Effects of ketogenic diet on epileptiform activity in children with therapy resistant epilepsy

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Summary
Purpose: The purpose was to quantify changes of epileptiform activity during ketogenic diet (KD) treatment in children with therapy resistant epilepsy, and evaluate how these changes are related to activity stage and to clinical effects on seizure frequency, seizure severity, attentional behaviour, quality of life (QOL), and beta-hydroxybutyrate (βOHb).
Methods: Eighteen children were investigated with 24 h ambulatory EEG monitoring 1 week prior to KD initiation and, after 3 months of KD treatment. Epileptiform activity was evaluated by automated spike detection. This data was compared with data presented in a previous study published in Epilepsia 2006, on sleep structure and different activity stages, clinical data on seizure frequency, seizure severity, QOL and attentional behaviour on the same children [Hallbök, T., Lundgren, J., Rosén, I., 2007. Ketogenic diet improves sleep quality in children with therapy resistant epilepsy. Epilepsia 48, 59–65].
Results: After 3 months of KD treatment the number of interictal epileptiform discharges (IEDs) was significantly reduced (p < 0.001). When considering the four activity stages separately, the reduction was significant during non-rapid eye movement sleep stage 2, slow wave sleep (SWS) and rapid eye movement (REM) sleep (p = 0.001, 0.001, 0.002), and not significantly so during awake (p = 0.07). Beta-hydroxybutyrate was significantly increased (p < 0.001). There was a significant correlation between the reduction in IEDs and clinical seizures (Spearman r = 0.6, p = 0.005) and between improvement in attentional behaviour and the increase in βOHb (Spearman r = 0.5, p = 0.03). There was no significant correlation between changes in attentional behaviour and IEDs or clinical seizures.
Conclusion: This study shows that KD reduces the number of IEDs, especially during sleep. It shows a correlation between reduction in epileptiform activity and clinical seizures. There were no correlations between reduction in epileptiform activity and clinical seizures and improvement in QOL or attention. The increase in βOHb correlated with improvement in attention.

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Introduction
Ketogenic diet (KD) is a high-fat, low-carbohydrate and low-protein diet. It has been used for childhood therapy resistant
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epilepsy since the 1920s. KD was developed to mimic the ketogenic state of starvation (Geyelin, 1921; Wilder, 1921). A standard approach to KD treatment includes a 2-year diet period with a 6–12 months wean (Freeman et al., 1994). Fasting and fall in blood glucose reduces plasma insulin production and stimulates lipolysis and production of fatty acids. Fasting is also up-regulating the MCT-1, monocarboxylic transport system of ketone bodies to the brain. Ketone bodies can pass directly into the neuronal mitochondria. Once in the mitochondria, beta-hydroxybutyrate (β-OHB) is converted to acetoacetate and acetoacetate-CoA for ATP production in the tricarboxylic acid cycle. Although no class I and II studies have been published concerning efficacy and safety, several studies present more than 50% reduction in seizure frequency in at least 50% of children with therapy resistant epilepsy (Anonymous, 1989; Freeman et al., 1998; Henderson, 2006; Keene, 2006; Lefevre and Aronson, 2000). The reported improvement in attention seems to be unrelated to the level of attained seizure control (Murphy and Burnham, 2006; Pulsifer et al., 2001). A number of previous studies have used animal models to examine the anticonvulsant effects of the KD (Bough et al., 2003, 2006; Hori et al., 1997; Likhodii and Burnham, 2003; Likhodii et al., 2003; Nylen et al., 2006; Thavendiranathan et al., 2003). As far as we know, no previous studies evaluating effects of KD on interictal epileptiform activity have been conducted.

The purpose of this study was to quantify interictal epileptiform activity following KD in children with therapy resistant epilepsy and to correlate possible alterations with different activity stages and changes in clinical effects on seizure frequency, seizure severity, QOL, and attention. Clinical effects on seizure frequency, seizure severity, QOL, and attention was also correlated with changes in β-OHB.

Methods

Subjects

Eighteen children (nine boys and nine girls) aged 2–15 years (median 7.5 years) with the diagnosis of therapy resistant epilepsy with developmental impairment, and absence of non-epileptic seizures or specific sleep disorders were started on KD.

Ketogenic diet

All children were admitted to the hospital and started gradually on the diet following a 12-h out-patient fast. The children were started on the classical KD. Fifteen received a 4:1 and, three a 3.5:1 ratio implying 4g or 3.5g of fat to 1g of combined protein and carbohydrates. Sixteen children were kept stable and two changed from ratio 4:1 to 3:5:1 during the 3 months because of nausea and problems with tolerance. The children also received the recommended daily intake of vitamins and minerals and were supplemented with calcium, magnesium, phosphorous, potassium and carnitine. The children were closely monitored to exclude intake of extra carbohydrates. In two children the diet was introduced via a gastrostomy tube, using Ketocal and a soy milk-based standard ketogenic formula.

