Received: 9 December 2019 | Revised: 26 February 2020 | Accepted: 13 March 2020

DOI: 10.1002/jimd.12233

ORIGINAL ARTICLE

JIMD SSIEM WILEY

Impact of enteral arginine supplementation on lysine metabolism in humans: A proof-of-concept for lysine-related inborn errors of metabolism

Zoe Schmidt[1] | **Gayathri Murthy**[1] | **Madeleine Ennis**[1] | **Sylvia Stockler-Ipsiroglu**[1,2,3] | **Rajavel Elango**[1,4]

[1]BC Children's Hospital Research Institute, BC Children's Hospital, Vancouver, British Columbia, Canada

[2]Division of Biochemical Genetics, BC Children's Hospital, Vancouver, British Columbia, Canada

[3]Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada

[4]School of Population and Public Health, University of British Columbia, Vancouver, British Columbia, Canada

Correspondence
Rajavel Elango, Rm170A, 950 West 28th Avenue, BC Children's Hospital Research Institute, Vancouver, BC V5Z 4H4 Canada.
Email: relango@bcchr.ubc.ca

Sylvia Stockler-Ipsiroglu, K3-204-4480 Oak Street, Vancouver, BC V6H 3V4, Canada.
Email: sstockler@cw.bc.ca

Funding information
B.C. Children's Hospital Foundation

Communicating Editor: Ina Knerr

Abstract

Patients with lysine-related inborn errors of metabolism (pyridoxine-dependent epilepsy [PDE] and glutaric aciduria type 1 [GA1]), follow a lysine-restricted diet with arginine-fortified lysine-free amino acid formula and additional oral arginine supplementation as a newer therapy for PDE. The rationale of arginine supplementation is based on arginine's ability to compete with lysine transport across cell membranes via shared transporter systems. Adequate doses of arginine required to competitively inhibit enteral lysine uptake has not been studied in humans This proof-of-concept study investigates the effect of incremental enteral arginine doses on whole-body lysine oxidation using an in vivo stable isotope tracer, L-[1-^{13}C] lysine, in healthy humans. Five healthy men completed six study days each consuming one dose of L-arginine HCl per study day; range = 50-600 mg/kg/d. Lysine intake was at DRI (30 mg/kg/d). Breath samples were analysed for L-[1-^{13}C] lysine oxidation to $^{13}CO_2$ using an isotope ratio mass spectrometer. Plasma amino acid concentrations were analysed using an amino acid analyser. Increasing doses of L-arginine HCl caused a linear decrease in whole-body lysine oxidation. Plasma arginine concentration increased, and plasma lysine concentration decreased below normal range with high arginine intakes. We provide the first empirical evidence of arginine-lysine antagonism in response to increasing oral arginine doses. Results suggest 300-600 mg/kg/d of L-arginine HCl and lysine intake restricted to DRI is needed to reduce enteral lysine uptake and systemic lysine oxidation. This could potentially lead to a recommended dose for arginine in lysine-related inborn errors of metabolism.

Abbreviations: AAA, amino acid analyser; α-AASA, α-aminoadipic semialdehyde; ALFA, arginine-fortified lysine-free amino acid formula; L-Arginine HCl, L-Arginine hydrochloride; APE, atoms percent access; BBB, blood brain barrier; BIA, bioelectric impedance analysis; DRI, dietary reference intakes; F$^{13}CO_2$, rate of appearance of ^{13}C labelled carbon dioxide in breath; GA, glutaric acid; GA-1, glutaric aciduria type 1; 3-OH-GA, 3-hydroxyglutaric acid; PDE, pyridoxine-dependent epilepsy; PLP, pyridoxal 5′-phosphate; P6C, L-D1-piperidine-6 carboxylate; RDA, recommended dietary allowance; REE, resting energy expenditure; UCD, urea cycle defect.

KEYWORDS

vitamin B6–dependent epilepsy, inborn errors of metabolism, triple therapy, ALDH7A1 related pyridoxine dependent epileptic encephalopathy, medical nutrition therapy, stable isotopes

1 | INTRODUCTION

Lysine is an essential amino acid needed for the synthesis of tissue protein, collagen and carnitine.[1] In humans, lysine is catabolised via two separate pathways; the saccharopine pathway mainly located in the liver, and the pipecolic acid pathway mainly located in the brain.[2] Both pathways converge at the level of α-aminoadipic semialdehyde (α-AASA) and its cyclic equivalent piperidine-6-carboxylic acid (P6C), yielding glutaryl-CoA downstream the pathway and acetyl-CoA as the ultimate end product of the lysine degradation (Figure 1).

