European Journal of Clinical Nutrition (2019) 73:961–965
https://doi.org/10.1038/s41430-018-0286-8

CASE REPORT

Ketogenic diet, a potentially valuable therapeutic option for the management of refractory epilepsy in classical neonatal nonketotic hyperglycinemia: a case report

Maina P. Kava[1,2,3] · Annie Robertson[4] · Lawrence Greed[5] · Shanti Balasubramaniam[2,3,6,7]

Received: 3 March 2018 / Revised: 2 July 2018 / Accepted: 27 July 2018 / Published online: 14 August 2018

Abstract

Nonketotic hyperglycinemia (NKH) is a devastating inborn error of glycine metabolism caused by deficient activity of the glycine cleavage enzyme. Classically, patients present with lethargy, hypotonia, myoclonic jerks, transient respiratory depression in the first week of life and often progress to death. Surviving infants have profound psychomotor retardation, refractory epilepsy and poor quality of life. Currently, no effective therapeutic avenues exist for severe NKH. Ketogenic diet (KD) has been trialled only in a small group of patients with neonatal NKH and early myoclonic encephalopathy, in whom significant improvements in seizure control were reported. We describe an infant with classical neonatal NKH who presented on the third day of life with hypotonia, poor feeding, respiratory insufficiency resulting in ventilatory support and seizures with burst-suppression pattern on electroencephalogram (EEG). KD initiated at age 6 months for intractable seizures, lead to a dramatic decrease in seizure frequency, EEG improvements, normalisation of plasma glycine levels, reduced spasticity and improved quality of life. KD may be a valuable treatment modality for refractory seizure control in classical NKH.

Introduction

Nonketotic hyperglycinemia (NKH) is a devastating autosomal recessive neurological disorder caused by defective glycine metabolism resulting in elevated amounts of glycine in body fluids, most notably in the central nervous system. The glycine cleavage system (GCS) comprises four protein components: P-, T-, H- and L-proteins, the first three of which are, respectively encoded by *GLDC* accounting for 70–75% of disease, *AMT* for approximately 20% of disease and *GCSH* for less than 1% of disease [1].

Glycine functions both as an excitatory and inhibitory neurotransmitter. Its neuromodulatory action with excitatory effect on cortical N-methyl-D-aspartate (NMDA) receptors causes intractable seizures. Conversely, activation of the glycinergic receptors in the brainstem and spinal cord result in inhibitory effects including recurrent apnoea, hiccups and diffuse hypotonia [2]. Biochemical diagnosis of NKH is suggested by elevated plasma and CSF glycine levels and abnormal CSF-to-plasma glycine ratio [>0.08 is consistent with typical NKH (reference values < 0.02), although mildly affected patients are described with ratios of 0.04–2.0] [1]. Definitive diagnosis is made by the demonstration of pathogenic mutations in the genes of GCS.

Phenotypic classification of the disease can broadly be categorised into classic and atypical or late-onset forms based on the onset and severity of presentation [3]. The majority of individuals present as neonates, typically manifesting as an acute encephalopathy with 85% displaying a severe form [profoundly affected developmental outcome

✉ Maina P. Kava
maina.kava@health.wa.gov.au

1 Department of Neurology, Princess Margaret Hospital for Children, Perth, WA, Australia

2 School of Paediatrics and Child Health, University of Western Australia, Perth, WA, Australia

3 Metabolic Unit, Department of Rheumatology and Metabolic Medicine, Princess Margaret Hospital, Perth, WA, Australia

4 Dietetics Department, Princess Margaret Hospital, Perth, WA, Australia

5 PathWest Laboratory Medicine WA, Princess Margaret Hospital, Perth, WA, Australia

6 Western Sydney Genetics Program, Children's Hospital at Westmead, Sydney, NSW, Australia

7 Discipline of Genetic Medicine, Sydney Medical School, University of Sydney, Sydney, NSW, Australia

and development quotient (DQ) less than 20], and 15% presenting with milder forms [1].

Patients with classical, severe neonatal NKH present in the first few days of life with hypotonia, progressive lethargy, seizures, hiccups, encephalopathy, recurrent apnoea and often progressing to death unless assisted ventilation is provided until restoration of spontaneous respiratory effort by age 3 weeks. Congenital brain malformations, including agenesis of the corpus callosum or retrocerebellar cyst with hydrocephalus have been reported in some patients [4]. Survivors almost universally have profound neurological sequelae, with severe disability, psychomotor retardation, seizure disorder with burst-suppression pattern that evolves into hypsarrhythmia, with or without multifocal spikes on electroencephalogram (EEG), quadriplegia, spasticity, irritability and poor quality of life. Of all children, 20% presenting in the neonatal period or infancy have a mild outcome, defined as DQ greater than 20 [1].

