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Effect of a ketogenic diet on EEG: Analysis of sample entropy

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Summary Although ketogenic diet (KD) is an effective alternative therapy for controlling intractable seizures, the anticonvulsant mechanism still remains unclear. Sample entropy (SampEn) provides a generalized measure of regularity in time-series data. To investigate the potential anticonvulsive mechanism of a KD, we analyzed the SampEn of electroencephalography (EEG) data in patients with intractable pediatric epilepsy before and after treatment with a KD. Seventeen pediatric patients with epilepsy who were treated with KD were enrolled in present study. Patients were classified as good responder and poor responder according to therapeutic responsiveness on KD. Thirty segments of 30-s epochs were selected before and after KD from each patient which were subject to SampEn. The KD increased the SampEn in the whole patient population; the SampEn increased significantly in all electrodes in the

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good responders, but the change in SampEn varied according to the electrode in the poor responders. Before the KD, the good responders had significantly lower SampEn values than the poor responders, but after the KD, SampEn values were higher in the good responders than in the poor responders. KD may have an anticonvulsive effect by decreasing the regularity of the EEG dynamics.

Introduction

The ketogenic diet (KD), a high-fat, adequate-protein, low-carbohydrate diet designed to mimic many of the biochemical changes associated with prolonged starvation,[1] is an effective alternative therapy for controlling intractable seizures. Researchers from a wide array of fields have investigated how systemic metabolic changes induced by a high-fat, low-carbohydrate diet, which results in prominent ketosis, can dampen neuronal excitability and hypersynchrony, the presumed end product(s) of the diet's action. The vast majority of studies on the anticonvulsive mechanisms of a KD have been focused mainly at the biochemical and microscopic morphological level.[2,3] However, the mechanisms underlying the KD have not been clarified through such bottom—up approaches.

Epilepsy is a state of increased neuronal excitability and abnormal hypersynchrony among neuronal networks even in the interictal state, which can be reflected in electroencephalography (EEG) as forms of interictal epileptiform discharges and abnormal slow waves.[4] EEG is a mesoscopic indicator of the collective behavior of neuronal assemblies.[5] It provides a top—down model that is often designed with the assistance of "black boxes" to make it easier to complete. While it is insufficient and irrelevant in understanding the elementary mechanisms, EEG can help elucidate the underlying therapeutic mechanisms of the KD if the EEG is analyzed carefully using mathematical methods, even without detailed information regarding the biochemical mechanisms.

To investigate the potential anticonvulsive mechanism of a KD, we calculated the sample entropy (SampEn) of EEG data in patients with intractable pediatric epilepsy and compared the results before and after treatment with a KD. We then correlated the results of the SampEn with therapeutic responsiveness according to the results of long-term follow-up in regard to the good responders versus poor responders, as differences in therapeutic responsiveness may reflect underlying, distinct dynamic changes during treatment. SampEn quantifies the regularity of subsequent amplitude values of EEG based on knowledge of previous amplitude values[6]; it is a revised version of the approximate entropy,[7] which has been widely used for quantifying signal regularity or the system complexity generating the signals, such as heart rate variability and EEG.

Methods

Subjects

The subjects of the present study were selected from a series of 199 patients who were treated for epilepsy at three university epilepsy centers. The detailed clinical characteristics of the patients and efficacy of the KD were reported elsewhere.[8] All the patients received a classic KD with a lipid-to-nonlipid ratio of 4:1 following either the Hopkins protocol or a revised protocol that did not include the initial fasting and fluid restriction. We tried to maintain "strong ketosis" as defined by $>3+$ (50—150 mg/dl urinary ketones) in routine urinalysis and a high level (3.6—5.6 mM) of serum β-hydroxybutyrate. Antiepileptic drugs were maintained at the same dosage as before the diet at least at the time of the EEG recording.

