SES) was rated according to postal area using the Australian Bureau of Statistics Socio-Economic Indexes for Areas (10). Parents also provided information on their child's developmental and school history (birth complications, motor and language milestones, academic performance, need for remedial classes, tutoring, or enrollment in special education setting).

The following data were collected at each assessment: height, weight, episodes of severe hypoglycemia, episodes of recurrent DKA, comorbidities, insulin

dose and regimen, and glycated hemoglobin. Severe hypoglycemia was defined as hypoglycemia with loss of consciousness and/or seizure or altered conscious state that required therapy. DKA was defined in the same way (serum pH <7.30) as at presentation. Insulin dose-adjusted A1C (IDAA1C) levels were calculated as an index of degree of residual endogenous insulin secretion (11).

## MRI

Brain imaging was performed at four time points using a Siemens Trio 3T scanner. Images were acquired using the standard quadrature head coil. Transverse, T2-weighted images were acquired using a turbo spin echo sequence (repetition time [TR]/echo time [TE] 6,400/93 ms, echo train length 17, slice thickness 3.5 mm, in-plane resolution  $0.51 \times 0.51$  mm). T1-weighted images were acquired using an magnetization-prepared rapid acquisition with gradient echo sequence with contiguous slices (TR/TE/inversion time 1,900/2.19/900 ms, flip angle 9°, contiguous slices 1 mm<sup>3</sup> isotropic spatial resolution). Diffusion tensor imaging data were acquired with a spin echo echoplanar imaging sequence using one of two image parameter sets. The first was TR/TE 6,000/97 ms, slice thickness 3 mm, in-plane resolution  $1.5 \times 1.5$  mm, and b amplitude  $1,000 \times 10^{-3}$  s/mm<sup>2</sup> with 20 directions. The second was TR/TE 4,000/89 ms, slice thickness 4 mm, in-plane resolution  $1.7 \times 1.7$  mm, and b amplitude  $1,000 \times 10^{-3}$  s/mm<sup>2</sup> with 24 directions plus five b = 0 acquisitions.

Cortical and volumetric segmentation were performed with FreeSurfer 4.4 (<http://surfer.nmr.mgh.harvard.edu>) using the T1-weighted images. Volumes were determined for both cortical and subcortical regions (12). Parcellated brain volumes were combined into larger regions matching the regions for diffusion analysis. These included frontal, temporal, and parietal regional volumes (Supplementary Table 1) and were expressed as a proportion of the total brain volume (TBV) to normalize for age-related variations in brain size.

Mean diffusivity (MD) and anisotropy were measured. MD is a measure of tissue water displacement. In the clinical setting, MD values are typically used to detect brain injury, with a reduction in MD associated with acute and subacute

injury such as stroke. Increases in MD values reflect increased tissue fluid content and/or more subtle cellular injury or loss. Diffusion anisotropy is a measure of the directional variation of water displacement, with values greater in highly ordered structures such as myelinated white matter. Anisotropy values may consistently change in the opposite direction of MD. It is likely that this inverse relationship is caused by tissue swelling, with the greater spacing of membrane boundaries associated with tissue swelling leading to an increase in MD and decrease in anisotropy. As a result of this close coupling between MD and anisotropy, the anisotropy values are relatively uninformative. Thus, the results and discussion in this article focus on MD, and anisotropy is not discussed in any detail.

Raw data from the diffusion acquisitions were phased using a Bayesian procedure (13). The images were aligned to the corresponding T2-weighted image data set. Diffusion encodings corrupted by participant motion were automatically identified and removed. Parametric maps were generated for MD and fractional anisotropy. Parameter sampling was performed with Analyze version 9.0 software (Mayo Clinic, Rochester, MN). A data analyst blinded to subject status placed regions of interest (ROIs) in the gray and white matter of the frontal, temporal, and parietal lobes.