Among the various inborn errors of metabolism described within the lysine catabolic pathway, glutaric aciduria type 1 (GA1) caused by glutaryl-CoA dehydrogenase (*GCDH*) deficiency [OMIM 231670], and pyridoxine-dependent epilepsy (PDE) caused by α-aminoadipic semialdehyde dehydrogenase (*ALDH7A1*) deficiency [OMIM 266100], are amenable to medical nutrition therapy with a lysine restricted diet. This diet has been shown to improve clinical outcomes and to reduce the accumulation of potentially toxic metabolites arising from the respective enzyme defects.[3,4] Provision of abundant arginine through an arginine-fortified lysine-free amino acid formula (ALFA) has been suggested as an additional treatment modality for GA1[5] and additional oral supplementation of L-arginine has been promoted as a newer therapy for PDE.[6] The rational for these therapies is based on a proposed lysine arginine antagonism effecting their cellular uptake.[1]

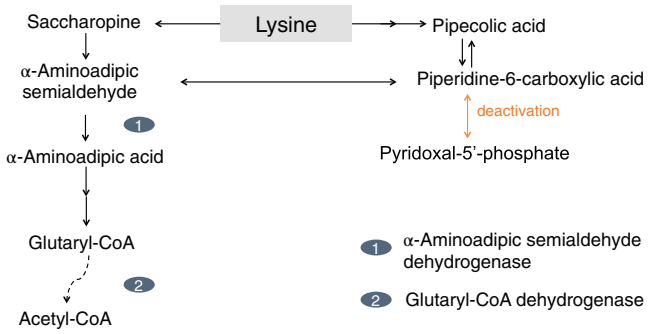

FIGURE 1 Lysine catabolic pathways. (1) Pyridoxine-dependent epilepsy (PDE) is characterised by ALDH7A1 encoding a-aminoadipic semialdehyde dehydrogenase, also known as antiquitin (ATQ), deficiency. Accumulation of α -aminoadipic semialdehyde (α-AASA)/piperidine-6 carboxylic acid (P6C) results in inactivation of pyridoxal phosphate (PLP). (2) Glutaric aciduria type I (GA1) is caused by deficiency of glutaryl-CoA dehydrogenase (GCDH) deficiency leading to accumulation of glutaric acid

Lysine and other basic cationic amino acids (CAA), such as arginine and ornithine, are transported across cell membranes via transport systems that are encoded by a variety of family 7 solute carrier (*SLC7*) genes.[7] System y^+ encoded by *SLC7A1*, *SLC7A2A*, *SLC7A2B*, and *SLC7A3* is the major route of entry for CAA into non-epithelial cells at the blood brain barrier (BBB)[8] and is selectively expressed at the basolateral membrane of epithelial cells.[7] Systems y^+L, $b^{0,+}$, and $B^{0,+}$ are expressed in epithelial cells of the small intestine and renal tubules, with system y^+L being primarily responsible for a high affinity transport across the apical membranes.[7] The lysine arginine antagonism has been proven in various animal experiments demonstrating a concentration dependent inhibition of enteral CAA uptake.[1,7]

In GA1, provision of ALFA and a lysine intake no higher than the DRI, has been associated with improved clinical outcomes in GA1 patients.[5] A similar regimen was shown to reduce neurotoxic metabolites in a GA1 mouse model.[9] In GA1, the ALFA itself provides the commonly recommended arginine dose of 100-150 mg/kg/d,[10] while in PDE, extra L-arginine supplements between 100 and 400 mg/kg/d have been given in addition to dietary lysine restriction and ALFA.[6,11-13]

Thus far, no studies have been conducted to examine to what extent increasing arginine supplements impact lysine metabolism in humans. There is no evidence of an ideal dose of arginine for treatment. Therefore, our objective was to examine, in the presence of lysine intakes at the age dependent estimated average requirements (Dietary Reference Intakes, DRI, 30 mg/kg/d), whether graded increases in L-arginine supplements (ranging from 50 to 600 mg/kg/d) had any impact on lysine catabolism. We achieved this by measuring changes in the oxidation of L-[1-^{13}C] lysine to $^{13}CO_2$, and plasma lysine concentrations in response to graded L-arginine intakes in healthy young men, as a proof-of-concept study.