We enrolled patients who fulfilled the following criteria: they were treated from February 1999 to March 2003, had an age when starting the KD of 12—36 months, had EEGs that were recorded just before the KD and at 3—6 months after starting the KD, and were followed for at least 12 months. Patients who had an underlying inherited metabolic disease or neurodegenerative disease were excluded from the study. Patients were classified into two groups, good responders and poor responders, according to our criteria. The good responders were defined as patients who were seizure-free or had a reduction in their seizure frequency of $>50\%$, and the poor responders were patients who experienced a reduction in their seizure frequency of $<50\%$.[1,9] The epileptic classifications were delimited by partial and generalized seizures because of the small number of patients in the present study.

EEG recordings and sample entropy

EEGs were acquired using a digital EEG system after patients fell asleep in a quiet room without the

administration of chloral hydrate to avoid any effect of sedatives. EEG recordings were made from 16 electrodes of a standard 10–20-electrode system referenced to the Pz electrode. Electrical impedance was maintained at 5 kΩ. The filter settings were 0.5–70 Hz, with a sampling rate of 256 Hz and 16-bit A-to-D precision. All recorded data were carefully reviewed for technical and biological artifacts. As most EEG records showed frequent paroxysmal generalized or multifocal high amplitude spikes or sharp waves intermixed with high amplitude delta slows, we were not able to select a quiet background EEG activity without epileptiform discharges. Therefore, three 30-s-long EEG segments, including background and abnormal activities for each patient, were selected during light sleep for analysis by a neurologist (JKY) who was blind to the clinical information. After transformation to an average reference, the EEG data were digitally filtered with a band pass between 0.5 Hz and 32 Hz.

The SampEn is the negative logarithm of the conditional probability that two template sequences with similar m points will remain similar at the next point. It is a corrected version of the approximate entropy, which has been widely used for quantifying signal regularity or the system complexity generating the signal. To compute the SampEn(m,r,N), three input parameters should be fixed (m, the length of the compared runs; r, the effective filter; and N, the length of the data points). In the present study, $m = 2$, $r = 0.2$, and $N = 7680$ (i.e., 30-s length).

Let each EEG data set be represented as $x(i)$. SampEn(m,r,N) = the average over i of $\ln[p \quad |x(j+m) - x(i+m)| \leq r$, given that $|x(j+k) - x(i+k)| \leq$ for $k = 0, 1, \ldots, m-1$, $i \neq j]$, where p is the conditional probability.

From $x(i)$, the vector sequences $u(1)$ through $u(N-m+1)$ are formed, defined by $u_m(i) = [x(i), \ldots, x(i+m-1)]$. Then, $u_{m+1}(i) = [x(i), \ldots, x(i+m)]$.

These vectors represent m and $m+1$ consecutive x values, commencing with the ith point, respectively. Define the distance $d[u_m(i), u_m(j)]$ between vector $u_m(i)$ and $u_m(j)$ as the maximum difference in their respective scalar components. Use the sequence $u_m(1)$, $u_m(2)$, …, $u_m(N-m+1)$ to construct for each $i \leq N-m+1$

$$B_i^m(r) = \frac{\text{numbers of } j \leq N-m+1 \text{ such that} d[u_m(i), u_m(j)] \leq r,\ j \neq i}{N-m-1} \qquad (1)$$

In Eq. (1), $j \neq i$ indicates the elimination of the self-matching strategy.

Define $B^m(r) = (N-m)^{-1} \sum_{i=1}^{N-m} B_i^m(r)$.

Similarly, $A_i^m(r) = (\text{the numbers of } j \leq N-m$ such that$d[u_{m+1}(i), u_{m+1}(j)] \leq r,\ j \neq i)/(N-m-1)$. In addition, $j \neq i$ indicates the elimination of the self-matching strategy.

Define $A^m(r) = (N-m)^{-1} \sum_{i=1}^{N-m} A_i^m(r)$. Then, SampEn($m$,$r$) = $-\ln[A^m(r)/B^m(r)]$.