#### MRS

Single-voxel, water-saturated spectra were acquired using a standard point resolved spectroscopy sequence. ROIs included the left-side frontal white matter, frontal gray matter, and left-side basal ganglia, each previously identified as vulnerable (7,14). The ROIs were positioned using T1-weighted scout images. Basal ganglia ROIs were positioned over the lentiform nuclei; frontal lobe white matter ROIs were positioned above the anterior tip of the left lateral ventricle; and frontal lobe gray matter ROIs were placed in the same plane as the white matter voxel, anterior to and at the level of the corpus callosum, within cingulate gray matter. Acquisitions comprised 80 transients recorded with a TE/TR of 30/3,000 ms. The voxel placed in the basal ganglia was  $20 \times 20 \times 10 \text{ mm}^3$ , and the frontal lobe voxels were  $25 \times 20 \times 15 \text{ mm}^3$ . All measurements were made

with the same Siemens 3T scanner using a quadrature head coil. Metabolite concentrations are presented relative to creatine concentration. The values for *N*-acetylaspartate (NAA) reported here include the NAA-glutamate component and are expressed as a ratio relative to creatine (reported as NAA). NAA is considered a marker of neuronal density. Myoinositol concentration is an endogenous osmolyte considered to be a marker of gliosis. Tissue metabolite content was determined using LCModel (15) with a basis set of 21 metabolites prepared using reference solutions.

#### Cognition

**Mental State.** At day 1, tests were limited to those that could be administered at the bedside to unwell children, which were the Mental State School-Years Screening Test for the Evaluation of Mental Status (SYSTEMS) (16) and a paired associate verbal learning task (17). The SYSTEMS is a child-appropriate alternative to the adult Mini-Mental State Examination (18) on which it is based. It was developed in a pediatric hospital to measure subtle changes in mental state and cognition in the context of both acute and chronic illness or brain injury. The SYSTEMS was administered at days 1 and 5 after admission. Responses were classified using age-specific cutoff scores (16).

**Memory.** Verbal memory was assessed at each time point using a paired associate learning task where children learned novel word pairs and recalled them after a delay (17). Alternative forms of the test were used to minimize practice effects. The number of words remembered after a delay was reported.

**Attention.** Four tasks (Sky Search [focused attention], Sky Search Dual Task [auditory/visual divided attention], Score Dual Task [auditory/auditory divided attention], and Walk/Don't Walk [sustained attention/impulsivity]) from the Test of Everyday Attention for Children (19) were administered at day 5, day 28, and 6 months. Age- and sex-standardized scores were reported.

#### Statistical Analysis

All data (Supplementary Tables 2–4) were analyzed using SPSS version 20 (IBM Corporation) statistical software. Groups (DKA and non-DKA) were compared using ANOVA and ANCOVA (continuous variables) and  $\chi^2$  (categorical

variables) analyses. Age and sex were added as covariates in all analyses involving nonage- and nonsex-adjusted variables. Change in neurologic outcome over time was investigated using repeated-measures ANCOVA. Relationships between clinical characteristics at presentation and neurologic outcomes were examined using multiple linear regression analyses with age and sex entered as additional predictors. Regression diagnostics were performed, and no multicollinearity among independent variables was identified. Data for this model are also presented in terms of percentage of variance accounted for by the individual predictor ( $sr^2$ ). Proportionate changes in brain measures at presentation were calculated as (measured value at day 5 – measured value at baseline)/value at day 5. Proportionate volume changes were correlated with cognitive variables at 6 months. All analyses were two-sided, and a Bonferroni correction was applied, resulting in an  $\alpha$  level of 0.01.

## RESULTS

Participant characteristics are described in Table 1. DKA/non-DKA participant numbers were 36/59, 29/46, 27/46, and 25/44 at the four time points of <48 h, day 5, day 28, and 6 months, respectively. There were no differences between the DKA and non-DKA groups in age, duration of symptoms, SES, or preexisting premorbid need for school assistance/remediation. After diagnosis, the groups did not differ in frequency of severe hypoglycemia, recurrent DKA, thyroid or celiac disease, insulin regimens, or glycated hemoglobin (raw or IDAA1C).

#### MRI and MRS Measures

##### Total Cerebral Volumes

Total supratentorial brain volumes did not differ with DKA at any time point (Fig. 1A) or change over time in either group.

##### White Matter

Relative total white matter volume was greater in the DKA group at baseline compared with the non-DKA group ( $F = 7.43$ ,  $P = 0.008$ ) (Fig. 1D) and decreased over 6 months ( $F = 25.72$ ,  $P < 0.001$ ). For regional analysis, relative frontal white matter volume was greater in the DKA group at baseline ( $F = 10.84$ ,  $P = 0.002$ , Fig. 2A) and decreased over 6 months

