Title page

An examination of biochemical parameters and their association with response to ketogenic dietary therapies

Natasha E Schoeler[1,2], Gail Bell[3], Alan Yuen[3], Adam D Kapelner[4], Simon J R Heales[5,6,7], J Helen Cross[1,8,9], Sanjay Sisodiya[2,3]

Author information:

[1]UCL Great Ormond Street Institute of Child Health, London, United Kingdom

[2]NIHR University College London Hospitals Biomedical Research Centre, Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, London, United Kingdom

[3]Epilepsy Society, Chalfont St Peter, United Kingdom

[4]Queens College, The City University of New York (CUNY), Department of Mathematics, New York, USA

[5]Genetics and Genomic Medicine, UCL Institute of Child Health, London, United Kingdom

[6]Chemical Pathology, Great Ormond Street Hospital for Children, London, United Kingdom

[7]Neurometabolic Unit, National Hospital for Neurology and Neurosurgery, London, United Kingdom

[8]Great Ormond Street Hospital for Children, London, United Kingdom

[9]Young Epilepsy, Lingfield, United Kingdom

Corresponding author: Dr. Natasha Schoeler. Address: Clinical Neurosciences, 4th Floor PUW, UCL Great Ormond Street Institute of Child Health, 30 Guilford Street, London WC1N 1EH. E-mail: n.schoeler@ucl.ac.uk

First author information: Dr Natasha Schoeler is a research dietitian whose principal research interest is in predicting response to ketogenic diets for epilepsy; she is also involved in ketogenic diet trials in children and adults.

Running title: Acetyl carnitine and response to the ketogenic diet

Key Words: Low-carbohydrate, high-fat, biochemistry, blood, epilepsy, predictor

Number of text pages: 18

Number of words: 3586 (including Summary, Acknowledgements and Disclosures of Conflicts of Interest)

Number of references: 40

Number of figures: 2

Number of tables: 3

Summary

Objective:

In the absence of specific metabolic disorders, accurate predictors of response to ketogenic dietary therapies (KDT) for treating epilepsy are largely unknown.

We hypothesised that specific biochemical parameters would be associated with the effectiveness of KDT in humans with epilepsy. The parameters tested were β-hydroxybutyrate, acetoacetate, non-esterified fatty acids, free and acylcarnitine profile, glucose and glucose-ketone index (GKI).

Methods:

Biochemical results from routine blood tests conducted at baseline prior to initiation of KDT and at 3-month follow-up were obtained from 13 adults and 215 children with KDT response data from participating centres. 132/228(57%) participants had some data at both baseline and 3-months; 52/228(23%) had data only at baseline; 22/228(10%) had data only at 3-months; 22/228(10%) had no data. KDT response was defined as ≥50% seizure reduction at 3-month follow-up.

Results:

Acetyl carnitine at baseline was significantly higher in responders ($p<0.007$). It was not associated with response at 3-month follow-up. There was a trend for higher levels of free carnitine and other acylcarnitine esters at baseline and at 3-month follow-up in KDT responders. There was also a trend for greater differences in levels of propionyl carnitine and in β-hydroxybutyrate measured at baseline and 3-month follow-up in KDT responders. No other biochemical parameters were associated with response at any time point.

Significance:

Our finding that certain carnitine fractions, in particular baseline acetyl carnitine, are positively associated with greater efficacy of KDT is consistent with the theory that alterations in energy metabolism may play a role in the mechanisms of action of KDT.

Key Words

Low-carbohydrate, high-fat, biochemistry, epilepsy, predictor

Key point box

- Pre-diet acetyl carnitine is associated with ketogenic diet response.
- There is a trend for higher levels of free carnitine and other acylcarnitine esters in responders.
- Acetyl carnitine is reversibly converted to acetyl-CoA. Acetyl-CoA may enter the citric acid cycle or be used to produce ketones.
- Alterations in mitochondrial energy metabolism may play a role in the anti-seizure effects of ketogenic diets.

Introduction

Ketogenic dietary therapies (KDT) can be an effective treatment for people with drug-resistant epilepsy [1-4]. Despite the effectiveness of these therapies, in the absence of specific metabolic disorders (glucose transporter type 1 deficiency syndrome and pyruvate dehydrogenase complex deficiency [5]) patient characteristics that accurately predict differential response are largely unknown. Evidence for association of favourable response, for example, in younger patients, those with higher pre-diet seizure frequency or specific EEG parameters, is inconsistent [6]. High response rates have been observed in certain epilepsy syndromes [7] and individuals with focal malformations [8,9], but these are merely indications; there are still non-responders within these groups and the question remains whether other patient demographics or clinical parameters allow response to KDT to be predicted. Prior to commencing KDT, individuals are screened with biochemical testing of blood and urine for disorders of fatty acid metabolism and organic acidurias. Blood levels of various vitamins, minerals and electrolytes (depending on local practice) are also measured and monitored throughout treatment.