No effective treatment currently exists for severe NKH. Therapies aimed at decreasing glycine levels (sodium benzoate and moderate protein restriction) and blocking its effect at the NMDA receptor site (dextromethorphan, ketamine and felbamate) may improve seizure control, respiration and alertness in neonatal severe NKH; however, development of severe mental retardation and spasticity is not prevented, suggesting prenatal and ongoing postnatal glycine induced neurotoxicity [5]. Recently, Ketogenic diet (KD) trialled in a small cohort of patients has demonstrated improvement in seizure control and quality of life [6, 7]. We describe the dramatic response to KD in a male infant presenting with classical neonatal NKH and epileptic encephalopathy [8].

Case study

A male infant was born in good condition at full term following an uneventful pregnancy to a nonconsanguineous couple of Maori and Caucasian ancestry. He was discharged the subsequent day on exclusive breast feeds but represented 2 days later with poor feeding and lethargy. Recurrent apnoeas necessitated intubation and ventilation. A 20 mg/kg loading dose of intravenous phenobarbitone was administered for seizures with protracted hiccups that lasted up to ten minutes per episode, and myoclonic jerks. He was clinically encephalopathic and profoundly hypotonic with depressed reflexes and extensor plantars.

A diagnosis of classical NKH was promptly made based on amino acid quantification, which showed elevated levels of plasma glycine—2358 µmol/L (reference range: 150–560 µmol/L), CSF glycine—535.3 µmol/L (reference range: 3.8–8 µmol/L), and urine glycine—6370 µmol/mmol creatinine (reference range: <1097). The CSF: plasma glycine ratio was significantly elevated—0.23 (reference range: <0.02). An EEG performed on day four of life was markedly abnormal with a discontinuous background and burst-suppression pattern. Magnetic resonance imaging of the brain at age 5 days displayed a thin corpus callosum and at 2 weeks of age displayed in addition, areas of vacuolating myelinopathy involving the posterior limbs of the internal capsule, posterior corona radiata, cerebral peduncles and dorsal brainstem. A high glycine peak was demonstrated within the spectra at 3.56 ppm bilaterally within the cerebral white matter on magnetic resonance spectroscopy. *GLDC* gene sequencing and dosage analysis, using a custom-designed CGH array confirmed the diagnosis of NKH with the detection of 2 novel mutations observed in a compound heterozygous state: c.395C > T p.(Ser132Leu) in exon 3, and c.256-?_334þ?del p.(Ser86Valfs_119), resulting in an out-of-frame deletion of exon 2. Details of the mutational analysis have been described previously [8].

The parents deferred the decision to withdraw ventilatory support despite comprehending the poor long-term neurodevelopmental outcome and transient nature of respiratory depression in survivors of classical neonatal NKH. The patient was initially treated with sodium benzoate (300 mg/kg/day), dextromethorphan (5 mg/kg/day) and mild dietary protein restriction. Levetiracetam and clonazepam were subsequently added in view of recurrent myoclonic jerks. He soon developed spasticity, brisk reflexes and clonus. Regular, spontaneous respiratory effort was gradually gained, and he was extubated at 21 days, and discharged home at 33 days of life. KD was considered during the neonatal period, however due to social constraints, it was deferred.

Despite being on antiepileptic medications, at 6 months of age, he presented with epileptic encephalopathy with several seizure types including flexor spasms, multifocal clonic and myoclonic seizures and severe spasticity. The repeat EEG was markedly abnormal and showed multifocal spike discharges. Plasma glycine levels had also further risen despite dose increment of sodium benzoate to 510 mg/kg/day and continued moderate dietary protein restriction of 1.1 gm/kg/day (infant formula + carbohydrate module). Further increments of sodium benzoate were precluded due to associated reflux oesophagitis and possible gastritis. KD was attempted, however due to difficulties in maintaining appropriate weight gain and euglycaemia; it was temporarily ceased after 25 days, despite improvement in seizure control. Seizure frequency increased soon after discharge requiring emergency seizure medication with midazolam up to three times a day, and hospital readmission. Seizure control remained suboptimal despite antiepileptic medications which included levetiracetam, clonazepam and clobazam. KD was recommenced after 5 weeks. The caloric