Statistical analysis

Statistical procedures were completed using the SPSS package (version 10.0; SPSS Inc., Chicago, IL). For the statistical significance of clinical variables between two groups, the chi-square test and Mann–Whitney rank-sum test were used. SampEn was analyzed with analysis of variance (ANOVA). These consisted of a "treatment" factor (before versus after the KD), "group" factor (good responders versus poor responders), and "location" factor (the 16 electrodes). All analyses were performed using an alpha level of 0.01 as the criterion for statistical significance.

Results

Electroclinical characteristics

Nineteen of the 199 patients with the KD fulfilled our selection criteria. Of these, two patients were excluded due to an inadequate quality of the EEG, which prevented further analysis. Therefore, 17 patients (13 males and 4 females) were included in the present study. Ten patients were classified as good responders, and seven patients were classified as poor responders. Fourteen patients had West syndrome and two had Lennox–Gastaut syndrome evolved from West syndrome. One patient was diagnosed with early infantile epileptic encephalopathy.

The age of seizure onset and age when beginning the KD were not significantly different between the good responder and poor responder groups (Table 1). However, the duration of the KD was significantly longer in the good responder group compared to the poor responder group (18.1 months versus 5.0 months, respectively, $p = 0.001$). Generalized seizures were much more common than partial seizures in both groups. No difference was observed in the number of antiepileptic drugs used between the two groups.

Sample entropy

In one patient (patient 14) from the poor responder group, only one EEG segment was available due to the presence of frequent movement artifacts. As a result, 30 EEG segments in the good responder group

Table 1 Clinical characteristics of the patients

	Good responders	Poor responders	*p*-Value
Number of patients	10	7	
Gender (male:female)	7:3	6:1	
Age of seizure onset (months)	4.9 ± 0.3	3.7 ± 0.2	NS[a]
Age at beginning the KD (months)	19.1 ± 0.5	22.1 ± 0.6	NS[a]
Duration on KD (months)	18.1 ± 0.6	5.0 ± 0.4	0.001[a]
Epilepsy syndrome			NS[b]
Generalized seizure	8	6	
Partial seizure	2	1	
Number of AEDs	2.5 ± 0.05	3.4 ± 0.2	NS[a]

AED, antiepileptic drug.
[a] Mann–Whitney rank-sum test.
[b] χ^2 test.

and 19 segments in the poor responder group were analyzed.

The KD significantly increased the mean SampEn, irrespective of the patient group (treatment effect, d.f. = 1, $F = 147.4$, $p = 0.000$; Table 2). The location also showed a significant effect on the SampEn (d.f. = 15, $F = 6.8$, $p = 0.000$). However, the effect of the group by location did not show a significant interaction. In the good responder group, SampEn values significantly increased in all electrodes after the KD (d.f. = 1, $F = 268.4$, $p = 0.000$; Fig. 1A). In contrast, in the poor responder group, the change in SampEn after the KD varied according to the electrode (d.f. = 1, $F = 5.7$, $p = 0.017$; Fig. 1B).

Before the KD, the mean SampEn revealed a significant between-group effect (d.f. = 1, $F = 35.2$, $p = 0.000$; Table 2). The good responder group had significantly lower SampEn values than the poor responder group (Fig. 1). The location effect and group × location interaction were also significant. After the KD, the mean SampEn was higher in the good responder group than in the poor responder group (group effect, d.f. = 1, $F = 33.1$, $p = 0.000$; Fig. 1). The location effect was also significant. The interaction between the group effect and location effect did not reach the level of significance (d.f. = 15, $F = 1.4$, $p = 0.113$).

Discussion

The objective of the present study was to investigate the effect of a KD on EEG dynamics in patients with drug-resistant pediatric epilepsy. We found that a KD increased SampEn in intractable pediatric epilepsy patients, and this was independent of therapeutic responsiveness. However, the increase in SampEn was more evident and extensive in good responders than in poor responders.