Previous studies have examined potential associations between KDT response and β-hydroxybutyrate (BHB), the primary circulating ketone body in response to starvation and/or KDT [10] but results have been inconsistent [11-13]. Other biochemical parameters that are already measured as part of routine clinical practice could theoretically be associated with KDT response based on their role in fat and carbohydrate metabolism. For example acetoacetate, akin to BHB, can be converted back to acetyl-CoA for entry into the citric acid cycle and thus used as an energy source; non-esterified fatty acids (NEFA) are precursors of ketone bodies and flux to the liver is increased in individuals following KDT [14]; carnitine and

acylcarnitine esters (measured by the acylcarnitine profile) are essential for mitochondrial uptake of long-chain fatty acids; reduced glucose levels (but stable and within physiological range) are a natural consequence of consuming a low-carbohydrate diet and are necessary for ketone production, which occurs in response to reduced hepatic glycogen stores. The relationship between glucose and BHB levels may also be important for KDT response: a lower glucose-ketone index (GKI), the ratio of blood glucose to ketones, has been found to be associated with greater efficacy of ketogenic diets (or calorie restriction) for brain tumour management in mice and humans [15].

There is very limited evidence suggesting lack of association between carnitine (unclear whether measured at baseline or follow-up) [13] and KDT response; evidence regarding KDT response and glucose, including measures at baseline, follow-up and differences between the two time points, is conflicting [16-19].No studies have examined the possible association between acylcarnitine esters, acetoacetate, NEFAs, or calculated GKI and the effectiveness of KDT in humans with epilepsy. We hypothesised that these parameters are associated with response to KDT.

Methods

Ethics and recruitment

Biochemical data were obtained from participants of a study investigating the genetic basis behind response to KDT ([20,21]). The project gained ethical approval through relevant ethics committees or institutional review boards. Written informed consent was obtained from all study participants or from their parents/carers in the case of minors or adults with intellectual disability.

Participants were recruited for the study from April 2011-December 2012 from the following sites: Great Ormond Street Hospital for Children, London; National Hospital for Neurology and Neurosurgery, London; Evelina Children's Hospital, London; St George's Hospital, London; Young Epilepsy (including Matthew's Friends clinics for Ketogenic Dietary Therapies), Surrey; Birmingham Children's Hospital, Birmingham; Addenbrooke's Hospital, Cambridge; Alder Hey Children's Hospital, Liverpool; Bristol Royal Hospital for Sick Children, Bristol, all in the UK.

Criteria for study inclusion were: individuals aged ≥3months who were either following KDT or who had followed KDT in the past for epilepsy. Exclusion criteria were: individuals who discontinued KDT before the 3-month point due to lack of tolerability (those who discontinued KDT before the 3-month point due to lack of response or seizure increase were included), individuals with progressive myoclonic epilepsies or other progressive neurological diseases.

Data collection

All participants underwent electroclinical phenotyping to establish seizure type and epilepsy syndrome. This involved evaluation of medical history, seizure semiology, examination and review of EEG and imaging studies. Demographic data were obtained from medical records. Biochemical results for BHB, acetoacetate, NEFA, free- and acylcarnitine profile and glucose conducted at baseline (prior to initiation of KDT) and at 3-month follow-up (three months after KDT was started) as part of routine clinical care were obtained from medical records and NHS Trust databases. The GKI was calculated using the GKI calculator using glucose and BHB levels measured at baseline and at 3-month follow-up [15].

KDT response was defined as a function of seizure frequency, as previously published ([20,21]). Response was estimated in 28 day epochs prior to starting the diet (baseline) and prior to 3-month follow-up after the start of KDT. Clinic letters and seizure diaries, where already used as part of clinical monitoring, were used to estimate seizure frequency at each time point. The calculation used to determine percentage reduction in seizure frequency was: [(a-b)/a]*100, where a = number of seizures in the 28 days prior to KDT initiation; b = number of seizures in the 28 days preceding the 3-month point. A case/control observational study design was adopted. Those with ≥50% seizure reduction were classified as 'responders'; those with <50% seizure reduction were 'non-responders'. Seizure freedom achieved at 3-month follow-up was also documented.

