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# The relationship between D-beta-hydroxybutyrate blood concentrations and seizure control in children treated with the ketogenic diet for medically intractable epilepsy

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## Summary

**Objective:** **The ketogenic diet (KD) is a proven treatment for drug-resistant (DR) seizures in children and adolescents. However, the relationship between seizure control and the most commonly measured metabolite of the diet, the ketone body D-beta-hydroxybutyrate (D-BHB), is controversial. This study was performed to clarify the relationship because specific ketone bodies may be useful as biomarkers of diet efficacy.**

**Methods:** **Families of children with DR seizures were approached for participation in this open-label, prospective study when they were referred for the KD at two western Canadian children's hospitals. Inclusion criteria included documentation of DR seizures without exclusion based on age, sex, seizure, or syndrome type. Patients were excluded if they were referred for treatment of a metabolic disorder independent of seizures. Seizures were quantified via parental report and standardized as seizure frequency per 28 days. Epilepsy syndromes were identified on the basis of the medical record. Blood D-BHB was determined by tandem mass spectrometry.**

**Results:** **A total of 23 patients were recruited from both sites. Data from five individuals were excluded because these seizures occurred in clusters, leaving 18 patients for the primary analysis. In the latter group, a clear positive correlation was present between measures of seizure frequency and D-BHB concentrations. However, this failed to reach statistical significance, likely because of the relatively small numbers.**

**Significance:** **A trend clearly exists between seizure frequency and D-BHB levels, so we should not be dissuaded by the lack of statistical significance because it possibly results from methodological techniques, especially sample size. These results call for a larger prospective study in which seizure frequency is assessed at the point of care in a standardized fashion so as to determine whether D-BHB can be used as a reliable biomarker of KD efficacy.**

**KEY WORDS:** **Ketogenic diet, Epilepsy, Child, Ketone, Beta-hydroxybutyrate.**

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## Key Points

- The ketogenic diet is an effective treatment for childhood epilepsy
- D-beta-hydroxybutyrate (D-BHB) levels tend to correlate with ketogenic diet (KD) efficacy
- A larger study is required to establish whether D-BHB levels can be used as a reliable measure of KD efficacy

The ketogenic diet (KD) was invented in 1921 based on the observation that fasting led to remarkable seizure control in individuals with epilepsy.[1] During KD treatment, the ketone bodies (KBs) D-beta-hydroxybutyrate, acetoacetate (ACA), and acetone[2–4] are all increased. However, it remains uncertain what contribution, if any, these metabolic substrates play in KD action, either individually or collectively.[5–7] If KBs indeed provide direct antiseizure effects, validating their clinical role and identifying their target(s) may lead to reliable clinical biomarkers and possibly the development of agents more effective than existing drugs.

It is important to define the relationship of KBs to seizure control for at least two reasons. The first is that there is currently no reliable way to track the clinical effectiveness of the KD other than parental reporting of seizure activity, a practice that is fraught with inaccuracies. The second is that an association between KB levels and seizure control would have implications for the development of new antiseizure medications to address the approximately 30–40% of children with drug-resistant epilepsy. Thus, the specific aim of this pilot study was to determine the relationship of blood levels of the clinically relevant D-(-)-stereoisomer of beta-hydroxybutyrate (BHB) to seizure control in children with medically intractable epilepsy on the KD.

## Methods

### Patient recruitment

Patients with drug-resistant epilepsy who were going to start the KD and who were seen in the Pediatric Neurology clinics at Alberta Children's Hospital and the British Columbia Children's Hospital were invited to participate.

### Inclusion criteria

Ages 0–18 years inclusive,[8] both sexes, all ethnicities, all seizure types, and all epilepsy syndromes. Drug-resistant epilepsy was defined as lack of full seizure control following adequate trials of two appropriate antiseizure medications.[9] All levels of intellectual and developmental status were included. The rationale for the broad inclusion criteria was twofold. First, the literature indicates that the KD is suggested to be efficacious across the pediatric age spectrum, across seizure types and epilepsy syndromes, and across developmental status.[10] The second is that it seems likely that the KD is effective independent of the etiology of the seizures by acting on a final common pathway involving energy metabolism.[11]

### Exclusion criteria

Any nonseizure indication for starting the KD (e.g., Glut1 deficiency, brain tumor) or any metabolic contraindication (e.g., carnitine deficiencies, beta-oxidation defects, pyruvate carboxylase deficiency, porphyria).