EEGs represent time-series data that are spatiotemporal summations of the electrochemical activities of several million neurons.[10] Interactions between neurons and synapses have nonlinear characteristics. Therefore, quantifying nonlinear dynamic structure of the EEG signal could provide insight into the characteristics and behavior of the underlying neurophysiological processes that generate the signals.[11] The SamEn(m,r,N) is precisely the negative natural logarithm of the conditional probability that two sequences within a tolerance r for m points remain within r of each other at the next point. SampEn provides a generalized measure of regularity in EEG time-series data. A deterministic signal with high regularity has a higher probability of remaining close for longer vectors of the series and hence has a very small SampEn value.

Table 2 Sample entropy before and after the ketogenic diet in both patient groups

	Before the KD			After the KD			[a]*p*-Value
	Mean ± SEM	95% CI		Mean ± SEM	95% CI		
		Lower	Upper		Lower	Upper	
Total patients	0.324 ± 0.268	0.318	0.329	0.386 ± 0.004	0.377	0.395	0.000
Good responders	0.312 ± 0.003	0.306	0.318	0.410 ± 0.006	0.398	0.421	0.000
Poor responders	0.343 ± 0.004	0.335	0.351	0.361 ± 0.006	0.349	0.373	0.017
[b]*p*-value	0.000			0.000			

SEM, standard error of the mean; KD, ketogenic diet; CI, confidence interval.
[a] ANOVA comparing sample entropy before the ketogenic diet to that after the ketogenic diet.
[b] ANOVA comparing good responders to poor responders.

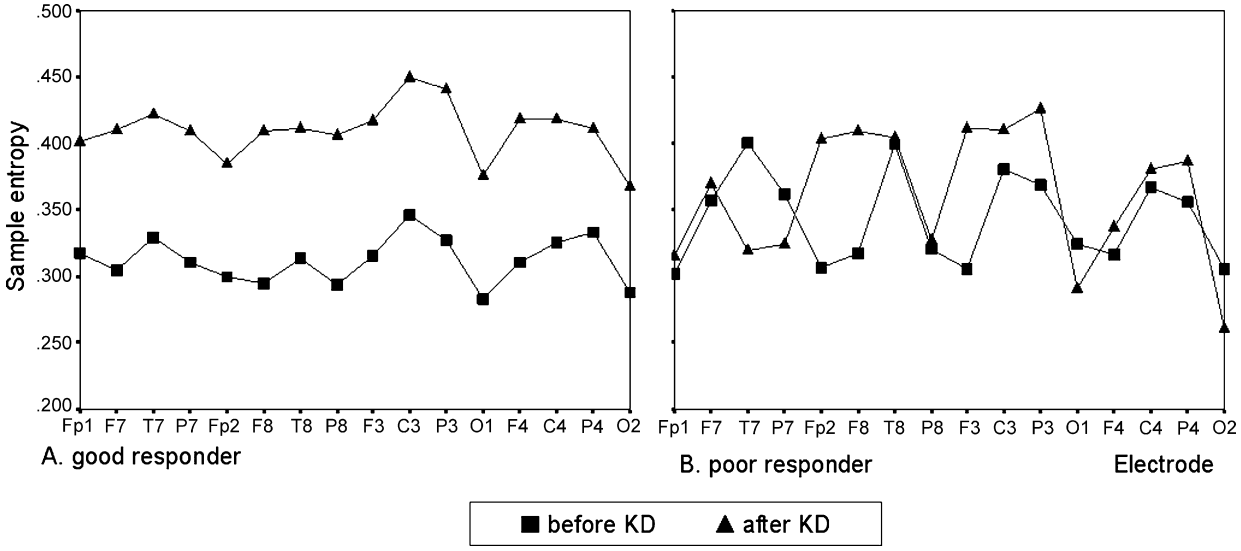

Figure 1 Change in sample entropy before and after the ketogenic diet in good responders (A) and poor responders (B). KD, ketogenic diet.

In contrast, a random signal has a very low regularity and produces a high SampEn value.