Statistical analyses

The effect of demographic parameters between groups with biochemical data at various time points (either with no biochemical data, some data at baseline and 3-month follow-up, data only at baseline, or data only at 3-month follow-up) was assessed using Fisher's exact or Kruskal-Wallis tests. The effect of demographic parameters on KDT response was assessed using Fisher's exact or Kruskal-Wallis tests.

Parametric tests were conducted to assess the relationship between KDT response at 3-month follow-up and blood test results that had ≥50 data points in each response group. Non-parametric analyses were used for blood test results with <50 data points in each response group. Analyses were conducted with biochemical results from baseline, at 3-month follow-up and also using the difference between the biochemical results taken at these two time points.

A significance threshold of 0.05 was applied, as this was an exploratory analysis. The Bonferroni-corrected significance threshold to account for the multiple testing of 14 tests was α=0.004. Note that this correction is too conservative, as our tests are not independent of each other.

All statistics were performed in Stata v13.1 (*Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP).

Results

Response data could not be collected for 4/232(2%) participants which is a small enough proportion to safely ignore dropout without fear of biasing our results. 117/228(51%) participants (113 children and 4 adults [aged ≥18 years at start of diet]) had ≥50% seizure reduction after 3 months of following KDT, of whom 10 were seizure-free (all aged < 18 years); 111/228(49%) (102 children and 9 adults) had <50% seizure reduction. There was no statistically significant difference in response between children and adults (Fisher's exact test p = 0.16).

132/228(58%) participants with KDT response data had some data at both baseline and 3-months; 52/228(23%) had data only at baseline; 22/228(10%) had data only at 3-months; 22/228(10%) had no data available.

A summary of the clinical and demographic parameters of the cohort are given in Supplementary Table 1. No participants received carnitine supplementation either at baseline or during KDT.

There was no difference in demographic parameters between groups with biochemical data at various time points, as shown in Supplementary Tables 2-9. No analyses were conducted

for cause of epilepsy or for epilepsy syndrome as, for most participants, the cause was 'unknown' and they had no syndromic diagnosis.

There was no difference in any demographic parameter between responders and non-responders to KDT when response was defined as 50% seizure reduction, as shown in Supplementary Table 10.

There was a trend for lower age at diet start in those who achieved seizure freedom compared to those who did not become seizure-free (median age=3.4 years in those who became seizure free; median age=5.7 years in those who did not achieve seizure freedom; p=0.02). There was no difference in any other demographic parameters when response was defined as seizure-freedom, as shown in Supplementary Table 10.

Acetyl carnitine at baseline was higher in KDT responders (uncorrected $p<0.003$; Table 1). This is significant, even when applying a Bonferroni-corrected significance threshold ($p<0.004$).

Acetyl carnitine data at baseline ranged from 2.74-40.7µmol/L for responders and 3.59-32.5µmol/L for non-responders. Figure 1 shows a trend for higher acetyl carnitine levels at baseline in responders although, at the lower end of the scale (acetyl carnitine levels approximately <7µmol/L), there is some overlap between responders and non-responders.

There were trends for higher free, propionyl, octanoyl and palmitoyl carnitine and β-hydroxybutyrate at baseline in KDT responders ($p<0.05$ but >0.004; Table 1), higher palmitoyl carnitine at 3-month follow-up in KDT responders ($p<0.05$ but >0.004; *p-values derived from t-tests

Table 2), and greater differences in levels of propionyl carnitine and in BHB measured at baseline and 3-month follow-up in KDT responders ($p<0.05$ but >0.004; *p-values derived from t-tests

Table 3).

There was a trend for higher NEFA levels at 3-month follow-up and a greater difference in BHB levels at baseline and at 3-month follow-up in participants who achieved seizure freedom at 3-month follow-up compared to those who were not seizure-free ($p<0.05$ but >0.004). These data are presented in Supplementary Tables 11-13.

Discussion

We have found that acetyl carnitine at baseline was significantly higher in KDT responders compared to responders, although the effect size is small and potential clinical significance unknown. This result is supported by a trend for higher levels of free carnitine and other acylcarnitine esters in KDT responders at baseline, 3-month follow-up or the difference in levels measured at these two time points. We are not aware of previously published reports of a relationship between acetyl carnitine, or any other acylcarnitine esters, and KDT response.