### Study protocol

This was a prospective, nonblinded investigation of patients who were referred to the respective study teams and screened for eligibility on the basis of the criteria noted above. Patients were seen for a study initiation visit at which time consent and assent and baseline laboratories were obtained and families instructed to keep a seizure diary. Baseline seizure frequency was assessed as the average frequency of each seizure type during the month prior to study initiation. The KD was initiated (54% in the outpatient setting), and the diet advanced to a 4:1 ratio (fats to carbohydrate plus protein, by weight) if tolerated. The physician adjusted the ratio for each individual on the basis of seizure frequency and tolerability. Patients were asked to return at 3, 6, and 12 months, and at each of these time points, their seizure frequency was assessed and blood testing was performed to measure the D-BHB isomer, in addition to standard-of-care comprehensive metabolic parameters, including acylcarnitine profile, prealbumin/albumin, lipid panel, urine calcium, urine creatinine, and urine organic acids. A post-KD discontinuation blood draw was also requested for the BHB isomer. Samples were obtained after fasting for 8–12 h. Standardized urine testing was not part of the protocol because families performed this with a variety of methods at times not directly correlated with obtaining blood for BHB levels. Ethics approval was obtained from the appropriate committee at both participating institutions.

### Seizure classification and frequency

Seizures and epilepsy syndromes were classified using the International League Against Epilepsy recommendations[12] and the information abstracted from the medical record for each patient. Because seizure frequencies were expressed in a nonstandardized fashion by each provider and often as a range (e.g., 1–3 per day, week, month), we decided to "normalize" the seizure frequency reports by converting all reports to seizures per 28 days.

The main analysis included 18 patients at baseline. Out of 18, 3 dropped out after 3 months and 3 after 6 months. At the end of the 12-month study, a total of 6 patients stopped the KD. The remaining 12 continued on the KD. No further analysis was done on the 5 patients with cluster seizures as a

result of the inherent problems with assessing seizure frequency in this group.

**D-BHB isomer determination**

A liquid-chromatography, electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) method was established to measure D-BHB levels in plasma. Plasma from whole blood was separated using standard procedures and stored at −80°C until analysis. D-BHB was derivatized with (S)(+)-1-(2-pyrrolidinylmethyl)-pyrrolidine (SPMP) according to a previously published method,[13] using deuterium-labeled D-BHB-d4 as an internal standard (IS). Briefly, 10 µl of diluted plasma was mixed with IS and 200 µl of acetonitrile, then deproteinized by filtration. The filtrate was reacted with SPMP and activation reagents at room temperature for 90 min. The reaction solutions were then dried under nitrogen gas at 45°C, followed by resuspension in 100 µl of 0.1% formic acid and again filtered. The collected filtrates (2 µl) were injected for LC/ESI-MS/MS analyses.

D-BHB was chirally separated on a Waters XBridge C18 column (35-µm particle size; 2.1 × 150 mm) using isocratic elution on a Waters Alliance 2795 and Quattro Ultima LC/MS/MS System. D-BHB was well resolved from other known hydroxybutyric acid isomers, including 2-hydroxybutyric and isobutyric acids, by optimizing the collision energy for the mass charge ratio 241.2 > 126.1 multiple reaction monitoring transition in positive mode.

The data were securely managed at the servers of the Clinical Research Unit, University of Calgary, using the REDCap (Research Data Electronic Capture) program (Vanderbilt University, Nashville, TN, U.S.A.).