**Table 1—Patient characteristics**

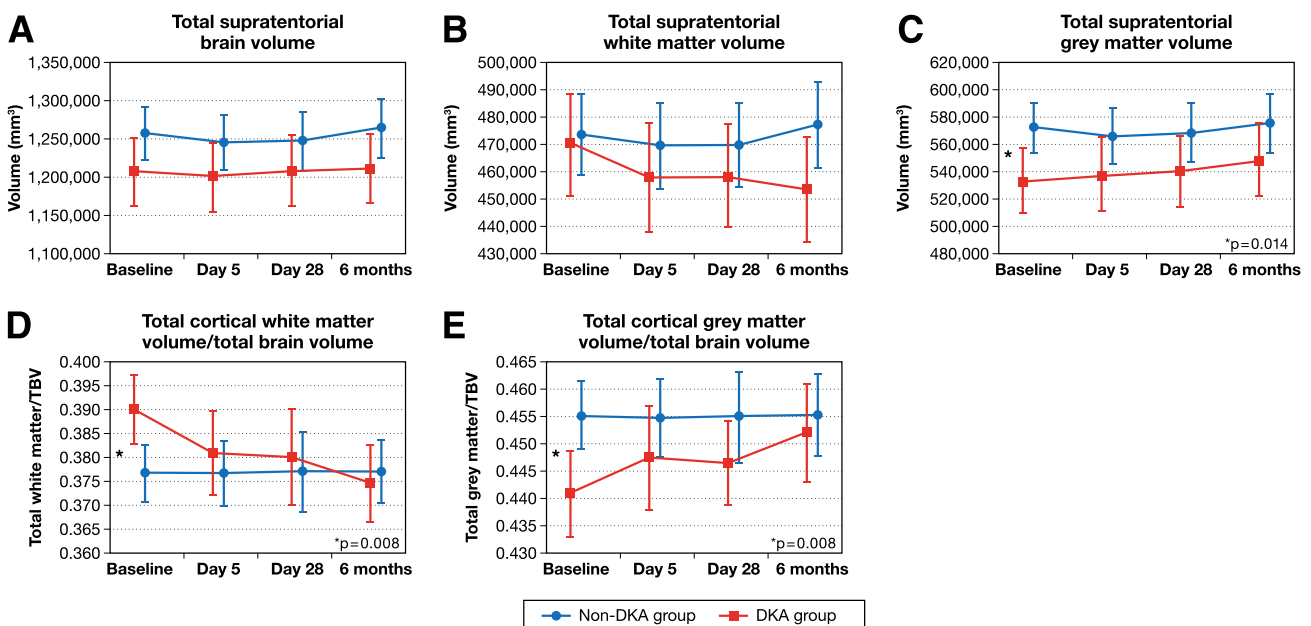
	Non-DKA group (n = 59)	DKA group (n = 36)	P value
Age (years)	11.52 (6.17–16.16)	11.52 (6.05–17.87)	0.99
Male	54.2	47.2	0.5
Duration of polyuria/polydypsia (days)	23.41 (2–168)	19.46 (1–56)	0.4
SES (percentile)	61.51 (5–99)	60.58 (4–100)	0.99
Requiring school assistance	15.8	16.7	>0.99
<b>At presentation</b>			
Lowest pH	7.37 (7.30–7.44)	7.12 (6.72–7.29)	<0.001
Highest blood glucose (mmol/L)	25.77 (13.00–45.10)	31.00 (16.00–81.00)	0.03
Highest corrected serum sodium (mmol/L)	139.69 (134–146)	147.99 (124–198)	<0.001
Lowest corrected serum sodium (mmol/L)	136.00 (133–141)	142.56 (128–169)	0.075
Highest urea (mmol/L)	5.15 (2–8)	6.21 (3–13)	0.006
Serum osmolality (mmol/L)	298.39 (285–318)	312.80 (264–394)	<0.001
Percent dehydration*	NA	7.84 (0–14)	NA
<b>At 6-month follow-up</b>			
Patients with one or more episodes of severe hypoglycemia	1.7	2.8	0.3
Patients with one or more episodes of subsequent DKA	0	0	—
Percentage of patients on			
Two insulin injections/day	78 (n = 46)	74 (n = 26)	0.7
Four insulin injections/day	22 (n = 13)	26 (n = 9)	
Total daily insulin dose per body weight (units/kg/day)	0.7 (0–1.4)	0.8 (0.3–1.7)	0.016
HbA <sub>1c</sub> (%)	7.52 (5–12)	7.40 (5–9)	0.617
HbA <sub>1c</sub> (mmol/mol)	59 (31–108)	57 (31–75)	
IDAA1C	10.2 (5.6–15.7)	10.6 (6.9–14.8)	0.205

Data are mean (range) or % unless otherwise indicated. NA, not applicable. \*Calculated as (weight at discharge – weight at presentation)/weight at discharge.

