Original article

Epileptic Disord 2019; 21 (4): 366-9

Absence of increased blood decanoic acid levels in children with epilepsy treated with classic ketogenic diet

Julie Enderlin [1], Anna Loussouarn [2], Jean-François Benoist [3], Blandine Dozières-Puyravel [2], Stéphane Auvin [1,2]

[1] Université Paris Diderot, Sorbonne Paris Cité, INSERM UMR1141, Paris
[2] AP-HP, Hôpital Robert Debré, Service de Neurologie Pédiatrique, Paris
[3] AP-HP, Hôpital Robert Debré, Service de Biochimie, Paris, France

Received February 16, 2019; Accepted June 06, 2019

ABSTRACT – *Aim*. Recently, decanoic acid (C10), a medium-chain fatty acid, was shown to be a direct inhibitor of the AMPA receptor. Accordingly, C10 has been suggested as a potential anticonvulsant factor in the ketogenic diet (KD) or the medium-chain triglyceride KD. Here, we tested whether C10 serum levels correlate with the response to KD in five children (1.5 ± 0.6 years of age) with epilepsy.
Methods. The serum levels of C10 were measured before and after KD initiation (*n*=2 at one month, *n*=3 at three months, and *n*=1 at six months after initiation) by gas chromatography-mass spectrometry.
Results. After three months on KD, two patients were found to be responders. The mean serum level before KD initiation was 63.2 μM. Only one patient, who was a non-responder, showed an increase (5%) in C10 serum level after a month of KD. The remaining four patients (two responders) showed a decrease in the C10 level from -5.3% to -75.5%.
Conclusion. Our preliminary data show that KD does not lead to an increase in C10 serum levels, suggesting that increased concentration of C10 might not be directly involved in the anticonvulsant effects of classic KD.

Key words: decanoic acid, epilepsy, fatty acid, ketogenic diet

Correspondence:
Stéphane Auvin
Service de neurologie pédiatrique
et des maladies métaboliques,
CHU Hôpital Robert Debré,
48, boulevard Sérurier,
75935 Paris cedex 19, France
<stephane.auvin@inserm.fr>

The ketogenic diet (KD) is commonly used to treat pharmacoresistant epilepsy (Doziéres-Puyravel *et al*., 2018; Kossoff *et al*., 2018). Despite clinical studies having established the efficacy of the KD, the underlying mechanisms of the KD remain unclear. Moreover, the significance of the correlation between blood ketone body concentrations and seizure frequency changes remains unclear with more studies reporting an absence of correlation between these two factors (Gilbert *et al*., 2000; Buchhalter *et al*., 2017; Dallerac *et al*., 2017; Augustin *et al*., 2018a). Years of experimental studies suggest that the KD is probably effective via multiple mechanisms (Rho, 2017).

Medium-chain fatty acids, in particular decanoic acid (C10), have shown anticonvulsant activity via

doi:10.1684/epd.2019.1079

direct inhibition of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor by binding to sites on the M3 helix of the AMPA-GluA2 transmembrane domain (Chang *et al*., 2016). C10 is also a peroxisome proliferator-activated receptor-γ (PPARγ) agonist and its effects mimic the mitochondrial proliferation seen upon KD treatment (Augustin *et al*., 2018a). The experimental evidence for the anticonvulsant effect of C10 is currently based only on *in vitro* studies (Augustin *et al*., 2018b). This has led to the hypothesis that a mechanism of action for the medium-chain triglyceride (MCT) KD may involve C10.

It is currently unknown whether the use of classic KD results in a significant change in serum C10 levels and whether such changes correlate with the anti-seizure effect in patients. Therefore, in this study, we examined the serum levels of C10 in epilepsy patients treated with classic KD, relative to any changes in seizure frequency.

Methods

This retrospective study took place at the Paediatric Neurology Department, in Robert-Debré University Hospital, Paris, France from January 2016 to July 2017. The inclusion criteria were: patients with pharmacoresistant epilepsy treated with the classic KD (not MCT KD), and availability of the remaining blood samples from the standard evaluation at KD initiation and during follow-up, allowing storage of at least 400 μL of serum.

Data collection

For the patients who had enough serum stored before and after KD initiation, we retrospectively collected the following clinical data: sex, age, age at epilepsy onset, epilepsy syndrome, underlying aetiology, antiepileptic drug (AED) prior to KD initiation, AED at KD initiation, and KD at one, three, and six months after the initiation of the diet. Change in seizure frequency was based on analysis of the diary used by patients at the time of KD initiation.