## Results

A total of 27 patients were initially enrolled (14 at the Alberta Children's Hospital and 13 at the British Columbia Children's Hospital) from June 6, 2012, to October 7, 2014, inclusive. Four patients were subsequently excluded because of the following reasons (one each): switching to a low-glycemic index therapy after 6 months, noncompliance with the KD, an indication other than seizures, no seizure frequency information provided by the family. Further, five more patients were excluded because of cluster seizures. Thus, the final study population included 18 patients.

Table 1 provides information regarding the clinical characteristics of the seizures and antiseizure medications. Patients were aged 2 months to 18 years, inclusive. Age at first seizure was 0–59 months (mean 17.8 months), and mean age at the time of diet initiation was 56.8 months (18–105 months). The small number of specific seizure types and syndromes precluded statistical analysis of either as individual entities.

There was a wide range for the reported seizure frequency per 28 days (0–672), D-BHB (5.1–7,307), and D/L (ratio)-BHB (7.20–250.14). It is apparent from Fig. 1 that there is a relation between the seizure frequency and median D-BHB levels. Spearman's rank correlation (ρ) established a moderate negative correlation between the seizure frequency per 28 days and the median D-BHB levels ($\rho = -0.429$, $p = 0.397$). However, at the 0.05 level of significance this correlation is not statistically significant.

## Discussion

The role of KBs as either mediators or indicators of seizure control is seriously questioned by the low-glycemic-index

**Table 1. Seizure characteristics, antiseizure drugs, and KD discontinuation rates**

| | |
|---|---|
| Seizure types at KD initiation | |
| Number of seizure types per patient at KD initiation | |
| 1 seizure type | 28% (5) |
| 2 seizure types | 50% (9) |
| 3 seizure types | 22% (4) |
| Mode of seizure onset | |
| Focal | 39% (7) |
| Generalized | 56% (10) |
| Focal/Generalized | 5% (1) |
| ASD therapy | |
| Number of previous therapies | 4 (SD = 2.81) |
| Number of ASDs at KD initiation | 1.92 (SD = 1.21) |
| Age at KD initiation | 4.74 (SD = 2.79) |
| KD discontinuation within 12 months of initiation | |
| Total patients discontinuing KD within 12 months of initiation | 33% (06) |
| Discontinuing at 3–6 months on diet | 50% (3) |
| Discontinuing at 6–12 months on diet | 50% (3) |
| Reasons for discontinuation | |
| Lack of efficacy and adverse events | 100% (6) |
| Seizure types | |
| Focal dyscognitive | 50% (9) |
| Bilateral convulsive | 33% (6) |
| Typical absence | 17% (3) |
| Atypical absence | 17% (3) |
| Myoclonic absence | 17% (3) |
| Atonic | 22% (4) |
| Tonic | 17% (3) |
| Tonic-clonic | 22% (4) |
| Myoclonic tonic | 11% (2) |
| Epileptic spasms | 33% (6) |
| Electroclinical syndromes | |
| Childhood absence | 11% (2) |
| Epilepsy with myoclonic absences | 17% (3) |
| Epilepsy with myoclonic atonic seizures | 11% (2) |
| Continuous spike wave during sleep | 06% (1) |
| Lennox-Gastaut syndrome | 11% (2) |
| Dravet syndrome | 06% (1) |
| West syndrome | 11% (2) |
| Malformation of cortical development | 06% (1) |
| Undefined | 50% (9) |

The percentages do not add up to 100% because patients may have had more than one seizure type.
ASD, antiseizure drug; KD, ketogenic diet.