Our finding that higher blood acetyl carnitine levels are associated with greater effectiveness of KDT is consistent with our knowledge of biochemistry: carnitine is needed to transport long-chain fatty acids into hepatic mitochondria to produce ketones and acetyl carnitine and acetyl coenzyme A (acetyl-CoA) plays a critical role in mitochondrial energy

metabolism. There is an equilibrium between acetyl carnitine (acetyl coA + carnitine) and acetyl-CoA. Acetyl carnitine is reversibly converted to acetyl-CoA by carnitine acetyltransferase (CAT) enzymes [22] (see Figure 2). This maintains acetyl-CoA levels for production of ketone bodies (acetyl carnitine then leaves the mitochondria with the ketones) and also for entry of acetyl-CoA into the citric acid cycle. Levels of acylcarnitines, including acetyl carnitine, are increased in patients following KDT [23]. These metabolic adaptations, triggered by PPARα, serve to favour the use of ketones as fuel [24].

Our findings may point to a targetable alteration in energy metabolism and acetyl CoA buffering/availability. Whilst the mechanisms responsible for this observation are unknown, this result may relate to our recent reports that decanoic acid, a component of the medium chain triglyceride (MCT) KD, is able to increase citrate synthase activity in both cultured neurons and fibroblasts [25,26]. This enzyme utilises acetyl CoA and catalyses the first reaction of the citric acid cycle (the condensation of acetyl-CoA and oxaloacetate to form citrate). We may have identified a subset of patients that responded to KDT due to improvement in mitochondrial energy metabolism. The fact that acetyl carnitine at 3-month follow-up was not associated with response to KDT in our study may reflect the fact that these patients' 'deficiencies' in mitochondrial bioenergetics have been 'corrected' through use of KDT. One could infer that acetyl carnitine is important for successfully starting KDT, but once ketosis has been achieved and/or mitochondrial energy metabolism is altered, carnitine levels are no longer associated with seizure control. However, it is unknown whether the serum biochemical parameters looked at in our study reflect cerebral energy metabolism.

One study previously investigated the relationship between carnitine (it is unclear whether this was free or total carnitine) and KDT response [13]; no statistically significant relationship

was found. Correlations have been identified between KDT response and other biochemical markers not usually measured as part of routine management in the UK. For example, responders to KDT were found to have larger absolute decreases in plasma phospholipid fatty acid 18:0 and smaller increases in 24:1 during dietary treatment [27] than non-responders; there are also reports of a positive correlation between reduction in seizure frequency and elevation in serum total arachidonic acid compared to pre-diet levels [28], higher serum palmitoleic acid in responders after one month, lower serum arachidonic acid in responders after one month, and lower serum arachidonic and docosahexaenoic in responders at 3-month follow-up [29]. However, no difference has been found in levels of other plasma fatty acids [27], monoamine or HVA/5HIAA [30], linoleic acid or alpha-linolenic acid [29], octanoic or decanoic acid [31] between responders and non-responders.

We found a trend for association between KDT response – both defined as ≥50% seizure reduction and seizure-freedom – and the difference in blood BHB levels at baseline and 3-month follow-up. This is a novel approach to analysing the relationship between BHB and KDT effectiveness. Some studies have previously reported a correlation between improved seizure control at various follow-up points and higher blood BHB levels in children [11,12,32,33], adolescents [11,12] and adults [12] following KDT. Others have found no correlation, either with blood BHB or urinary ketosis in all KD variants, with use of the classical diet, MCT diet, Modified Atkins diet/Modified Ketogenic Therapy and the Low Glycaemic Index Treatment (see [6] for a comprehensive list of references). BHB has been shown to have direct [34] and indirect [35] neuroprotective and/or anticonvulsant effects, and ketone esters have displayed anticonvulsant properties in animal seizure models [36-38], which supports the premise that the production of ketones is necessary for effectiveness of KDT. On the other hand, recent studies found that medium chain fatty acids, specifically decanoic acid, displayed anti-

seizure activity (although this has not yet been tested in humans), whereas BHB did not [25,26,39], pointing towards alternative therapeutic mechanisms of KDT. We also found a trend for higher NEFA levels at 3-month follow-up in participants who achieved seizure freedom at 3-month; no other studies were found assessing the relationship between total NEFA and KDT response.

We did not find that GKI, either at baseline or at 3-months, was associated with KDT effectiveness. We hoped to investigate the difference in the GKI values taken at the two time points, but the baseline results were much higher; thus, it would have mainly reflected the effect of the baseline values. Furthermore, no relationship was seen with glucose alone, showing that the GKI trends are driven by BHB levels. A more accurate and consistent measurement of GKI could be obtained by measuring blood glucose and BHB twice a day, 2-3 hours post-prandially, at approximately the same time of day for each patient, as suggested in the manuscript that originally discussed the concept of GKI [15].