EEG monitoring and spike detection

Eighteen children were investigated with 24h EEG monitoring 1 week prior to KD initiation and, after 3 months of KD treatment. The recordings were ambulatory with the children in their natural surroundings. Meals, naps, other activities, time of sleep and seizure events were registered in a diary. Via the Embla A10 FlagaMedcare digital data recorder, using sampling rate 200Hz with 16 bits resolution, data were recorded on a PC memory flash card. EEG was recorded with a standard ambulatory montage, with 11 scalp electrodes and a referential electrode (F3, F4, C3, C4, T3, T4, P3, P4, O1, O2, Cz, Ref.) according to the 10–20 International System. The digitalized data were converted to Nervus Tauрагрейнинг EEG format (Value format). The epileptiform activity was counted in comparable assessment periods according to time of day and activity stage. The assessment periods were the same for all children except in three children where the periods were selected, never more then 30 min, earlier or later than stipulated because of movement artefacts. Seizure events or disturbances in the recordings due to movement artefacts or technical problems were not selected. In one child (#11) selected channels with continuous artefacts were excluded. Because of fragmented and scattered daytime sleep the periods of sleep during daytime were not selected. The spike counting was based on automatic spike detection, PersystEEG Suite Spike Detector system version 01-26-2006. In an earlier article, a single blind pilot study was performed comparing visual spike detection and the PersystEEG Suite Automatic Spike Detector system (Hallbook et al., 2005). The automatic spike detection was performed in its most sensitive setting. All events were visually evaluated and edited for false detections. The investigation was blinded for the patient’s clinical data and order of investigation, baseline or treatment (Hallbook et al., 2005; Wilson et al., 1999).

Monitoring

Data on sleep structure and different activity stages from polysomnographic recordings, clinical data on QOL, seizure frequency, seizure severity scored with the National Hospital Seizure Severity Scale (NHSS), and attentional behaviour assessed with Ponsford and Kinsella’s visual analogue rating scale on these children, are analyzed in a previous study published in Epilepsia 2006 (Hallbook et al., 2007). Fasting (morning) β-OHB (μmol/l) was measured in whole blood 1 week prior to KD initiation and after 3 months of KD. Capillary β-OHB (μmol/l) via a semi-quantitative analysis for instant results was also performed daily during initiation of KD. These results are not presented. Follow-up assessments were performed after 3 months of KD.

Statistical evaluation

Wilcoxon signed rank test was used for comparison of IEDs. Wilcoxon signed rank test was also used for comparison of the clinical data on seizure frequency, seizure severity, QOL, attention and β-OHB before KD initiation and 3 months later. Spearman rank correlation coefficient (r) was used to calculate the correlation between IEDs and clinical effects. The level of significance was set at p < 0.05.

The present study was conducted at the Paediatric Department, University Hospital, Lund, during 1999–2003. The study was accepted by the Ethics Committee of the Faculty of Medicine, Lund University. Written informed consent from the parents and, when possible, from the patients was obtained.

Results

The type of epilepsy and the types of seizures were classified according to the International League Against Epilepsy classification 1981, 1989 (Anonymous, 1989, 1981). Three children had GTCS, five tonic–clonic with two generalized seizures, five had tonic generalized, three atonic drop, four tonic drop, three atypical absences and three myoclonic
Table 1  Clinical features and demographic information