Blood samples and C10 blood level measurements of the patients

Sample preparation: lipids were extracted from 200 μL of serum mixed with a known amount of internal standard (decanoic acid-1-C^{13} [Sigma-Aldrich, France]) using the Folch method (chloroform/methanol: 2/1 v/v). Briefly, the samples were stirred and centrifuged at 14,000 rpm for 15 min at 4°C; the lower chloroform phase was collected and dried under nitrogen. Fatty acid methyl esters (FAMEs) were formed using a one-step trans-esterification method by adding 1.7 mL of methanolic acetyl-chloride (10%) and 100 μL of acetyl chloride at 80 °C for 35 minutes. The FAMEs were then extracted using 2 mL of hexane and directly analysed.

Decanoic acid quantification: the FAMEs were analysed by gas chromatography coupled to mass spectrometry (Focus-DSQII, Thermo, les Ulis, France) on a dedicated capillary column in split injection mode. The decanoic acid methyl ester was quantified by isotopic dilution using the single ion monitoring mode.

Statistical analysis

Data were analysed using Prism 5 software (GraphPad, San Diego, CA, USA). Statistical analysis was performed using the Mann-Whitney test (mean level of C10 before KD versus C10 after one month of KD or C10 after three months of KD). *P* values < 0.05 were considered significant.

Results

We included five patients (1.5 ± 0.6 years of age [mean ± SEM]) who were treated with the KD for pharmacoresistant epilepsy; four patients were treated for infantile spasm syndrome and one for epilepsy with myoclonic atonic seizures (*table 1*). Among the five patients, three were found to be responders (≥50% reduction of seizure frequency compared with baseline) after one month of KD; of these, two of them remained responders after three months of KD (Patient 2 and 3) (*table 1*). Patient 4 and 5 improved after six months of KD and additional antiepileptic drug treatment (*table 1*).

C10 blood levels were measured in the five cases before and in at least one sample after KD initiation (*table 2*). The mean serum level in the five patients before initiation was 63.2 μM. C10 measurements were performed at one month after KD initiation for two patients, at three months for three patients, and at six months for one patient (*table 2*). Only one patient had a 4.9% increase in C10 serum level after one month of KD. However, this patient did not show improvement under KD. The remaining four patients showed a decrease in C10 serum level, ranging from -5.3% to -75.5%, compared with the serum concentration before KD initiation (*table 2*). The two patients who were responders to the KD showed a mean decrease of 18.5% of C10 serum level after three months of KD compared with baseline (*table 2*). Patient 3, who improved with KD, also showed a decrease in the C10 serum concentration when comparing the levels at three months (-28.4%) and six months (-38.8%) after KD initiation (*table 2*).

Table 1. Clinical characteristics and response to the ketogenic diet of the five included patients.

Pt	Sex	Epilepsy syndrome / aetiology	Age at epilepsy onset / age at KD onset	No. AEDs before KD	AEDs at KD initiation	M1 Sz change / no. AEDs	M2 Sz change / no. AEDs	M3 Sz change / no. AEDs	M6 Sz change / no. AEDs
1	M	EMA / unknown	3Y4M / 3Y6M	5	VPA+LTG	≥50%/2	≥50%/3	<50%/4	≥50%/4
2	F	ISS / *CDKL5* mutation	0.5M / 5.5M	3	VGB +steroid +LVT	≥50%/3	>90%/3	>90%/2	>90%/1
3	F	ISS / polymicrogyria	4M / 1Y1M	5	VGB+ZNS +CNZ	≥50%/3	≥50%/2	≥50%/3	≥50%/3
4	M	ISS-FOS / cortical malformation	2M / 5M	4	VGB +steroid +LVT+VPA	0%/4	<50%/3	<50%/3	≥50%/4
5	M	ISS / *ARX* deletion	4M / 10M	3	VGB+ZNS	<50%/2	0%/3	0%/2	≥50%/3

AED: antiepileptic drug; CNZ: clonazepam; EMA: epilepsy with myoclonic atonic seizure; FOS: focal onset seizure; KD: ketogenic diet; LTG: lamictal; LVT: levetiracetam; M:month; ISS: infantile spasm syndrome; Sz: seizure; VGB: vigabatrin; VPA: valproate; Y:year; ZNS: zonisamide.

Table 2. Blood level of decanoic acid as serum concentration (μM) and evolution from the initiation of the ketogenic diet.