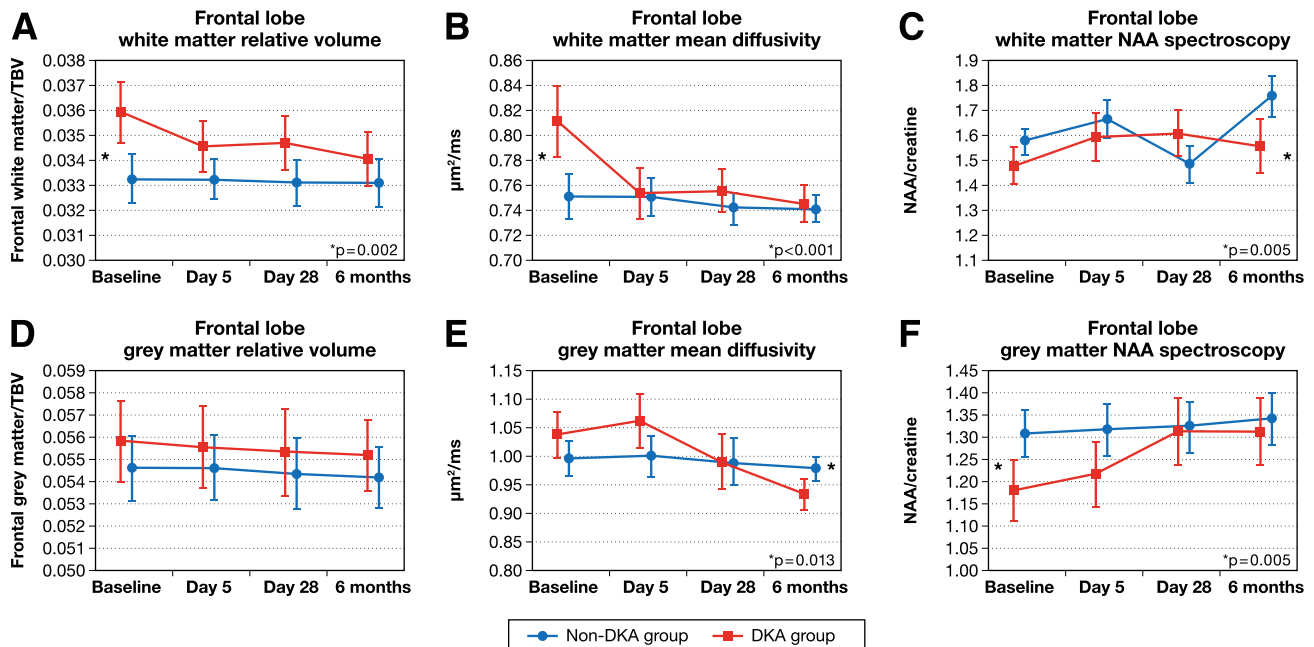
( $F = 16.56, P = 0.001$ ). A decrease in relative parietal white matter volume also occurred over 6 months in the DKA group only ( $F = 9.19, P = 0.008$ ). For diffusion

measures, within the DKA group, white matter MD at baseline was higher in the frontal lobe ( $F = 18.78, P < 0.001$ ) (Fig. 2B), temporal lobe ( $F = 18.12, P < 0.001$ ),

and parietal lobe ( $F = 13.42, P < 0.001$ ). Reductions in white matter MD occurred over 6 months with DKA in the frontal ( $F = 11.08, P = 0.006$ ) (Fig. 2B), temporal



**Figure 1—Group differences (DKA vs. non-DKA) for TBVs.** Data are presented as the estimated mean and 95% CI at each time point. A: Total supratentorial brain volumes. B: Total supratentorial white matter volume. C: Total supratentorial gray matter volume; \* $P = 0.014$ . D: Total cortical white matter volume/TBV; \* $P = 0.008$ . E: Total cortical gray matter volume/TBV; \* $P = 0.008$ .



**Figure 2**—Group differences (DKA vs. non-DKA) for frontal lobe volumes, diffusivity, and spectroscopy. Data are presented as the estimated mean and 95% CI at each time point. A: Frontal lobe white matter relative volume; \* $P = 0.002$ . B: Frontal lobe white matter diffusivity; \* $P < 0.001$ . C: Frontal lobe white matter NAA spectroscopy; \* $P = 0.005$ . D: Frontal lobe grey matter relative volume. E: Frontal lobe grey matter diffusivity; \* $P = 0.013$ . F: Frontal lobe grey matter NAA spectroscopy; \* $P = 0.005$ .