<table>
<thead>
<tr>
<th>Pat. no.</th>
<th>Age at KD-onset (years)</th>
<th>Age at epilepsy-onset (years)</th>
<th>Etiology</th>
<th>Mret</th>
<th>Epilepsy type/syndrome</th>
<th>AED</th>
<th>Seizure type</th>
<th>Changes in seizure frequency at 3 months (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>3</td>
<td>Unknown</td>
<td>M</td>
<td>Generalized</td>
<td>SUX CLON</td>
<td>AD GTCS</td>
<td>Decreased 99%</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>5</td>
<td>Unknown</td>
<td>M</td>
<td>MAE</td>
<td>VPA LTG CLON</td>
<td>Tonic/Gen</td>
<td>Decreased 90%</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.13</td>
<td>Unknown</td>
<td>S</td>
<td>IS, Lennox-G</td>
<td>LTG Sulth CLOB Nitra</td>
<td>AA GTCS</td>
<td>Decreased 72%</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>0.25</td>
<td>Asphyxia</td>
<td>S</td>
<td>IS, Lennox-G</td>
<td>LTG VPA CLON</td>
<td>TC/2 GTCS</td>
<td>Unchanged 60%</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>2.5</td>
<td>Encephalitis</td>
<td>S</td>
<td>Generalized, Lennox-G</td>
<td>OXC LTG</td>
<td>AA AD TD</td>
<td>Decreased 90%</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0.75</td>
<td>Unknown</td>
<td>Mild</td>
<td>Partial</td>
<td>VPA CBZ</td>
<td>Tonic/Gen</td>
<td>Decreased 96%</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>5</td>
<td>Unknown</td>
<td>S</td>
<td>Partial</td>
<td>AZT</td>
<td>TC/2 GTCS</td>
<td>Increased 50%</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>Neonatal</td>
<td>Asphyxia</td>
<td>S</td>
<td>Generalized</td>
<td>LTG Nitra</td>
<td>TD MC</td>
<td>Decreased 100%</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>2</td>
<td>Encephalitis</td>
<td>S</td>
<td>Generalized</td>
<td>VPA OXC</td>
<td>TC/2 GTCS</td>
<td>Decreased 22%</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>Neonatal</td>
<td>Asphyxia</td>
<td>S</td>
<td>IS, Partial</td>
<td>LTG GBP CLOB</td>
<td>Tonic/Gen MC</td>
<td>Decreased 72%</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>Neonatal</td>
<td>Asphyxia</td>
<td>S</td>
<td>IS, Partial</td>
<td>VGB VPA CLON</td>
<td>AA TD MC</td>
<td>Decreased 40%</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>0.67</td>
<td>Unknown</td>
<td>M</td>
<td>Partial</td>
<td>LTG Sulth CLOB</td>
<td>Tonic/Gen</td>
<td>Decreased 100%</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>Neonatal</td>
<td>Asphyxia</td>
<td>S</td>
<td>IS, Lennox-G</td>
<td>VPA Nitra</td>
<td>Tonic/Gen</td>
<td>Decreased 30%</td>
</tr>
<tr>
<td>14</td>
<td>9</td>
<td>Neonatal</td>
<td>Unknown</td>
<td>S</td>
<td>Partial</td>
<td>LTG TPA</td>
<td>TC/2 GTCS</td>
<td>Decreased 80%</td>
</tr>
<tr>
<td>15</td>
<td>14</td>
<td>6</td>
<td>Sp tumor, HC</td>
<td>M</td>
<td>Partial</td>
<td>OXC CLON</td>
<td>TC/2 GTCS</td>
<td>Unchanged 0%</td>
</tr>
<tr>
<td>16</td>
<td>13</td>
<td>2.5</td>
<td>CMV</td>
<td>S</td>
<td>Partial</td>
<td>TPA</td>
<td>GTCS</td>
<td>Decreased 67%</td>
</tr>
<tr>
<td>17</td>
<td>6</td>
<td>1</td>
<td>Asphyxia</td>
<td>S</td>
<td>Lennox-G</td>
<td>LTG Sulth CLOB</td>
<td>TD MC</td>
<td>Decreased 100%</td>
</tr>
<tr>
<td>18</td>
<td>15</td>
<td>0.5</td>
<td>Glut-1 deficiency</td>
<td>M</td>
<td>Generalized</td>
<td>LTG</td>
<td>AD</td>
<td>Decreased 100%</td>
</tr>
</tbody>
</table>

Abbreviations: AA, atypical absence; AD, atonic drop; AED, antiepileptic drugs; AZT, acetazolamide; CBZ, carbamazepine; CLOB, clobazepam; CLON, clonazepam; CMV, cytomegalovirus; G, gastaut; GBP, gabapentin; GTCS, generalized tonic–clonic seizures; HC, hydrocephalus; IS, infantile spasms; LTG, lamotrigine; M, moderate; MAE, myoclonic astatic epilepsy; MC, myoclonic; Mret, mental retardation; Nitra, nitrazepam; OXC, oxcarbazepine; S, severe; Sp, spinal; Sulth, sulthiame; SUX, suxinutin; TC, tonic–clonic; TD, tonic drop; TPA, topiramate; VPA, valproate.
Table 2  Effects of ketogenic diet on epileptiform activity