Our findings are not affected by differences in demographic parameters between KDT responders and non-responders, nor between groups with biochemical data at various time points. Age at diet start was lower in those who achieved seizure-freedom, but there were only 10 participants in this group, compared with 218 who did not achieve seizure-freedom. This correlation may have been related to epilepsy syndrome; many participants did not have a syndromic diagnosis and, for those who did, the numbers within each syndrome group were too small for statistical analysis. Although some studies have reported a more favourable response in younger children or infants, the majority have found that age makes no difference (see [6] for a detailed list of references).

We may have been underpowered to detect the small effect sizes of certain biochemical parameters (in particular other acylcarnitine esters, BHB and NEFA), leading to non-significant results when accounting for multiple testing. Although large effect sizes would be more beneficial when considering translation into clinical practice, factors with small effect sizes may provide insight into the mechanisms of action of KDT. There were missing data in our cohort and very few participants achieved seizure-freedom. Missing data may have been because some blood tests were performed at local hospitals and were not recorded on participating centres' databases. Despite missing data, with 56 responders and 56 non-responders with baseline acetyl carnitine data, any concentration error estimates in the laboratory values should be spread evenly within the two groups. Our results are worth following up in a larger cohort, particularly with a larger number of seizure-free patients.

Another limitation of the study is that KDT response data were collected retrospectively, predominantly from clinic letters. Ideally, seizure diaries would be used throughout the 3-month period to allow for the natural variability of seizures over time, rather than relying on 28 day epochs. Seizure recording throughout the entire follow-up period may be crucial for individuals whose seizures occur in weekly/monthly clusters; seizure reduction on the diet may not be accurately reflected by using 28 day epochs, as the cluster may occur just before the 28 days prior to baseline and so, according to the seizure diary, the patient would be seizure-free before starting the diet. This was not an issue for any of our participants, as they all had seizures in the 28 days prior to baseline. Completion of a seizure diary for any prolonged length of time, however, can be burdensome for patients and/or carers. A 3-month baseline period before starting KDT may also be prohibitively long for some patients. Prospective data collection may have resulted in fewer missing data points and have allowed the possibility of ensuring a more consistent procedure for timing of blood

collection - this would be particularly important for glucose and BHB levels. However, this would be difficult to achieve in an outpatient setting.

There are possible analyses that could be performed in a follow-up study. If seizure diaries were used for all participants, we could use percentage seizure frequency change directly as a response variable; much information is lost when using binary outcomes. We could also perform a multivariable regression of our biochemical markers, as many of our measurements were correlated.

Conclusion

We have found a previously-unreported relationship between acetyl carnitine at baseline and KDT response. This provides supporting evidence for the role of altered mitochondrial energy metabolism in the mechanisms of action of KDT. We also report a trend for association with free- and other acylcarnitine esters – some at baseline, others at 3-month follow-up, or the difference between results at the two time points. Our findings also add to the conflicting evidence that blood BHB levels are associated with KDT response. These results are worth following-up in a larger cohort; although the relationship between baseline acetyl carnitine and KDT response is significant, the effect size is small and thus the finding has limited clinical meaning until followed up with a more controlled prospective study. Confirming the association would aid our understanding of the mechanisms of action of KDT and could potentially be used to target resources towards patients who are more likely to response favourably to KDT.

Acknowledgements

This work was undertaken at UCLH/UCL, and a proportion of funding was received from the Department of Health's NIHR Biomedical Research Centres funding scheme. NS was

supported by a UCL Impact Studentship in conjunction with Epilepsy Society. Funding was also received from the Wellcome Trust (084730).

Disclosures of Conflicts of Interest

JHC has received funds to the department for research into the ketogenic diet from Vitaflo. Honoraria for speaking have also been made to the department on her behalf from Nutricia. JHC has co-written a cookery book, 'Ketocooking', funds from the sale of which will be donated to the department. ADK owns a share in Kuma Shake, Inc., an American food startup developing a vegan 4:1 ketogenic shake. SMS has received meeting support or honoraria from Vitaflo and Nutricia. SJRH has received research funding and honoraria from Vitaflo.The remaining authors have no conflicts of interest in relation to this work.

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.