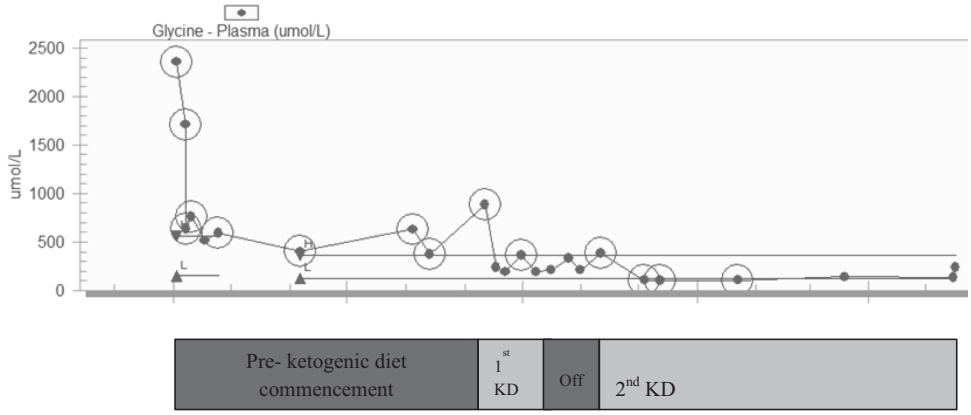

Fig. 1 Snapshot of Glycine levels over the course of treatment

and protein content of feeds were stringently titrated until optimal ketosis, glycaemic control and normal plasma glycine levels were attained (Fig. 1).

Significant reduction in seizure frequency occurred a week post-KD recommencement, whilst decrement in spasticity and amelioration of clonus, over the next couple of weeks. EEG displayed corroborating significant improvements in the background activity and reduction in epileptiform discharges. The patient was discharged on continuous nasogastric feeds of 3.5:1 ratio KD, comprising 8.5 g protein/day (1.8 g/kg/day), 77.9 g fat/day, (16 g/kg/day) and 13.7 g carbohydrate/day (3 g/kg/day). The feeds incorporated Ketocal, fat and carbohydrate modules (Calogen & Carb Plus), vitamin and mineral supplementation (Paediatric Seravit). After initiation of KD from 7 months to the current age of 3.5 years, his inpatient stay had significantly decreased. He had six brief hospitalisations, totalling 13 days, of which four were unrelated to primary seizure control and two for intercurrent infection related seizure exacerbation. No dosage increments of anti-convulsants were required. The patient has successfully stayed out of hospital for the last 15 months. Prior to the commencement of KD, the patient had four admissions for status epilepticus and a total of 85 days in hospital.

Currently, at 3.5 years of age, the patient has global developmental delay and mild-hypertonia, but no contractures. He coos and occasionally babbles. He rolls to both sides, but cannot sit. He smiles and interacts with his parents. Figure 2a–d describe his clinical improvement prior to commencement of KD at 6 months of age and sequentially post-second attempt. He displayed increased alertness, happier disposition and improved muscle tone. He receives regular dietary review and adjustments of caloric, protein intake and KD ratio based on his plasma glycine, weight, blood ketones and blood sugar levels. He remains nil by mouth, receiving his full nutritional requirements via percutaneous endoscopic gastrostomy. His KD ratio is 3:1 (9.3 g protein/d (1.7 g/kg/day), 88.6 g fat/day, 18.5 g carbohydrate/day). His weight *z*-score has improved from −5.53 on KD commencement to −3.03 at 3.5 years of age. Antiepileptic medications include levetiracetam and clobazam. His parents appreciatively report the profound impact KD had on enhancing his quality of life with reduction of seizure frequency, spasticity and hospital admissions.

Discussion

Currently, there is no effective treatment for severe NKH, even when initiated early. Oral sodium benzoate administration at doses of 250–750 mg/kg/day is aimed at reducing plasma glycine levels to low normal range (between 120 and 300 µmol/L) to observe beneficial effects, including increased wakefulness and enhanced seizure control in severe phenotypes. However, even early initiation of treatment with high doses of benzoate will not preclude the natural progression towards profound intellectual disability and seizure disorder in classical, severe NKH [1]. Protein or glycine-restricted diet assists in normalising plasma glycine levels in severely affected patients who require high doses of benzoate. NMDA receptor antagonists including dextromethorphan (5–15 mg/kg/day), ketamine, and felbamate have been used to improve seizure control. In our patient, refractory seizures persisted despite therapy with dextromethorphan, sodium benzoate, standard anti-convulsants and moderate dietary protein restriction. Hence, based on two previous studies describing the clinical effectiveness of KD in NKH [6, 7], we attempted this dietary intervention in our patient, with resultant normalisation of plasma glycine levels, dramatic reduction in seizures with associated improvements in EEG, decreased spasticity, hospital admissions and improved quality of life.