<table>
<thead>
<tr>
<th>Activity stage (18 children a)</th>
<th>Median (range) (no. of spikes/h)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before KD</td>
<td>After 3 months of KD</td>
</tr>
<tr>
<td>Wake</td>
<td>99.5 (3—5591)</td>
<td>17.5 (0—780)</td>
</tr>
<tr>
<td>Sleep stage 2</td>
<td>521 (33—4689)</td>
<td>186 (9—2140)</td>
</tr>
<tr>
<td>Slow wave sleep</td>
<td>888 (36—5153)</td>
<td>103.4 (32—2399)</td>
</tr>
<tr>
<td>REM</td>
<td>424 (17—2580)</td>
<td>71 (0—2457)</td>
</tr>
<tr>
<td>Total</td>
<td>2171 (117—18013)</td>
<td>682 (68—6300.4)</td>
</tr>
</tbody>
</table>

a 17 in sleep stage 2. Child #4 did not present any sleep stage 2.

KD, ketogenic diet; REM, rapid eye movement sleep.

seizures. Additional clinical features of the patients and additional demographic information are given in Table 1. For further clinical information see Hallbook et al. (2007).

The epileptiform activity consisted of focal spike or sharp-wave complexes in five children and bilateral or generalized spike-wave complexes in 13. All children but two had abundant epileptiform activity. All patients remained on stable antiepileptic drug (AED) medication for at least 3 months prior to the KD initiation and, during the follow up. Plasma concentrations of AEDs were taken 1 week before KD initiation and, after 3 months of KD. They were not changed. Epilepsy surgery had been performed in one patient (#4) and found not applicable in the others.

After 3 months of KD the IEDs were significantly reduced (p < 0.001). When considering the four activity stages separately the reduction was significant during non-REM sleep stage 2, SWS and REM sleep (p = 0.001, 0.001, 0.002) but not significantly so in awake (p = 0.07) (Table 2). As has already been reported in an earlier article on the effects of KD on sleep (Hallbook et al., 2007), there was a significant reduction in seizure frequency (p = 0.001). Eight children (44%) showed 90% or more reduction in seizure frequency, four of them (22%) became seizure free, four (22%) had a 50–90% seizure reduction, five (28%) less than 50% seizure reduction and one (6%) increased in seizure frequency. Seizure severity, QOL, and attentional behaviour were significantly improved (p < 0.001, p < 0.001, p = 0.006). Beta-hydroxybutyrate was significantly increased (p < 0.001). These data are presented in Table 3. There was a significant correlation between the reduction in epileptiform activity and clinical seizures (Spearman r = 0.6, p = 0.03). There was no correlation between reduction in epileptiform activity and the improvement in QOL and attentional behaviour.

Side effects

No severe side effects were seen. Child #18 with glucose transporter type 1 (Glut-1) deficiency showed an increased ataxia and lethargy (Gordon and Newton, 2003). She discontinued the diet after 3 months although she became seizure free and had a decrease in epileptiform activity. Two children had a slight increase in liver enzymes in combination with Valproic acid. One had a loss of hair in combination with Valproic acid. One boy with mental retardation and autism had persistent acidosis and increased outbursts of rage in combination with Sulthiame. This boy became seizure free and had a decrease in epileptiform activity. In addition, increased blood lipids, LDL and apolipoprotein B and, a decreased carotid distensibility were seen (Clarkson et al., 1996; Kwiterovich et al., 2003; Liuba et al., 2003).

Discussion

The frequency of IEDs and seizures is modulated by unknown factors as well as known factors such as activity stage, REM—non-REM sleep, sleep-waking cycle, real time of day, AEDs, time since last seizure and, environmental factors. Although efforts are made to control for known influencing factors, the variability of the amount of IEDs between consecutive days is considerable and larger in generalized than in focal epilepsies (Camfield et al., 1995; Gotman, 1991; Martins da Silva et al., 1984; Mizrahi, 1996). In our
study we used 24 h recordings in order to minimize spontaneous variability. We selected long artifact-free recordings with comparable periods during the same real time of day. Over-night sleep recordings were done for quantification of sleep in order to get comparable periods in the different activity stages. AEDs were kept stable from 3 months before and throughout the study, plasma concentrations were taken 1 week before initiation and after 3 months of KD treatment. The 24 h EEG recordings were ambulatory, with the children in their natural surroundings. We based the identification of epileptiform discharges on the commonly used criteria (Pedley, 1980). As pointed out by Gotman, the definition of a spike is not completely defined (Gotman and Yves, 1997). Automatic computerized spike detection methods have developed. Even though many improvements have been made the number of false negative or false positive detections are unknown. On the other hand, electroencephalographers differ when asked to independently identify spikes in the same EEGs (Hostetler et al., 1992; Wilson et al., 1996).