2 | METHODS

2.1 | Subjects

Five adult males ranging in age 22-25 years (Table 1) were studied and participated in six study days each.

All subjects were selected after completing a preliminary study where medical history, dietary habits, and activity levels were collected; weight, height, body composition, and resting energy expenditure (REE) were measured. Participants were excluded from the study if they had the following: a history of smoking, chronic diseases/illnesses, a BMI outside normal range, and/or a vegetarian/vegan diet. Participants that took part in the preliminary study were assessed to ensure they could adhere to the diet and exercise protocols prior to each study day. Body composition was measured by bioelectrical impedance analysis (Quantum IV; RJL Systems, Detroit, Michigan). REE was measured by continuous, open-circuit indirect calorimetry (Vmax Encore, Viasys, California) performed on 10-12 hour fasted subject. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg using a digital scale.

Informed Consent: All subjects provided informed written consent before participating in the study. Subjects were offered financial compensation for their time at the end of each study day. All procedures were approved by the Research Ethics Board at the University of British Columbia Children's and Women's Research Ethics Board (H15-01151). The trial was registered at Clinical Trails.Gov (NCT02499926).

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

2.2 | Study principle

The study design was based on the oxidation of a stable isotope tracer L-[1-^{13}C] lysine to $^{13}CO_2$ to examine lysine metabolism in response to graded increases of oral arginine intake. The principle of the test is based on quantifying the amount of the carboxyl labelled ^{13}C tracer (L-[1-^{13}C] lysine) and its oxidation, which would release as $^{13}CO_2$ in breath. We have previously used this test either as an indirect measure of whole-body protein synthesis,[14] or to test the upper limit of oxidative capacity of essential amino acids.[15]

TABLE 1 Subject characteristics[1]

Characteristic	Value
Age (y)	23.6 ± 1.14
Weight (kg)	79.0 ± 11.9
Height (cm)	179 ± 6.6
BMI (kg/m^2)	24.5 ± 2.0
Lean body mass (kg)	59.7 ± 7.6
Fat mass (%)	24.2 ± 2.3
REE (kcal/day)	1768.4 ± 275.2

Notes: Values are mean ± SD (n = 5).
Abbreviations: REE, resting energy expenditure; BMI, body mass index.

2.3 | Experimental design

2.3.1 | Arginine doses

Study days took place at the Clinical Research Evaluation Unit at BC Children's Hospital, Vancouver. The subjects were fasted and had not participated in vigorous exercise for 10-12 hours prior to a study day. Each study day was separated by at least 2 weeks to eliminate any carry over effect. Subjects participated in six study days each, consuming one graded dose of L-arginine on each study day. Doses of L-arginine HCl (Ajinomoto Inc, Japan) were 50, 100, 150, 200, 250, 300, 400, 500, and 600 mg/kg/d. These doses correspond to 41, 82, 123, 164, 206, 247, 329, 411, 493 mg/kg/d of free L-arginine, due to the molecular mass of HCl, L-arginine HCl is ~17.8% heavier than L-arginine. Two subjects participated in 50, 100, 150, 200, 250, and 300 mg/kg/d studies. Three subjects participated in 100, 200, 300, 400, 500, and 600 mg/kg/d studies.

We used 50 mg/kg/d L-arginine HCl as the starting reference point to determine the dose of arginine that will competitively inhibit lysine uptake. For an adult weighing 79 kg (our study subject's average weight), the starting arginine dose will account to about 3.95 g/d of L-arginine HCl. The mean daily intake of arginine is 4.2 g/d reported from the 1993 to 1994 NHANES III. At the highest arginine dose, 600 mg/kg/d will account to 47.4 g/d for a 79 kg individual.

2.3.2 | Diet

Two days before the study day, subjects followed a prescribed standardised diet to stabilise protein intake (1.0 g/kg/d) and meet energy needs (1.7 × REE). The subjects' energy requirement was determined based on REE (kcal/d) measured at preliminary study assessment. During the preliminary study, a nutrition history was obtained via a 2-day food record to determine nutritional adequacy of macro and micronutrients and current lysine intake. The prescribed diet was tailored to each subject based on the 2-day food record. The subjects also provided a 2-day

food record prior to each study day which was used to measure compliance, determine protein intake, and better tailor the prescribed diet for the next study day.