The KD, a high-fat, low-carbohydrate and protein restricted diet was developed in the 1920s to replicate the beneficial effects of fasting on seizure management by activating ketone body biogenesis through the substitution of carbohydrates with fats as a primary energy source. KD has long been used as a nonpharmacologic therapy for

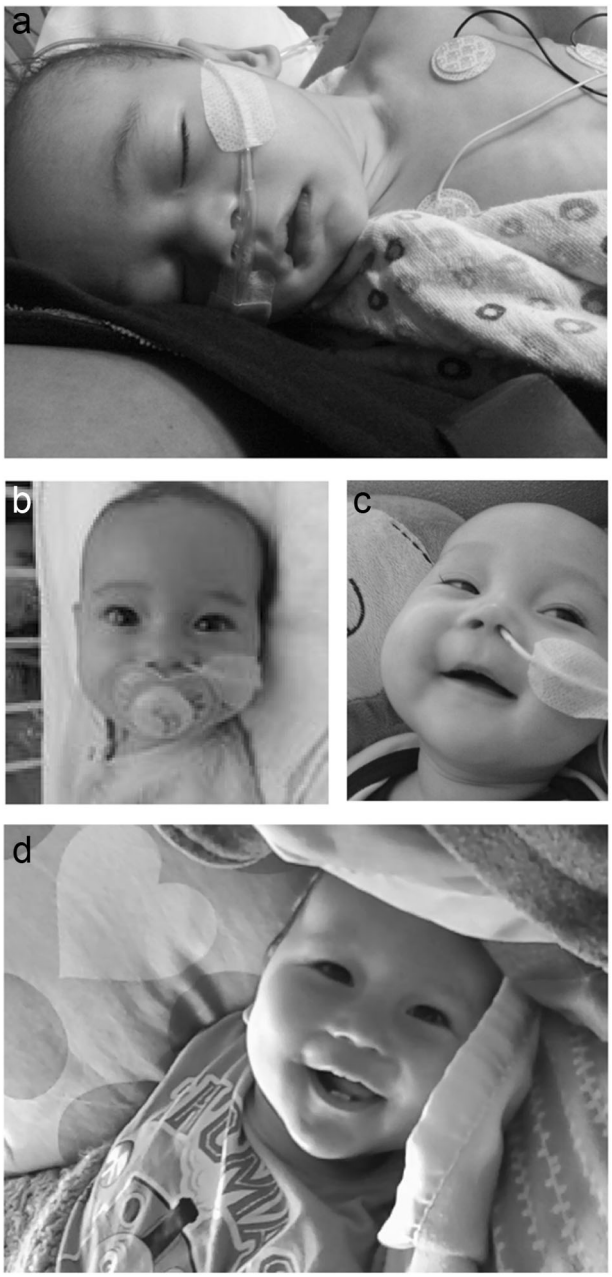

Fig. 2 a–d Serial images illustrating his clinical improvement from baseline (pre-KD) to post-KD intervention

intractable paediatric epilepsy [9]. KD has been recognised as first-line therapy in epilepsies secondary to inborn errors of metabolism including pyruvate dehydrogenase (E1) deficiency and glucose transporter protein 1 (GLUT1) deficiency. Positive responses have also been described in glycogenosis type V, phosphofructokinase deficiency and mitochondrial respiratory chain complex 1 disorders [6].

The mechanisms of the KD's efficacy in the treatment of epilepsy, is still not completely understood. A complex interplay of alternative biological pathways involving lactate, pyruvate, glucose, ketone bodies and amino acids may underlie the therapeutic potential of KD. The neuroprotective features of KD is explained by the dramatic enhancement of brain energy production through upregulation of several genes involved in energy metabolism (33 of 34 transcripts were up-regulated, of which 21 genes are involved in oxidative phosphorylation), mitochondrial biogenesis (approximately 46% increase in the density of neuronal mitochondria, with upregulation of 39 of 42 genes encoding mitochondrial proteins), and increasing energy reserves (stored as phosphocreatine). KD-induced energy generation modified amino acid metabolism through enhanced GABA production, and GABAergic inhibition, elevated the seizure threshold by stabilising the membrane potential in neurons, explaining the anti-convulsant effects [10].