( $F = 42.20$ ,  $P < 0.001$ ), and parietal ( $F = 16.63$ ,  $P = 0.002$ ) lobes. Fractional anisotropy values were highly correlated with MD values in all areas/analyses, changing in the opposite direction of MD. Frontal white matter NAA levels were lower with DKA at 6 months ( $F = 8.51$ ,  $P = 0.005$ ) (Fig. 2C). There was a trend for an increase in frontal white matter NAA levels over 6 months in the non-DKA group ( $F = 6.186$ ,  $P = 0.02$ ) but no change in the DKA group.

#### Gray Matter

Relative total cortical gray matter volume was lower with DKA on day 1 ( $F = 7.64$ ,  $P = 0.008$ ) (Fig. 1E) and increased over 6 months ( $F = 10.18$ ,  $P = 0.005$ ). Regionally, the relative temporal cortical gray matter volume was lower with DKA on day 1 ( $F = 8.66$ ,  $P = 0.005$ ), with a trend to increase over 6 months ( $F = 6.22$ ,  $P = 0.02$ ). For diffusion, parietal lobe gray matter MD was higher with DKA at baseline ( $F = 10.2$ ,  $P = 0.002$ ). Gray matter MD in the frontal lobes decreased over 6 months with DKA ( $F = 50.23$ ,  $P < 0.001$ ) (Fig. 2E) and trended lower at 6 months compared with the non-DKA group ( $F = 5.80$ ,  $P = 0.02$ ). For spectroscopy, frontal gray matter NAA levels were lower at baseline in the DKA group ( $F = 8.73$ ,  $P = 0.005$ ) (Fig. 2F),

whereas myoinositol levels decreased over 6 months ( $F = 14.827$ ,  $P = 0.002$ ).

#### Hippocampus and Basal Ganglia

Relative hippocampal and basal ganglia volumes did not differ with DKA at any time point. There was no change in either volume over 6 months. In the left-side basal ganglia, NAA levels trended lower at baseline ( $F = 5.24$ ,  $P = 0.03$ ) and day 5 ( $F = 7.29$  and  $P = 0.01$ ) with DKA.

#### Cognition

The mental state score was lower in the DKA group at baseline ( $F = 10.14$ ,  $P = 0.002$ ) (Fig. 3A), with both groups showing similar improvement by day 5 (DKA:  $F = 28.77$ ,  $P < 0.001$ ; non-DKA:  $F = 21.42$ ,  $P < 0.001$ ). Memory scores were lower in the DKA group at baseline ( $F = 14.58$ ,  $P < 0.001$ ) but improved by 6 months ( $F = 8.22$ ,  $P = 0.009$ ) (Fig. 3B). No differences in memory and divided or sustained attention were noted with DKA at 6 months. Focused attention improved between day 5 and 6 months in the non-DKA group ( $F = 13.73$ ,  $P = 0.001$ ) (Fig. 3C) but not in the DKA group.

#### Clinical Parameters on Admission and MRI and Cognitive Outcomes

Lowest pH level and age were the strongest predictors of MRI/MRS and cognitive measures. Lower baseline pH level