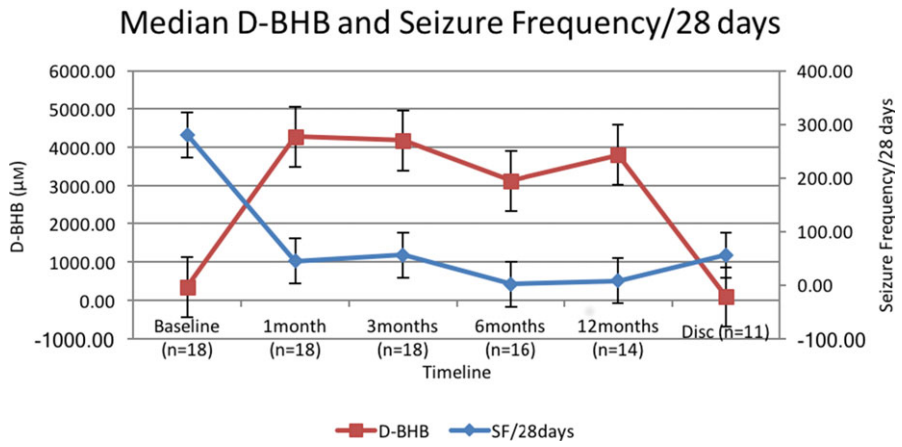


**Figure 1.**
Concentration of D-BHB versus seizure frequency/28 days.
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diet, which has no apparent relationship to levels of KBs, yet appears to have efficacy equivalent to that of the classic KD.[14–16] At present, the specific role of KBs in epilepsy remains unknown.[17] Studies that report the relationship between ketones and seizures are limited. A study of 44 patients on the classic KD revealed correlations between seizure control and blood BHB levels at 3 and 6 months, with regression coefficients of 0.47 and 0.42, respectively.[18] Another study of 33 patients (on different ketone-inducing diets) showed that the KD was effective in only 24% of patients and, of those, the correlation coefficient between BHB levels and seizure control was only 0.37.[14] There was no correlation found between seizure control and urine ketones, the most commonly used clinical marker. Although both studies suggest a meaningful correlation, the data do not provide strong evidence for linking seizure control with KBs.

Despite the clear trends in our data suggesting a direct relationship between reduction of seizure frequency and increase in the D stereoisomer of BHB, the aforementioned statistical analysis did not support a significant relationship. The most likely reason for this finding is the wide range of values of BHB levels. This is not entirely surprising given the variation in how individuals process substrates as well as the inability to control for identical diets in each child. The measured levels of substrates can be influenced by such factors as relationship to mealtimes, circadian influences, levels of exogenous stress, and glucose levels. Furthermore, our study had a relatively small sample size. Importantly, patients involved in this study who trended toward a positive therapeutic response to the KD were the most difficult of drug-resistant patients as indicated by being started on metabolic therapy.

As detailed in recent reviews,[19,20] the antiseizure effects of the KD were demonstrated in rodents[21] using the classic pentylenetetrazol model[22] and later the 6-Hz stimulation and kindling models of epilepsy. Interestingly, similar results were found using ketone esters alone.[23] Subsequent studies have shown that BHB and ACA may be important mediators of antiseizure activity. Specifically, BHB and ACA have been shown to affect neuronal excitability by altering synaptic transmission, excitatory neurotransmitter release,[24] augmentation of GABAergic neurons,[25] and indirect activation of $K_{ATP}$ channels.[26] In addition to KBs, several other metabolic changes have been implicated in KD action, including glycolytic restriction, elevations in fatty acids, and increases in bioenergetic reserves, among others.[27] Recently, experiments conducted in the *Kcna1*-null mouse model of epilepsy provided a plausible molecular mechanism for KD effects[28]—specifically, through a ketone-induced elevation in the threshold for brain mitochondrial permeability transition (mPT).[29] Thus, therapies (such as cyclosporine A, which is a known inhibitor of mPT) could be developed to exploit such a novel molecular target.

In summary, we found a trend in the relationship between seizure frequency and D-BHB levels; specifically, in our medically intractable population of pediatric patients with epilepsy who were treated with the KD, D-BHB levels were higher when seizure frequency was lower. Despite the lack of a statistically significant inverse correlation, the question of whether ketone levels are accurate bio- or surrogate markers of seizure control while on the KD remains to be definitively resolved. The lack of statistical significance was likely the result of methodology noted above, especially sample size. Independent of this finding, measure of D-BHB levels provides a direct measure of adherence to the diet. Our data indicate a need for a larger prospective study in which seizure frequency is quantified in a rigorous, standardized fashion to determine whether D-BHB is in fact an accurate biomarker of KD efficacy.

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## Disclosure

The authors of this manuscript have no conflicts of interests to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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