2.3.3 | Study day diet

The diet on the study day consisted of one of the test L-arginine dose and all other amino acids based on the profile of egg protein. Amino acids were in the form of crystalline L-amino acids. Lysine was provided at 30 mg/kg/d on all study days. Protein was provided at 1.0 g/kg/d; energy was provided at 1.7 × REE (to ensure caloric needs are met), with 35% from fat, 55% from carbohydrate and 10% from protein. The study day diet was divided into eight meals in the form of a liquid 'shake' (Protein-Free Powder, PFD1, Mead Johnson Nutrition; Tang and Kool-Aid, Kraft Foods) and protein-free cookies. The eight meals were consumed hourly and represented 1/12 of the daily requirements. Subjects were not allowed to consume anything other than water throughout the study day. Thus, our study design represents responses to arginine doses in the presence of adequate nutrients and calories.

During a study day, subjects consumed 8 hourly complete meals. Each meal had one twelfth of the daily dose of arginine. This study design is to ensure that we reach steady state in isotope with the doses of arginine provided. Seven breath samples (three at baseline and four at isotopic steady state) and one blood sample were collected during each study day. VCO_2 measured by continuous, open-circuit indirect calorimetry (Figure 2) after the fifth meal was used to calculate rate of CO_2 production on each study day.

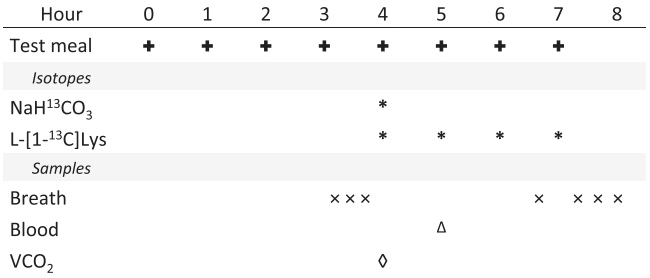

FIGURE 2 Study day protocol. The experimental complete diet (with supplemental arginine dose) was given as a test meal hourly for 8 hours. Lysine was provided at 30 mg/kg/d on all study days. Each meal was isocaloric and isonitrogenous and represented one-twelfth of each subject's daily nutrient requirement. Thus, our study represents responses to L-arginine HCl when a complete diet, with lysine restriction is followed. Priming doses of the isotopes $NaH^{13}CO_3$ and L-[1-^{13}C] lysine were given at the fifth meal; an hourly dose of L-[1-^{13}C] lysine was given simultaneously and continued throughout the remaining 4 hours. Baseline breath samples were collected before the tracer protocol began, and plateau breath samples were collected at isotopic steady state every 30 minutes, beginning 2.5 hours after the tracer protocol began. The carbon dioxide production rate (VCO_2) was measured by indirect calorimetry after the fifth hourly meal. Blood samples were collected between the fifth and sixth meal

2.3.4 | Isotope protocol

The isotopic labelled tracers were $NaH^{13}CO_3$ and L-[1-^{13}C] lysine:2HCL (verified 99% enrichment by Cambridge Isotope Laboratories, Woburn, Massachusetts). The oral tracer protocol began at the fifth meal while the four previous meals allowed for stabilisation of natural background ^{13}C, and collection of baseline breath samples (Figure 2). The subject's weight was measured at the beginning of each study day for isotope dose accuracy. Priming doses of $NaH^{13}CO_3$ (2.023 mmol/kg) and L-[1-^{13}C] lysine (6.36 mmol/kg) was given with the fifth meal. $NaH^{13}CO_3$ was only given during the fifth meal at the start of isotope infusion to achieve a whole-body steady state in $^{13}CO_2$ quickly.[16] L-[1-^{13}C] lysine isotope (3.6 mmol/kg) was given at the fifth meal and as a continuous dose with the remaining four meals. The priming and continuous doses of isotope were directly added to the liquid 'shake'. This primed-constant infusion of isotopes with hourly lysine intakes will ensure steady state isotope kinetics and will allow the calculation of the in-vivo metabolic capacity to catabolise lysine with graded increase in arginine intakes. The amount of L-[1-^{13}C] lysine given during the isotope tracer protocol was subtracted from the dietary provision of lysine to provide the total daily intake of 30 mg/kg/d.