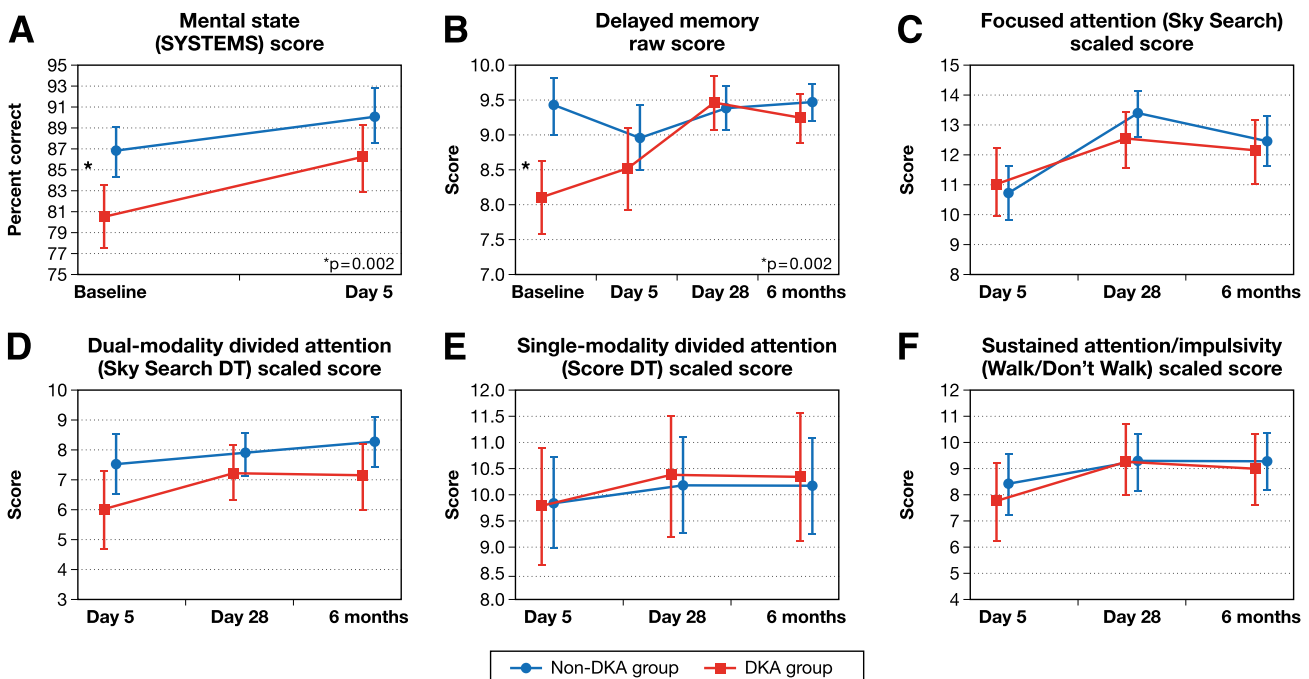
was positively related with change in gray matter volume by day 5 ( $sr^2 = 7.51\%$ ,  $P = 0.006$ ); negatively associated with baseline white matter diffusivity in the frontal ( $sr^2 = 41.3\%$ ,  $P < 0.001$ ), temporal ( $sr^2 = 33.03\%$ ,  $P < 0.001$ ), and parietal ( $sr^2 = 24.07\%$ ,  $P < 0.001$ ) regions; but positively associated with 6-month frontal gray matter diffusivity ( $sr^2 = 11.9\%$ ,  $P = 0.008$ ). There were positive associations between baseline pH level with baseline frontal gray matter NAA levels ( $sr^2 = 12.8\%$ ,  $P = 0.005$ ) and baseline memory ( $sr^2 = 17.47\%$ ,  $P < 0.001$ ) such that the lower the pH level, the lower the NAA level and poorer the memory score.

Age at presentation was positively related to diffusion measures at baseline in frontal and parietal gray matter MD ( $sr^2 = 22.84\%$ ,  $P < 0.001$ , and  $sr^2 = 21.87\%$ ,  $P < 0.001$ , respectively) and negatively associated with baseline frontal and temporal white matter MD ( $sr^2 = 10.36\%$ ,  $P < 0.001$ , and  $sr^2 = 11.46\%$ ,  $P = 0.001$ , respectively). Age at presentation was positively associated with 6-month frontal ( $sr^2 = 16.0\%$ ,  $P = 0.002$ ) and parietal ( $sr^2 = 17.1\%$ ,  $P = 0.003$ ) gray matter MD.

#### MRI Measures in the First Week and 6-Month Cognitive Outcomes

The reduction between baseline and day 5 in total white matter volume (an





**Figure 3**—Group differences (DKA vs. non-DKA) for cognitive outcomes. Data are presented as the estimated mean and 95% CI at each time point. A: Mental state (SYSTEMS) score; \* $P = 0.002$ . B: Delayed memory raw score; \* $P = 0.002$ . C: Focused attention (Sky Search) scaled score. D: Dual-modality divided attention (Sky Search DT) scaled score. E: Single-modality divided attention (Score DT) scaled score. F: Sustained attention/impulsivity (Walk/Don't Walk) scaled score. DT, dual task.

indirect measure of swelling) was negatively associated with performance on dual-modality divided ( $r = -0.46$ ,  $P = 0.002$ ) and sustained ( $r = -0.40$ ,  $P = 0.007$ ) attention at 6 months. Frontal white matter volume reduction was negatively associated with dual-modality divided attention ( $r = -0.43$ ,  $P = 0.004$ ) at 6 months, temporal white matter volume reduction was negatively associated with memory ( $r = -0.49$ ,  $P = 0.0009$ ) at 6 months, and parietal white matter volume reduction was negatively associated with sustained attention ( $r = -0.47$ ,  $P = 0.002$ ) at 6 months. Parietal gray matter volume increase was negatively associated with sustained attention ( $r = -0.45$ ,  $P = 0.002$ ).