An increased SampEn value as a result of undergoing the KD in the whole patient group indicates that the regularity of the underlying neurodynamics was decreased by this unique treatment. Epileptic activity requires that large aggregates of neuronal activity synchronize and recruit surrounding neurons progressively,[12] which results in increased regularity. Most previous studies using nonlinear analysis methods[13] and various entropy measures[14,15] confirmed decreased complexity during ictal state in patients with epilepsy. The loss of complexity[16,17] or irregularity[14,15] has been demonstrated even in interictal and preictal states when compared to normal subjects or normal hemispheres.

Decreased regularity may be related to reduction of increased neuronal synchrony and/or synaptic interaction in the epileptic state. Previous studies demonstrated that anticonvulsive mechanisms of antiepileptic drugs were associated with increased complexity of brain dynamics. Lehnertz and Elger found a significant inverse relationship between the loss of complexity and serum level of carbamazepine in primary epileptogenic areas.[18] They assumed that the anticonvulsive mechanism of carbamazepine was attributable to an inhibition of sustained high-frequency firing of bursting neurons. Kim et al. found increased brain complexity after treatment with valproate in patients with partial epilepsy.[17] Our results are in agreement with these previous studies.

Although the KD increased SampEn in the whole patient population, the degree of change was somewhat different when considering the therapeutic responsiveness to the diet. It is remarkable that all clinical variables except duration on KD were not significantly different between two groups. Before the KD, however, the patients with good therapeutic responses (good responder group) showed significantly lower SampEn values than patients with poor responses (poor responder group). This difference in SampEn between the two groups was reversed after treatment with the KD. In other words, the good responders revealed a marked increase in SampEn in all locations of the brain, while in the poor responders SampEn increased slightly in only some locations of the brain. Jung et al. showed that a lower level of correlation dimension in the late period of status epilepticus was associated with a good response to diazepam in pilocarpine-induced status epilepticus rats.[19] These findings suggest that susceptibility to the changes in regularity that result from the KD increases as the brain dynamics become more regular. Furthermore, it appears that patients may show a better response to therapy as the increase in SampEn becomes larger.

It could be possible that EEG can be changed either by natural course of epileptic syndrome as patient grows or by the effect of ketogenic diet. The mean age of our patients at beginning the KD was 19—22 months old. The development of brain in this age is not as fast as in infant. In addition, the interval between two EEG data was less than 6 months. Therefore, it can be thought that EEG change is attributed to the KD rather than to the epileptic syndrome itself.

Electrophysiological studies from rats treated with the KD showed reduced CA1 excitability in vitro and decreased mossy fiber sprouting which suggests that anticonvulsive mechanism of KD may involve long-term changes of excitability at the level of synaptic networks.[20,21] Taken together, it can be hypothesized that KD may have anticonvulsive mechanisms by which disturb synchronizing activities and disconnect synaptic networks ensuing decrease regularity of EEG dynamics.

We used sample entropy instead of approximate entropy to calculate general regularity of EEG dynamics in present study. In contrast to approximate entropy, which calculates probabilities in a template-wise fashion,[22] SampEn(m,r,N) calculates the negative logarithm of a probability associated with the time-series as a whole and self-matches are not included in calculating the probability. Thus, it is largely independent of data length and displays relative consistency under circumstances when approximate entropy does not.[6] In addition, the statistic of sample entropy is a robust quantifier of complexity suited for short physiological signals such as the EEG which results in detecting subtle changes in the brain state.[23]

There are some limitations of present study. EEG data were collected retrospectively and the number of patients was small. It is demanded a large cohort of patients who will be treated with the KD to substantiate our results and to identify long-term effect of KD.

Conclusions

The present study revealed that a KD may have an anticonvulsive effect by decreasing the regularity of the EEG in patients with drug-resistant pediatric epilepsy. Nonlinear analysis of EEG may help us understand the pathophysiological mechanisms of therapeutic modalities.

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