References

1 Neal EG, Chaffe H, Schwartz RH, et al. The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. Lancet Neurol 2008; 7: 500-506.
2 Henderson CB, Filloux FM, Alder SC, et al. Efficacy of the ketogenic diet as a treatment option for epilepsy: meta-analysis. J Child Neurol 2006; 21: 193-198.
3 Keene DL. A systematic review of the use of the ketogenic diet in childhood epilepsy. Pediatr Neurol 2006; 35: 1-5.
4 Payne NE, Cross JH, Sander JW, et al. The ketogenic and related diets in adolescents and adults - a review. Epilepsia 2011; 52: 1941-1948.
5 Kossoff EH, Zupec-Kania BA, Amark PE, et al. Optimal clinical management of children receiving the ketogenic diet: recommendations of the International Ketogenic Diet Study Group. Epilepsia 2009; 50: 304-317.
6 Schoeler NE, Cross JH, Sander JW, et al. Can we predict a favourable response to Ketogenic Diet Therapies for drug-resistant epilepsy? Epilepsy Res 2013; 106: 1-16.
7 Nangia S, Caraballo RH, Kang H-C, et al. Is the ketogenic diet effective in specific epilepsy syndromes? Epilepsy Res 2012; 100: 252-257.
8 Thammongkol S, Vears DF, Bicknell-Royle J, et al. Efficacy of the ketogenic diet: Which epilepsies respond? Epilepsia 2012; 53: e55-59.
9 Jung DE, Kang H, Kim H. Long-term outcome of the ketogenic diet for intractable childhood epilepsy with focal malformation of cortical development. Pediatrics 2008; 122: e330-e333.

10 Paoli A. Ketogenic diet for obesity: friend or foe? International journal of environmental research and public health 2014; 11: 2092-2107.
11 Neal EG, Chaffe H, Schwartz RH, et al. A randomized trial of classical and medium-chain triglyceride ketogenic diets in the treatment of childhood epilepsy. Epilepsia 2009; 50: 1109-1117.
12 van Delft R, Lambrechts D, Verschuure P, et al. Blood beta-hydroxybutyrate correlates better with seizure reduction due to ketogenic diet than do ketones in the urine. Seizure 2010; 19: 36-39.
13 Dressler A, Stocklin B, Reithofer E, et al. Long-term outcome and tolerability of the ketogenic diet in drug-resistant childhood epilepsy - The Austrian experience. Seizure 2010; 19: 404-408.
14 Nordli DR, Vivo DC. Effects of the Ketogenic Diet on Cerebral Energy Metabolism. In Stafstrom CE, Rho JM (Eds) Epilepsy and the Ketogenic DietSpringer Science+Business Media New York, 2004.
15 Meidenbauer JJ, Mukherjee P, Seyfried TN. The glucose ketone index calculator: a simple tool to monitor therapeutic efficacy for metabolic management of brain cancer. Nutr Metab (Lond) 2015; 12: 12.
16 Bergqvist A, Schall J, Richard E, et al. Predictive power of first morning glucose and the ketogenic diet Neuropediatrics 2007; 38: 193-196.
17 Numis AL, Yellen MB, Chu-Shore CJ, et al. The relationship of ketosis and growth to the efficacy of the ketogenic diet in infantile spasms. Epilepsy research 2011; 96: 172-175.
18 Coppola G, Aniello AD, Messana T, et al. Low glycemic index diet in children and young adults with refractory epilepsy: First Italian experience. Seizure 2011; 20: 526-528.
19 Muzykewicz DA, Lyczkowski DA, Memon N, et al. Efficacy, safety, and tolerability of the low glycemic index treatment in pediatric epilepsy. Epilepsia 2009; 50: 1118-1126.
20 Schoeler NE, Cross JH, Drury S, et al. Favourable response to ketogenic dietary therapies: undiagnosed glucose 1 transporter deficiency syndrome is only one factor. Dev Med Child Neurol 2015; 57: 969-976.
21 Schoeler NE, Leu C, White J, et al. Variants in KCNJ11 and BAD do not predict response to ketogenic dietary therapies for epilepsy. Epilepsy Res 2015; 118: 22-28.
22 Pettegrew JW, Levine J, McClure RJ. Acetyl-L-carnitine physical-chemical, metabolic, and therapeutic properties: relevance for its mode of action in Alzheimer's disease and geriatric depression. Molecular psychiatry 2000; 5: 616-632.
23 Neal E. Carnitine. In Neal E (Ed) Dietary Treatment of Epilepsy: Practical Implementation of Ketogenic TherapyJohn Wiley & Sons, Ltd, 2012.
24 Flanagan JL, Simmons PA, Vehige J, et al. Role of carnitine in disease. Nutr Metab (Lond) 2010; 7: 30.
25 Hughes SD, Kanabus M, Anderson G, et al. The ketogenic diet component decanoic acid increases mitochondrial citrate synthase and complex I activity in neuronal cells. Journal of neurochemistry 2014:
26 Kanabus M, Fassone E, Hughes SD, et al. The pleiotropic effects of decanoic acid treatment on mitochondrial function in fibroblasts from patients with complex I deficient Leigh syndrome. J Inherit Metab Dis 2016; 39: 415-426.
27 Dahlin M, Hjelte L, Nilsson S, et al. Plasma phospholipid fatty acids are influenced by a ketogenic diet enriched with n-3 fatty acids in children with epilepsy. Epilepsy Res 2007; 73: 199-207.
28 Fraser DD, Whiting S, Andrew RD, et al. Elevated polyunsaturated fatty acids in blood serum obtained from children on the ketogenic diet. Neurology 2003; 60: 1026-1029.
29 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: 359-364.
30 Dahlin M, Mansson JE, Amark P. CSF levels of dopamine and serotonin, but not norepinephrine, metabolites are influenced by the ketogenic diet in children with epilepsy. Epilepsy Res 2012; 99: 132-138.
31 Sills MA, Forsythe WI, Haidukewych D. Role of octanoic and decanoic acids in the control of seizures. Archives of disease in childhood 1986; 61: 1173-1177.