2.4 | Sample collection and analysis

Breath samples were collected in disposable Exetainers (Labco Limited, United Kingdom) using a collection mechanism that allows the removal of dead-air space (QuinTron Instrument Company, Inc., Milwaukee, Wisconsin). Breath samples were stored at room temperature until analysed. Enrichment of ^{13}C in breath was analysed by multi-flow continuous-flow isotope ratio mass spectrometry (CF-IRMS IsoPrime100 Limited; Elementar Americas). All analyses were performed in triplicate. Enrichments were expressed as atoms % excess (APE) compared with a reference standard of compressed carbon dioxide gas. The rate of $^{13}CO_2$ released by L-[1-^{13}C] lysine tracer oxidation in breath samples was then calculated for each study day using the tracer kinetics equation:

$$F^{13}CO_2 = \left[\frac{FCO_2 \times ECO_2}{W}\right]\left[\frac{44.6 \times 60}{0.82 \times 100}\right]$$

where FCO_2 is the CO_2 production rate (mL/min); ECO_2 is the $^{13}CO_2$ enrichment in expired breath at isotopic steady state (APE); and W is the weight (kg) of the subject. The constants 44.6 (μmol/mL) and 60 (min/h) were used to convert FCO_2 to μmol/h. The factor 0.82 is the correction for CO_2 retained in the bicarbonate pool of the body in the fed state. A factor of 100 changes the APE to a fraction. CO_2 production was measured during each study day for 20 minutes with an indirect calorimeter (Vmax Encore, Viasys, California). $F^{13}CO_2$ is a rate expressed as μmol/kg/h.

Blood samples were collected at the Core Lab at BC Children's Hospital in heparinised tubes. Blood samples were stored on ice promptly until centrifuged at 3000*g* at 4°C for 10 minutes and the separated plasma stored at −80°C. Plasma free amino acids concentrations were determined by ion exchange chromatography with post-column ninhydrin derivatization using an Amino Acid Analyser (AAA) (Hitachi L8900, Tokyo, Japan) using a modified procedure as previously described.[17] Fifty microliters of plasma was added to 100 μL of 6% TCA and let stand for 5 minutes, followed by centrifugation for 15 minutes at 8000*g* at 4°C. The resulting supernatant was removed and 20 μL was injected into the AAA and amino acids separated using an ion exchange column (Hitachi Packed Column #2622 6.0 × 40 mm Li Type, Tokyo, Japan). Plasma amino acids were separated and analysed against an amino acid standard mix (Sigma, St. Louis, Missouri). The areas under the peaks were integrated using the EZChrom Elite software (version 3.3.2 SP2; Agilent, Ontario, Canada).

2.5 | Statistical analysis

Subject characteristics are expressed as the mean ± SD. The effect of graded arginine intake on lysine oxidation as $F^{13}CO_2$ was tested using linear regression analysis (GraphPad Prism 8). Plasma amino acid concentrations (mean ± SD) on each study day, in response to graded arginine intakes were tested using a linear regression analysis (GraphPad Prism 8). Significance was set at $P \leq .05$.

3 | RESULTS

3.1 | Subject characteristics

Characteristics of the five subjects who participated in the study are shown in Table 1. All participants were healthy adult men and within normal range values for BMI and body composition.

3.2 | L-[1-^{13}C] lysine oxidation

The primary outcome of lysine oxidation, which is presented as a fraction of the label tracer oxidised ($F^{13}CO_2$, μmol/kg/h) is shown in Figure 3. Whole-body lysine oxidation decreased significantly ($P = .0005$) with a negative slope ($y = -0.0006x + 0.7302$, $R^2 = 0.36$) with increasing intakes of arginine.

3.3 | Plasma concentrations of arginine and lysine in response to oral L-arginine supplementation

Plasma arginine concentrations (μmol/L) (Figure 4) significantly increased with increasing intakes of arginine ($y = 0.4698x + 55.67$, $R^2 = 0.85$, $P = <.0001$). Plasma lysine concentrations (μmol/L) (Figure 4) significantly decreased with increasing intakes of arginine ($y = -0.1043x + 163.1$, $R^2 = 0.31$, $P = .0017$).

