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# Complementary dietary treatment using lysine-free, arginine-fortified amino acid supplements in glutaric aciduria type I — A decade of experience

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ABSTRACT

The cerebral formation and entrapment of neurotoxic dicarboxylic metabolites (glutaryl-CoA, glutaric and 3-hydroxyglutaric acid) are considered to be important pathomechanisms of striatal injury in glutaric aciduria type I (GA-I). The quantitatively most important precursor of these metabolites is lysine. Recommended therapeutic interventions aim to reduce lysine oxidation (low lysine diet, emergency treatment to minimize catabolism) and to enhance physiologic detoxification of glutaryl-CoA via formation of glutarylcarnitine (carnitine supplementation). It has been recently shown in *Gcdh*$^{-/-}$ mice that cerebral lysine influx and oxidation can be modulated by arginine which competes with lysine for transport at the blood–brain barrier and the inner mitochondrial membrane [Sauer et al., Brain 134 (2011) 157–170]. Furthermore, short-term outcome of 12 children receiving arginine-fortified diet showed very promising results [Strauss et al., Mol. Genet. Metab. 104 (2011) 93–106]. Since lysine-free, arginine-fortified amino acid supplements (AAS) are commercially available and used in Germany for more than a decade, we evaluated the effect of arginine supplementation in a cohort of 34 neonatally diagnosed GA-I patients (median age, 7.43 years; cumulative follow-up period, 221.6 patient years) who received metabolic treatment according to a published guideline [Kölker et al., J. Inherit. Metab. Dis. 30 (2007) 5–22]. Patients used one of two AAS product lines during the first year of life, resulting in differences in arginine consumption [group 1 (Milupa Metabolics): mean = 111 mg arginine/kg; group 2 (Nutricia): mean = 145 mg arginine/kg; $p<0.001$]. However, in both groups the daily arginine intake was increased (mean, 137 mg/kg body weight) and the dietary lysine-to-arginine ratio was decreased (mean, 0.7) compared to infants receiving human milk and other natural foods only. All other dietary parameters were in the same range. Despite significantly different arginine intake, the plasma lysine-to-arginine ratio did not differ in both groups. Frequency of dystonia was low (group 1: 12.5%; group 2: 8%) compared with patients not being treated according to the guideline, and gross motor development was similar in both groups. In conclusion, the development of complementary dietary strategies exploiting transport competition between lysine and arginine for treatment of GA-I seems promising. More work is required to understand neuroprotective mechanisms of arginine, to develop dietary recommendations for arginine and to evaluate the usefulness of plasma monitoring for lysine and arginine levels as predictors of cerebral lysine influx.



*Abbreviations:* 3-OH-GA, 3-hydroxyglutaric acid; AAS, amino acid supplement; ANOVA, analysis of variance; BBB, blood–brain barrier; CAT1, cationic amino acid transporter 1; GA, glutaric acid; GA-I, glutaric aciduria type I; GCDH, glutaryl-CoA dehydrogenase (EC 1.3.99.7); NO, nitric oxide; ORNT1 and 2, mitochondrial ornithine transporters 1 and 2; SDS, standard deviation score.

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## 1. Introduction

Glutaric aciduria type I (GA-I) is a rare cerebral organic acid disorder first described in 1975 [1]. The disease is caused by inherited deficiency of the homotetrameric mitochondrial flavoprotein glutaryl-CoA dehydrogenase (GCDH; EC 1.3.99.7), which is encoded by the *GCDH* gene mapping to human chromosome locus 19p13.2 [2]. More than 200 disease-causing mutations have been identified so far [3,4]. GCDH catalyzes the oxidative decarboxylation of glutaryl-CoA to crotonyl-CoA in the final degradative pathways of L-lysine, L-hydroxylysine, and L-tryptophan [5]. Deficiency of this enzyme results in accumulation of glutaryl-CoA, glutaric acid (GA), 3-hydroxyglutaric acid (3-OH-GA), and glutarylcarnitine. Natural protein consists of 2–9% lysine and 0.6–2% tryptophan and thus L-lysine is considered the quantitatively most important precursor for GA, 3-OH-GA, and glutarylcarnitine [6]. The concentrations of these compounds determined in body fluids of patients and body fluids and tissues of Gcdh-deficient mice, and fruit-eating bats showed a positive correlation to protein and lysine intake [1,7–11]. The initial clinical presentation of affected neonates is non-specific. The majority of untreated patients develop striatal injury acutely during catabolic conditions or insidiously between 3 and 36 months of age [7,12–14]. Striatal injury results in a complex movement disorder with predominant dystonia [15,16]. Antidystonic treatment is often unsatisfactory, and life expectancy is limited in severely disabled children [17,18]. The major aim of all therapeutic strategies is to prevent brain injury.