32 Huttenlocher PR. Ketonemia and seizures: metabolic and anticonvulsant effects of two ketogenic diets in childhood epilepsy. Pediatric research 1976; 10: 536-540.
33 Gilbert DL, Pyzik PL, Freeman JM. The ketogenic diet: seizure control correlates better with serum beta-hydroxybutyrate than with urine ketones. J Child Neurol 2000; 15: 787-790.
34 Rahman M, Muhammad S, Khan MA, et al. The beta-hydroxybutyrate receptor HCA2 activates a neuroprotective subset of macrophages. Nat Commun 2014; 5: 3944.
35 Hrynevich SV, Waseem TV, Hebert A, et al. beta-Hydroxybutyrate supports synaptic vesicle cycling but reduces endocytosis and exocytosis in rat brain synaptosomes. Neurochemistry international 2016; 93: 73-81.
36 Viggiano A, Pilla R, Arnold P, et al. Anticonvulsant properties of an oral ketone ester in a pentylenetetrazole-model of seizure. Brain research 2015; 1618: 50-54.
37 Hashim SA, VanItallie TB. Ketone body therapy: from the ketogenic diet to the oral administration of ketone ester. Journal of lipid research 2014; 55: 1818-1826.
38 Ciarlone SL, Grieco JC, D'Agostino DP, et al. Ketone ester supplementation attenuates seizure activity, and improves behavior and hippocampal synaptic plasticity in an Angelman syndrome mouse model. Neurobiology of disease 2016; 96: 38-46.
39 Chang P, Augustin K, Boddum K, et al. Seizure control by decanoic acid through direct AMPA receptor inhibition. Brain 2016; 139: 431-443.
40 Schroeder MA, Atherton HJ, Dodd MS, et al. The cycling of acetyl-coenzyme A through acetylcarnitine buffers cardiac substrate supply: a hyperpolarized 13C magnetic resonance study. Circulation. Cardiovascular imaging 2012; 5: 201-209.

Table 1: Relationship between KDT response at 3-month follow-up with biochemical parameters at baseline

	Non-responders (<50% seizure reduction)	**Responders (≥50% seizure reduction)**	**p-value**
Acetoacetate (mmol/L)	N=16 Median 0.03	N=18 Median 0.036	0.82
Glucose (mmol/L)	N= 47 Median 4.5	N= 60 Median 4.5	0.85
β-hydroxybutyrate (mmol/L)	N= 61 Median 0.07	N= 65 Median 0.09	0.028*
Glucose-ketone index	N=35 Median 84	N=41 Median 62.96	0.06
Non-esterified fatty acids (mmol/L)	N= 56 Median 0.303	N= 59 Median 0.4	0.40*
Free carnitine (µmol/L)	N= 70 Median 29.5	N= 72 Median 34	0.03*
Acetyl carnitine (µmol/L)	N= 56 Median 12.15	N= 56 Median 15.05	0.003*
Propionyl carnitine (µmol/L)	N= 39 Median 0.78	N= 50 Median 1.08	0.018
Butyryl carnitine (µmol/L)	N= 39 Median 0.22	N= 50 Median 0.25	0.70
Isovaleryl carnitine (µmol/L)	N=39 Median 0.16	N = 49 Median 0.17	0.48
Hexanoyl carnitine (µmol/L)	N=32 Median 0.06	N=44 Median 0.07	0.47
Octanoyl carnitine (µmol/L)	N=40 Median 0.075	N=50 Median 0.09	0.046
Tetradecenyl carnitine (µmol/L)	N=29 Median 0.07	N=40 Median 0.07	0.63
Palmitoyl carnitine (µmol/L)	N=31 Median 0.6	N=44 Median 0.8	0.02