## CONCLUSIONS

This study highlights the common nature of transient focal cerebral edema and associated impaired mental state at presentation with new-onset type 1 diabetes in children. We demonstrate that alterations occur most markedly in cerebral white matter, particularly in the frontal lobes, and are most prominent in the youngest children with the most dramatic acidemia. Given the somewhat arbitrary nature of a serum

pH  $< 7.30$  as the cut point to define DKA, we undertook regression analyses using pH as a continuous variable. We found that although changes in MRI measures over the first week after diagnosis resolved, these early brain changes were associated with persisting alterations in attention and memory 6 months later. Children with DKA did not differ in age, sex, SES, premorbid need for school assistance/remediation, or postdiagnosis clinical trajectory. Earlier diagnosis of type 1 diabetes in children may avoid the complication of DKA and the neurological consequences documented in this study and is worthy of a major public health initiative.

This study used several MRI techniques to define cerebral structure and biochemistry. All regional brain volume data were normalized to account for differences in absolute volume related to varying participant age. Thus, the measures from baseline showed a relative increase in white matter volume and decrease in gray matter volume. With DKA, white matter volume expansion was greatest within the frontal white matter, but a more widespread reduction in parietal and frontal white matter volume was seen over the 6 months following

diagnosis. Diffusion values were not normalized because they are comparatively stable throughout childhood (20). Diffusion values in the DKA group were high on day 1 throughout all white matter regions evaluated but returned to non-DKA levels by day 5. We postulate that increases in both volume and diffusivity within the white matter on initial presentation were due to increased tissue water, and this is supported by strong correlations between the reduction in volume and decrease in diffusivity. It is notable that diffusivity appeared to be more sensitive than volume because an increase in white matter volume was detected only for frontal white matter. This finding also suggests a greater vulnerability for the frontal white matter to DKA, which may represent a maturational vulnerability because the frontal white matter remains immature at this period of cerebral development.

We propose two explanations, which are not necessarily mutually exclusive, for the increased volume and altered diffusion that is acutely prominent in the white matter: 1) the osmotic effects caused by relatively rapid restoration of blood glucose (and osmolarity) in the

face of slower loss of idiogenic osmoles in axons and myelin, leading to cell swelling, and/or 2) the breakdown of the blood-brain barrier, leading to extravasation of fluid into the white matter interstitium with extracellular (vasogenic) edema. These disturbances in brain water balance can be viewed as being analogous to those associated with extrapontine myelinolysis, which is typically found in conjunction with fluid/electrolyte disturbances and has been associated with diabetes (21,22). The large U.K. case-control series of DKA-associated cerebral edema did not show an association with any changes in blood glucose after therapy (6). Furthermore, in a series of youth aged 6–17 years with new-onset and established diabetes and DKA, two magnetic resonance (MR) scans performed within 12–24 h and 36–72 h after insulin therapy showed that the majority of patients had a 2–5% increase in whole-brain diffusivity between the two time points (23). There was an associated decrease in mean transit time and no change in relative cerebral blood volume, suggesting a vasogenic process. The present findings are also consistent with other studies that suggested brain water shifts associated with DKA (9), including increased diffusion in the frontal white matter in the first hours of management (14). Further evidence of white matter pathology was demonstrated in two atypical comatose pediatric DKA patients without cerebral edema in whom subcortical white matter microhemorrhage and inflammation were noted (24).

Additional insight is provided from the MRS. Acutely in the DKA cohort, frontal gray matter demonstrated a reduction in NAA levels, which recovered. This may reflect neuronal dysfunction, emphasizing neuronal impact from DKA. Over the first 6 months after diagnosis, the greatest alterations on spectroscopy were reductions in frontal white matter NAA levels. Although a typical maturational increase in NAA occurs in children without ketoacidosis (25), this was not seen in the DKA group. Reductions in frontal white matter and basal ganglia NAA levels have been associated previously with impaired cognition (26) and DKA (27–29). Lactate was present in ~10% of both DKA and non-DKA participants in any voxel. This low incidence of elevated lactate level may

relate to the delay in imaging after initiation of therapy.