4 | DISCUSSION

Using in-vivo stable isotope labelling of lysine in healthy young adult males, we could show that increasing doses of enteral L-arginine HCl were associated with a decrease

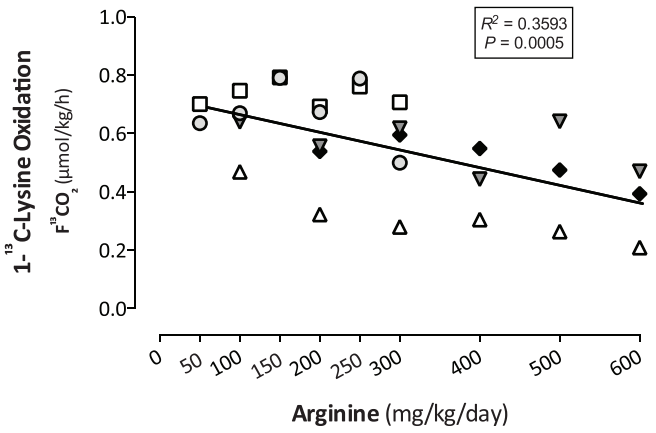

FIGURE 3 Oxidation of L-[1-^{13}C] lysine in response to graded oral supplements of L-arginine HCl. Five (n = 5) participants with individual study day oxidation values (N = 30) are presented. Two subjects participated in 50, 100, 150, 200, 250, and 300 mg/kg/d studies. Three subjects participated in 100, 200, 300, 400, 500, and 600 mg/kg/d studies. Oxidation of ^{13}C-Lysine decreased significantly ($P = .0005$) with increasing arginine intakes

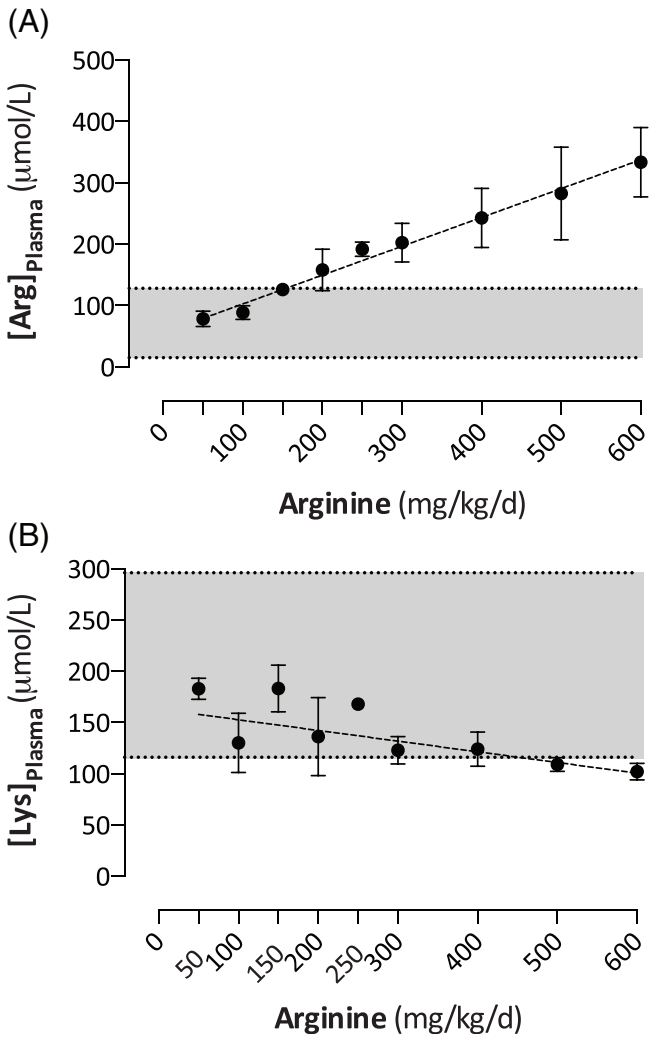

FIGURE 4 Plasma concentrations of arginine, A, and lysine, B, in response to graded oral supplements of L-arginine HCl. Values are presented as means ± SD; n = 2-5, per mean. Shaded areas within the dashed lines represent normal plasma concentration ranges

in whole-body lysine oxidation and plasma lysine concentrations as well as an increase in plasma arginine concentrations. While the reduced oxidation rates indicate a reduced availability of lysine for catabolic degradation at the systemic level, the concomitant changes in plasma lysine and arginine concentrations are suggestive of a reduced lysine and an increased arginine uptake in the gut. To achieve lysine arginine antagonism strong enough to result in plasma lysine levels lower than normal range, L-arginine dosages as high as 400-600 mg/kg/d were needed which produced plasma arginine concentrations as high as 250-350 μmol/L.