*p-values derived from t-tests

Table 2: Relationship between KDT response at 3-month follow-up with biochemical parameters at 3-month follow-up

	Non-responders (<50% seizure reduction)	Responders (≥50% seizure reduction)	p-value
Acetoacetate (mmol/L)	N=10 Median 1.11	N=19 Median 1.06	0.61
Glucose (mmol/L)	N= 37 Median 3.7	N= 51 Median 3.8	0.91
β-hydroxybutyrate (mmol/L)	N= 59 Median 2.56	N= 65 Median 2.82	0.13*
Glucose-ketone index	N= 31 Median 1.27	N= 41 Median 1	0.17
Non-esterified fatty acids (mmol/L)	N= 53 Median 0.89	N= 61 Median 0.93	0.99*
Free carnitine (µmol/L)	N= 46 Median 29.5	N= 58 Median 38	0.054
Acetyl carnitine (µmol/L)	N= 44 Median 24.15	N= 49 Median 27.2	0.08
Propionyl carnitine (µmol/L)	N= 32 Median 0.51	N =43 Median 0.47	0.74
Butyryl carnitine (µmol/L)	N= 32 Median 0.28	N= 43 Median 0.27	0.49
Isovaleryl carnitine (µmol/L)	N=32 Median 0.16	N=43 Median 0.19	0.77
Hexanoyl carnitine (µmol/L)	N=28 Median 0.09	N=40 Median 0.10	0.75
Octanoyl carnitine (µmol/L)	N=32 Median 0.14	N=43 Median 0.12	0.53
Tetradecenyl carnitine (µmol/L)	N=27 Median 0.10	N=34 Median 0.095	0.47
Palmitoyl carnitine (µmol/L)	N=28 Median 0.80	N = 39 Median 1	0.049

*p-values derived from t-tests

Table 3: Relationship between KDT response at 3-month follow-up with the difference in biochemical parameters at baseline and 3-month follow-up

	Non-responders (<50% seizure reduction)	Responders (≥50% seizure reduction)	p-value
Acetoacetate (mmol/L)	N=8 Median 0.66	N=10 Median 1.02	0.20
Glucose (mmol/L)	N=23 Median -0.5	N=32 Median -0.8	0.84
β-hydroxybutyrate (mmol/L)	N=38 Median 2.2	N=45 Median 2.93	0.02
Non-esterified fatty acids (mmol/L)	N=35 Median 0.51	N= 42 Median 0.415	0.76
Free carnitine (µmol/L)	N=35 Median -2	N=42 Median 2.5	0.99
Acetyl carnitine (µmol/L)	N= 33 Median 10.6	N= 35 Median 11.9	0.54
Propionyl carnitine (µmol/L)	N =24 Median -0.31	N =32 Median -0.69	0.014
Butyryl carnitine (µmol/L)	N = 24 Median 0.075	N = 32 Median -0.015	0.09
Isovaleryl carnitine (µmol/L)	N=24 Median 0.04	N=31 Median 0.02	0.46
Hexanoyl carnitine (µmol/L)	N=22 Median 0.035	N=29 Median 0.03	0.89
Octanoyl carnitine (µmol/L)	N=25 Median 0.05	N=32 Median 0.05	0.99
Tetradecenyl carnitine (µmol/L)	N=21 Median 0.02	N = 27 Median 0.05	0.28
Palmitoyl carnitine (µmol/L)	N=21 Median 0.1	N=29 Median 0.1	0.87

Figure 1: Box and whisker plot of acetyl carnitine (µmol/L) at baseline for ketogenic diet responders and non-responders

The box and whisker plot shows the distribution of data for responders and non-responders divided into quartiles, highlighting the mean (x), median (-) and outliers

Figure 2: Acetyl carnitine and acetyl CoA, adapted from Figure 1 [40]