Although the underlying pathophysiology of NKH has not been fully established, it has been postulated that the neurological damage observed in NKH may be attributed to glycine induced excitotoxicity. Glycine acts as a co-agonist on NMDA receptor, enhancing excitatory glutamate neurotransmission which may account for the intractable seizures. In addition, mitochondrial dysfunction has been implicated in the neuropathogenesis of brain injury in NKH [11]. In vitro studies of glycine toxicity in the brain of young rats have demonstrated severely impaired brain bioenergetics resulting from a cascade of deleterious intracellular effects due to NMDA receptor overstimulation, including impairment of the Kreb's cycle function due to inhibition of mitochondrial enzyme citrate synthase, reduced electron transport chain flow secondary to decreased respiratory chain enzyme activities and inhibition of mitochondrial isoform of creatine kinase and Na+, K+-ATPase [11]. In vivo intrastriatal administration of glycine in rats induced oxidative stress through decreased antioxidant defences and lipid peroxidation [12].

These findings suggest that KD and, possibly exogenous administration of ketone, in particular β-hydroxybutyrate, could be beneficial for the management of seizures secondary to metabolic abnormalities through its neuroprotective effect rather than a direct anti-convulsant mechanism, as proposed by an experimental, animal model of chronic ketosis [13]. KD, in combination with pharmacological therapy has successfully been attempted in four NKH patients from two reports, with marked effect on decreasing seizure frequency and severity, improved alertness and quality of life [6, 7].

In our patient, we observed a dramatic reduction in seizure severity and frequency, corroborated by improvements in EEG, plasma glycine normalisation, decreased spasticity and enhanced patients' and families' quality of life. Despite maintenance of seizure reduction, he continued to manifest a severe neurological phenotype, characterised by profound psychomotor delay, which may be due to intrauterine onset

of glycine induced neurotoxicity which was irreversible. Our experience corroborates the findings of previous reports in that KD may be a valuable treatment option for refractory seizure management in classical NKH, however, more robust studies are required to further validate its beneficial effects.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Van Hove J, Coughlin C II, Scharer G. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. *GeneReviews®* [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2017. Updated 11 July 2013.
2. Ohya Y, Ochi N, Mizutani N, Hayakawa C, Watanabe K. Non-ketotic hyperglycinemia: Treatment with NMDA antagonist and consideration of neuropathogenesis. Pediatr Neurol. 1991;7:65–68.
3. Hennermann JB, Berger JM, Grieben U, Scharer G, Van Hove JL. Prediction of long-term outcome in glycine encephalopathy: a clinical survey. J Inherit Metab Dis. 2012;35:253–61.
4. Swanson MA, Coughlin CR, Scharer GH, Szerlong HJ, Bjoraker KJ, Spector EB, et al. Biochemical and molecular predictors for prognosis in nonketotic hyperglycinemia. Ann Neurol. 2015;78:606–18.
5. Jaeken J, de Koning T, van Hove J. Disorders of GABA, glycine, serine and proline. In: Blau N, Duran M, Blaskovics ME, Gibson KM, editors. Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases. 2nd ed. Berlin: Springer; 2002. p. 123–40.
6. Cusmai R, Martinelli D, Moavero R, Dionisi Vici C, Vigevano F, Castana C, et al. Ketogenic diet in early myoclonic encephalopathy due to non ketotic hyperglycinemia. Eur J Pediatr Neurol. 2012;16:509–13.
7. Bzduch V, Behulova D, Kolnikova M, Payerova J, Fabriciova K. Ketogenic diet in nonketotic hyperglycinemia. J Inherit Metab Dis. 2010;33(S1):S31.
8. Nickerson SL, Balasubramaniam S, Dryland PA, Love JM, Kava MP, Love DR, et al. Two novel GLDC mutations in a neonate with nonketotic hyperglycinemia. J Pediatr Genet. 2016;5:174–80.
9. Nylen K, Likhodii S, Burnham WM. The ketogenic diet: proposed mechanisms of action. Neurotherapeutics. 2009;6:402–5.
10. Bough K. Energy metabolism as part of the anti-convulsant mechanism of the ketogenic diet. Epilepsia. 2009;49:91–93.
11. Busanello ENB, Moura AP, Viegas CM, Zanatta A, da Costa Ferreira G, Schuck PF, et al. Neurochemical evidence that glycine induces bioenergetical dysfunction. Neurochem Int. 2010;56:948–54.
12. Seminotti B, Knebel LA, Fernandes GC, Amaral AU, da Rosa MS, Eichler P, et al. Glycine intrastriatal administration induces lipid and protein oxidative damage and alters the enzymatic antioxidant defences in rat brain. Life Sci. 2011;89:276–81.
13. Samoilova M, Weisspapir M, Abdelmalik P, Velumian AA, Carlen PL. Chronic in vitro ketosis is neuroprotective but not anti-convulsant. J Neurochem. 2010;113:826–35.