Two cross-sectional retrospective studies highlighted a negative impact of ketoacidosis on cognition in school-aged children with existing diabetes (30,31). For the current study, the strength of examining patients with new-onset diabetes has allowed the window of dysglycemia to be simplified to a single period of hyperglycemia with or without one episode of ketoacidosis. Of note, the present findings replicate a similar unidimensional model of cognitive performance in rats after one episode of DKA (32). Another cross-sectional MR study investigating the effects of severe hyperglycemia associated with ketosis with or without acidemia in youth aged 9–22 years with a mean duration of 9.5 years showed that a history of one or more episodes of severe hyperglycemia was associated with increased diffusivity in the superior parietal lobule and hippocampus (33).

In relation to clinical risk factors, the degree of acidosis and younger age appeared to be the greatest risk factors for alterations in cerebral structure. A greater degree of cognitive impairment in younger children with type 1 diabetes has been reported previously (2), but these studies have included children exposed to various aspects of diabetes dysglycemia, including hypoglycemia. Specific studies of DKA in children have been limited thus far to cerebral edema. In these studies, the risk of cerebral edema was associated only with elevated serum urea level and low  $P_{ACO_2}$  (34). In children and adults with DKA without cerebral edema, the degree of acidosis was the determinant of impaired conscious state (6,35).

Finally, cerebral volume changes in the frontal, temporal, and parietal regions in the first week after diagnosis were associated with lower attention and memory scores 6 months later, suggesting that functional information processing difficulties persist after resolution of tissue water increases in cerebral white matter. These findings have not been reported to date but are consistent with the growing concern over academic performance in children with diabetes (2).

This study has several limitations to note. First, the lack of premorbid data on duration of preadmission hyperglycemia/acidemia, MR brain volumes, diffusivity,

and cognition is unavoidable. Currently, no screening programs practically identify children and adolescents destined to develop type 1 diabetes in low-risk populations. If there were such screening programs, it would be unethical to allow DKA to develop. However, given the fact that the DKA and non-DKA groups did not differ on any demographic variables or previous need for school assistance/remediation and that variations in brain morphology aligned temporally and statistically to the episode of acidosis, we surmise that the likelihood of significant premorbid group differences in brain structure and function is low. The second limitation relates to the period of hyperglycemia and DKA before admission. No clinical tool can retrospectively define duration of hyperglycemia alone; however, a baseline  $HbA_{1c}$  may have indicated a measure of degree and duration of preadmission hyperglycemia. Given the severity of the clinical phenotype of DKA, any variation in duration would be a matter of hours rather than days and is to some extent reflected by the degree of acidosis, but exact duration remains unknown. A third limitation relates to the short follow-up period of 6 months. However, the longer the follow-up period, the greater the likelihood of postdiagnosis glycemic events contributing to brain injury. We had to balance the need for allowing for recovery with avoiding additional clinical confounders; thus, we chose 6 months as the follow-up period to provide the best picture of the neurological sequelae of the initial DKA insult. A fourth limitation relates to the fact that only three-quarters of the participants completed all data collection, which may introduce some potential bias to the later evaluations. Finally, although the purpose of this study was to compare newly diagnosed diabetic patients with and without DKA, a nondiabetic control group may have been helpful for comparative purposes because normative data from some of the MR measures (e.g., MD) are scant (33,36). To overcome a lack of MR normative data, analyses were adjusted for age and/or sex. The volumetric analyses were also expressed as a proportion of TBV to normalize for age-related variations in brain size. Diffusion parameters appear to be relatively constant over the age range studied (33), so we believed it reasonable to compare participants with one another. Nevertheless, we also looked at diffusion parameters

as a fractional change from baseline in a fashion analogous to that used for the volume data. This had no effect on the final results, so we have simply reported nonnormalized diffusion parameter values.

### Clinical Implications

This study demonstrates common and widespread alterations in brain structure and chemistry in new-onset type 1 diabetes with ketoacidosis in a pediatric and adolescent cohort from a socioeconomically and ethnically diverse population. There are several implications that arise from these findings. First is the imperative to avoid ketoacidosis in children through improved public and professional awareness. Reductions in the rates of ketoacidosis at diagnosis are possible, as has been demonstrated with public health campaigns in Italy and Australia (37,38). Second, any neuroprotective strategy developed in the future must be prioritized at initial diagnosis. Finally, we should focus greater attention on neuropsychological evaluation of children with diabetes by both performing a brief mental state examination in all newly diagnosed patients and providing cognitive follow-up. It would appear sensible for clinicians to defer educational activities in patients with a suboptimal mental state for at least 1–2 weeks. Brain injury should no longer be considered a rare complication of DKA. This study has shown that it is both frequent and persistent.

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and editing of the manuscript. F.J.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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