Importantly, the observed changes occurred at a lysine intake at the DRI (30 mg/kg/d for young male adults), which is significantly lower than the average intake of healthy males who consume 5.3 g of lysine per day (70 mg/kg/d in a 75 kg male). However, the intake of lysine provided to the subjects in the current study falls within the degree of lysine restriction as recommended for GA1 and PDE[3,18]:30-45 mg/kg/d of lysine for >7 years, 45-80 mg/kg/d for 1-7 years, 70-100 mg/kg/d for <1 year of age.[4,18]

The current recommendation for arginine intake in the treatment of GA1 is based on theoretical calculations of lysine arginine antagonism at the BBB in the presence of a diet providing arginine and lysine at a ratio of 0.5-0.8.[5] Such a ratio is provided when infants with GA1 receive their protein requirements via a combination of breastmilk and an ALFA containing 90 mg/g protein of arginine (vs 34 mg/g in breast milk) and providing 119-137 mg/kg/d of total arginine (vs 51-54 mg/kg/d with breast milk alone).[5] Because of empirical benefits in preventing encephalopathy, provision of ALFA along with a lysine restricted diet is considered to provide sufficient arginine without additional arginine supplements being required for the nutritional management of GA1.[10]

While the recommended arginine intake for GA1 patients is considerably lower than the intake needed to reduce lysine oxidation in our study, the amounts of arginine recommended for PDE patients comes closer to our findings. Administration of 150 mg/kg/d of L-arginine, along with arginine from natural food and ALFA resulted in a total arginine intake of 230 and 355 mg/kg/d total arginine in six patients published by Coughlin et al[6] In combination with a lysine-restricted diet, these amounts of L-arginine resulted in a reduced accumulation of lysinergic metabolites such as α-AASA and P6C in blood and urine.[6] Similar effects were observed in other studies with L-arginine supplementation at 100-400 mg/kg/d.[6,11-13,19,20] An improved clinical and biochemical outcome was even observed in a 12-year old PDE patient who began L-arginine supplementation at 400 mg/kg/d (15 g/d), but neither followed a lysine restricted diet nor took ALFA.[20]

The safety profile of L-arginine supplements has been established mainly through their use in the treatment of various metabolic disorders. Doses of 200-400 mg/kg IV L-arginine HCl administered as boluses over 90-120 minutes, and similar dosages given orally, have been safe in the acute and chronic treatment of UCDs.[21] In MELAS intravenous and oral L-arginine HCl dosages at 500 mg/kg IV have been safely used in the treatment and prevention of metabolic strokes.[22] Finally, based on long term use in adult probands, dosages of 30-40 g/d (equivalent to 400-600 mg/kg/d in the average male)[23-25] were shown to be safe, while gastrointestinal side effects, diarrhoea mainly, were found when similar amounts of L-arginine were given as boluses. Based on this evidence, we are confident that the dosages needed to induce lysine arginine antagonism in our probands were within

the safety limits. However, for patients who may be consuming supplements much longer than the reported study periods our results needs to be interpreted with caution. The recent guidelines for management of urea cycle disorders,[21] recommends maintaining plasma arginine concentrations under 120 μmol/L, which is much higher than the plasma values observed in our study. It will be important to monitor patients on arginine therapy with routine measurement of plasma amino acids, ammonia and urea to prevent toxicity.

In natural protein, arginine occurs as DL-racemic compound. L-Arginine is the biologically active isomer and supplements used for medical purposes are available as purified L-arginine product on prescription. Arginine as a nutritional supplement is mostly available as L-arginine HCl because of its higher stability compared to the free amino acid. In our study we used L-arginine HCl to achieve our doses of 50, 100, 150, 200, 250, 300, 400, 500, and 600 mg/kg/d, which correspond to 41, 82, 123, 164, 206, 247, 329, 411, and 493 mg/kg/d of free L-arginine. Given the molecular mass of HCl, L-arginine HCl is ~17.8% heavier than L-arginine provided as a free amino acid. Readers must be aware of these differences when comparing earlier studies, as studies differ in the supplement forms.