As far as we know, no previous studies evaluating effects of KD on IEDs have been conducted. Bough et al. showed in an animal study, that KD might have both anticonvulsive and antiepileptogenetic effects. They showed enhancement of short-time inhibition in evoked response and, in a model of epileptogenesis, the rate of increase of electrophysiological seizure duration after repeated stimuli was markedly reduced (Bough et al., 2006). In our study there was a significant effect on both IEDs and clinical seizures. The concomitant reduction in IEDs and in clinical seizures, indicate that KD influences the epileptic process at different functional levels. In pharmacological studies, concomitant suppression of seizures and IEDs has been demonstrated in generalized epilepsy (Binnie et al., 1986). In partial epilepsies, lamotrigine and levetiracetam have been shown to reduce IEDs (Eriksson et al., 2001; Stodieck et al., 2001).

Dahlin et al. found significantly increased GABA and decreased glutamate levels in CSF in a study of KD in children with refractory epilepsy (Dahlin et al., 2005). Sub-regions within the thalamic ventrolateral preoptic nucleus (VLPO), containing GABA and galanin, are specialized for the control of REM versus non-REM sleep. KD mimics starvation, and the high-fat intake decreases leptin and insulin levels (Thio et al., 2006) and subsequently increases the expression of galanin and NPY. One could speculate that the increase in REM sleep is induced by changes in GABAergic and, galaninergic functioning (Kokaia et al., 2001; Mazarati et al., 2000; Sherin et al., 1998; Wang et al., 1998). Influences of sleep on epilepsy are well known. The different sleep stages have a different effect on IED production. In non-REM sleep, particularly in sleep stage 2, a synchronizing effect is shown with an increase and a spreading of the interictal abnormalities and seizures. In contrast, REM sleep, with its asynchronous cellular discharge patterns and skeletal motor paralysis, increases the resistance to propagation of epileptic EEG discharges and to clinical motor accompaniment (Mazarati et al., 2000; Mendez and Radtke, 2001; Murphy and Burnham, 2006; Shouse et al., 2000). In this study we saw an increase in REM sleep. This might be one of several contributing factors to the antiepileptic effects of KD.

The reported improvement in QOL was not correlated to the antiepileptic effect. In the earlier article on the effects of KD on sleep (Hallböök et al., 2007) we inferred that the increased REM sleep was a marker for improved sleep quality. The increased REM sleep was significantly correlated to the improvement in QOL. The reported improvement in attention was also unrelated to the level of attained seizure control. There was a significant correlation between the improvement in attentional behaviour and the increase in βOHB. These findings confirm earlier studies (Murphy and Burnham, 2006; Pulsifer et al., 2001). Murphy et al. showed in a rat study that KD decreases activity level but not anxiety level. These findings indicate that KD might be useful in the treatment of ADHD (Murphy and Burnham, 2006).

The group was too small for comparing the effects on IED between children with generalized and focal epileptiform activity. The 13 children with generalized or multifocal epileptiform activity had a reduction of IED in all sleep stages (p < 0.01), not significantly so in wake (p < 0.09). The five children with focal epileptiform activity only showed reduction of IED in REM sleep (p < 0.04). More children need to be analyzed to verify this trend.

After 3 months of KD seven children discontinued the diet. Four of these discontinued because of insufficient antiepileptic effect. One of them had an increase in seizure frequency, two were unchanged and one had 22% seizure reduction. They all had partial epilepsy with tonic–clonic and secondarily generalized seizures. Three children discontinued because of deficient compliance with the diet. Of the 12 children with >50% seizure reduction 11 children had GTCS, tonic generalized, atonic drop, tonic drop, atypical absences and myoclonic seizures. One had partial epilepsy with tonic–clonic and secondarily generalized seizures. The child with Glut-1 deficiency discontinued the diet after 3 months. Despite a significant reduction in seizure frequency, ataxia and lethargy deteriorated. This is not in line with other studies on KD and Glut-1 deficiency (Coman et al., 2006; Friedman et al., 2006; Fujii et al., 2007; Gordon and Newton, 2003; Wang et al., 2005). This could due to the late age of KD initiation. Treatment effects are known to be better the earlier you start the KD treatment. Extensive coffee drinking in our patient could be a related factor. Coffee, theophylline and phenobarbiturates decrease glucose-transportation and consequently might deteriorate neurological symptoms.

In conclusion, this study shows that KD reduces the number of IEDs, especially in sleep and it shows a correlation between the reduction in epileptiform activity and clinical seizures. This study also shows improvement in QOL and attention and, that improvement in attention correlates with the increase in βOHB. The improvement in QOL and attention do not correlate with the reduction in clinical seizures and epileptiform activity.

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