Several studies highlight the role of GA, 3-OH-GA, and glutaryl-CoA in the pathogenesis of this disease. Precipitation of excitotoxicity and oxidative stress, and impairment of cerebral energy metabolism via inhibition of the tricarboxylic acid cycle and the dicarboxylic acid shuttle between astrocytes and neurons are considered mechanisms [19–24]. In addition, disturbance of cerebral hemodynamics is thought to synergize with these mechanisms [25]. We have recently identified the blood–brain barrier (BBB) to play a central role by trapping intracerebrally produced neurotoxic dicarboxylates due to low BBB permeability [21,26–28]. Cerebral GA and 3-OH-GA concentrations of untreated patients are 100–1000fold higher than those in plasma. Strikingly, patients with complete loss of GCDH activity (i.e. high excreters) and those with residual enzyme activity (i.e. low excreters) reveal similar brain concentrations of GA and 3-OH-GA and share the same risk for brain injury [13,29–32]. *Gcdh*$^{-/-}$ mice, an animal model with complete loss of Gcdh activity [33], demonstrate the same steep plasma-to-brain gradient of neurotoxic dicarboxylates [21]. Based on these findings it has been suggested that lowering the cerebral lysine influx and lysine oxidation is a potential neuroprotective strategy.

In neonatally diagnosed patients, the frequency of brain injury was reduced to 0–36% compared to 90–95% in historical cohorts [7,12,18,34–37]. Evidence-based treatment recommendations include (1) a low L-lysine diet (including the use of lysine-free, tryptophan-reduced amino acid supplements [AAS]), (2) L-carnitine supplementation, and (3) emergency treatment during intercurrent illness [38,39]. Recently, we demonstrated that treatment according to the published guideline was associated with a favorable outcome (5% dystonia, n = 37 patients) in a newborn screening cohort [40]. In contrast, deviations from emergency treatment (100% dystonia, n = 6 patients) and metabolic maintenance treatment (i.e. low lysine diet and carnitine supplementation; 44% dystonia, n = 9 patients) were associated with a poor outcome.

In *Gcdh*$^{-/-}$ mice, the application of arginine, ornithine, and homoarginine all lowered the cerebral concentrations of GA and 3-OH-GA [41–43]. In analogy, the effect of low lysine diet was amplified by add-on therapy with arginine. This can be explained by competition of lysine and arginine at the BBB (cationic amino acid transporter 1, CAT1, which is one of three CATs that are called system $y^+$) and the inner mitochondrial membrane (mitochondrial ornithine carriers, ORNT1 and ORNT2) [43]. We have hypothesized that arginine supplementation might also be useful for optimizing dietary treatment in GA-I patients [44]. In fact, short-term outcome in 12 children receiving low lysine diet fortified with arginine supports this notion [45]. The major aim of this study is to specifically evaluate the effect of variation of dietary arginine intake on neurological outcome and concentrations of plasma amino acids in a prospectively followed newborn screening cohort.

## 2. Patients and methods

### 2.1. Study population

Thirty-eight children with confirmed diagnosis of GA-I who were identified by newborn screening between 2000 and 2011 and received metabolic treatment according to a published guideline [38,39] were evaluated. Their neurological outcome has been published previously [40]. Four children of this cohort who had not received either the amino acid mixture manufactured by Milupa Metabolics or Nutricia AAS were excluded from the analysis. Dietary, biochemical, anthropometrical, and neurological parameters were evaluated in the remaining 34 study patients. The study was approved by the local ethics committees of all study centers. Written informed consent was given by all parents of study patients.

### 2.2. Calculation of dietary arginine intake and lysine-to-arginine ratio

The arginine content of commercially available lysine-free, tryptophan-reduced, arginine-fortified AAS given to study patients was determined according to the manufacturer information (Table 1). SHS LT-AM infant had the same high arginine content (i.e. 90 mg arginine/g protein) as GlutarAde Junior GA-1 drink mix, an AAS previously investigated [45]. All other AAS applied to study patients had a lower arginine content (i.e. 48–59 mg arginine/g protein) which is similar to that of human milk (i.e. 51 mg arginine/g protein) [6]. AAS did not contain ornithine. The amount of AAS consumed was prospectively documented for all study patients from which the daily $\text{arginine}_{\text{AAS}}$ supplementation and the nutritional $\text{lysine-to-arginine}_{\text{AAS}}$ ratio were calculated. The effect of dietary treatment on biochemical and anthropometrical parameters was analyzed between ages 0 and 36 months, i.e. during the most vulnerable period for the development of striatal injury.

For breastfed infants (i.e. as long as human milk or adapted infant formula was the only natural protein source), we also calculated the total daily arginine intake along with other dietary and anthropometric parameters in analogy to a previous study [45]. When supplementary food is added, the amount of total arginine intake is dependent on the varying arginine content of natural foods (Suppl. Table 1). To estimate the total arginine intake and the nutritional $\text{lysine-to-arginine}_{\text{total}}$ ratio in infants and children receiving more than one natural protein source, we used standard dietary protocols at ages 8 months and 3 years for our calculations (Suppl. Tables 2 and 3). In addition, dietary intake was regularly adjusted every 3–6 months by specialized metabolic dietitians. Average lysine and arginine contents of natural foods were taken from "Bundeslebensmittelschlüssel" [6], a nutritional database provided by the German Ministry of Nutrition, Agriculture and Consumer Protection containing average contents of approximately 15,000 food items.

### 2.3. Calculation of standard deviation scores for anthropometrical parameters

Standard deviation scores (SDS) of anthropometrical data (height, weight, head circumference) were calculated using the LMS method [46]. This method provides a way of obtaining normalized growth centile standards values. Skewed distributions of the measurements are approximated to normal distributions by power transformation. The distribution of each covariate is summarized by three parameters, the Box–Cox power $\lambda$, the mean $\mu$, and the coefficient of variation $\sigma$.