Patient	Serum concentration (μM)				Evolution from before KD to M1 KD	Evolution from before KD to M3 KD	Evolution from before KD to M6 KD
	Before KD	M1 KD	M3 KD	M6 KD			
1	105 μM	25.7 μM	NA	NA	-75 %	NA	NA
2	35.6 μM	NA	33.7μM	NA	NA	-5%	NA
3	52.1 μM	NA	37.3 μM	31.9 μM	NA	-28%	-39%
4	43.4 μM	NA	39.7μM	NA	NA	-9%	NA
5	80 μM	83.9 μM	NA	NA	+5%	NA	NA

KD: ketogenic diet; M: month; NA: not available.

Discussion

This is the first report, since the description of its anti-convulsant properties, of measurement of C10 concentration in the blood of patients treated with classic KD for pharmacoresistant epilepsy. We observed a decrease in the serum C10 concentration after initiation of KD in four out of five patients. This decrease in serum C10 concentration was also observed in the patients who were responders to the KD treatment.

Our observation suggests that increased blood levels of C10 are not associated with the efficacy of the classic KD. The lack of an increase in blood level does not support the hypothesis that C10 could be active in the brain via an increase in brain concentration after crossing the blood brain barrier. However, C10 might act via an indirect mechanism that would not be reflected by an increase in blood concentration. It has been shown that C10 can bind PPARγ, which has been described as an anti-seizure target (Simeone *et al*., 2017), suggesting that the decrease in serum C10 levels might correlate with a lower availability of C10 for binding PPARγ.

However, these results are too preliminary to evaluate whether the change in C10 concentration correlates with clinical response to KD. As we previously reported, the serum level of arachidonic acid (C20:4 n-6) was also found to be lower in responders to classic KD or a modified Akins diet (Porta *et al*., 2009).

The restricted number of patients and the absence of detailed information on patients' food intake further limit this study. It would be of interest to further study the blood concentration of C10 in patients with epilepsy with various dietary treatments, including controls and both classic and MCT KD.

In summary, although our data do not support a correlation between C10 level and classic KD, this does not exclude the hypothesis that C10 may increase and correlate with anti-seizure activity in patients with MCT KD or that oral administration of C10 could result in anti-seizure activity. □

Acknowledgements and disclosures.

This work was supported by INSERM, Association 'INJENO' and 'Association Kemil et ses amis'. Stéphane Auvin has served as a consultant or gave lectures for Eisai, GW Pharma, Novartis, Nutricia, Shire, UCB Pharma, Ultragenyx, and Zogenix. Stéphane Auvin is also Associate Editor for Epilepsia. All other authors have no conflict of interest to declare.

References

Augustin K, Khabbush A, Williams S, *et al*. Mechanisms of action for the medium-chain triglyceride ketogenic diet in neurological and metabolic disorders. *Lancet Neurol* 2018a; 17: 84-93.

Augustin K, Williams S, Cunningham M, *et al*. Perampanel and decanoic acid show synergistic action against AMPA receptors and seizures. *Epilepsia* 2018b; 59: e172-8.

Chang P, Augustin K, Boddum K, *et al*. Seizure control by decanoic acid through direct AMPA receptor inhibition. *Brain* 2016; 139: 431-43.

Dallerac G, Moulard J, Benoist JF, *et al*. Non-ketogenic combination of nutritional strategies provides robust protection against seizures. *Scientific Rep* 2017; 7: 5496.

Doziéres-Puyravel B, Francois JF, de Lucia S, *et al*. Ketogenic diet therapies in France: state of the use in 2018. *Epilepsy & Behav* 2018; 86: 204-6.

Kossoff EH, Zupec-Kania BA, Auvin S, *et al*. Optimal clinical management of children receiving dietary therapies for epilepsy: updated recommendations of the International Ketogenic Diet Study Group. *Epilepsia Open* 2018; 21: 175-92.

Porta N, Vallée L, Boutry E, *et al*. Comparison of seizure reduction and serum fatty acid levels after receiving the ketogenic and modified Atkins diet. *Seizure* 2009; 18: 359-64.

Rho JM. How does the ketogenic diet induce anti-seizure effects? *Neurosci Lett* 2017; 637: 4-10.

Simeone TA, Matthews SA, Samson KK, Simeone KA. Regulation of brain PPARgamma2 contributes to ketogenic diet anti-seizure efficacy. *Exp Neurol* 2017; 287: 54-64.