The following considerations should be taken into account in the interpretation of our results. First, in GA1 and PDE the primary site of pathophysiology is localised in the brain and toxic metabolites produced by the catabolism of lysine directly in the brain is considered the major offending mechanism. In our experimental setting we could determine the effects of L-arginine HCl supplementation on whole-body lysine arginine antagonism, but we were not able to selectively determine its effects at the BBB. Whether the antagonism occurs at the brain level needs to be explored in future. Second, in our study, L-arginine HCl dosages as high 400-600 mg/kg/d were needed to effectively reduce lysine oxidation at a lysine intake limited to the DRI. It is likely that much higher dosages of arginine would be needed to achieve a similar effect in the presence of higher lysine intakes, and individuals need to be monitored for arginine toxicity.[21] We conducted a pilot study (unpublished) using a lysine intake at 75 mg/kg/d. Based on a reported habitual consumption of 5.3 g of lysine per day in North America,[26] this lysine intake represents the average intake of healthy adult males weighing 75 kg. At the increased lysine intake, arginine supplementation at 300 mg/kg/d was not sufficient to induce a reduction in lysine oxidation.

Another limitation of our results is that our study was conducted in a well-controlled repeated measure design following an acute dosing strategy, while the effects of chronic arginine dosing (as used in clinical practice) might be different. In our study setting, arginine was provided in multiple dosages during an 8 hour-test period to achieve steady state in isotope kinetics, which is also different from real life practice where arginine is taken at larger doses, but with longer intervals. Finally, the results of our study refer to healthy male adults and therefore cannot be generalised to a paediatric patient population, women or to patients with PDE and GA1.

In the future, we plan to use this experimental design to study lysine oxidation in response to a therapeutic dose of arginine and concomitant lysine restriction in patients with PDE. Additional assessment of biochemical markers α-AASA in plasma and urine will provide clinical evidence on the efficacy of arginine supplementation on reduction of metabolite accumulation. Ultimately, findings will inform ideal dosing of arginine for patients with PDE.

ACKNOWLEDGMENTS

We thank all the participant volunteers who participated in the study. We thank all nurses and phlebotomists who collected blood samples. This work was supported in part by funding from the B.C. Children's Hospital Foundation (first Collaborative Area of Innovation) to S. S.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Zoe Schmidt performed the study, analysed data, and wrote the manuscript. Gayathri Murthy wrote the Research Ethics Board application and performed parts of the study. Madeleine Ennis performed sample analysis. Sylvia Stockler-Ipsiroglu initiated the research, contributed to manuscript writing and edited the manuscript. Rajavel Elango developed the study protocol, supervised the study and the data analysis, and edited the manuscript.

ORCID

Rajavel Elango https://orcid.org/0000-0002-9380-1725

REFERENCES

1. Ball RO, Urschel KL, Pencharz PB. Nutritional consequences of interspecies differences in arginine and lysine metabolism. *J Nutr*. 2007;137(6):1626S-1641S.
2. Pink DBS, Gatrell SK, Elango R, et al. Lysine α-ketoglutarate reductase, but not saccharopine dehydrogenase, is subject to substrate inhibition in pig liver. *Nutr Res*. 2011;31(7):544-554.
3. Boy N, Mühlhausen C, Maier EM, et al. Proposed recommendations for diagnosing and managing individuals with glutaric aciduria type I: second revision. *J Inherit Metab Dis*. 2017;40(1):75-101.
4. van Karnebeek CDM, Hartmann H, Jaggumantri S, et al. Lysine restricted diet for pyridoxine-dependent epilepsy: first

evidence and future trials. *Mol Genet Metab*. 2012;107(3):335-344.
5. Strauss KA, Brumbaugh J, Duffy A, et al. Safety, efficacy and physiological actions of a lysine-free, arginine-rich formula to treat glutaryl-CoA dehydrogenase deficiency: focus on cerebral amino acid influx. *Mol Genet Metab*. 2011;104(1):93-106.
6. Coughlin CR, van Karnebeek CDM, Al-Hertani W, et al. Triple therapy with pyridoxine, arginine supplementation and dietary lysine restriction in pyridoxine-dependent epilepsy: neurodevelopmental outcome. *Mol Genet Metab*. 2015;116(1):35-43.
7. Fotiadis D, Kanai Y, Palacín M. The SLC3 and SLC7 families of amino acid transporters. *Mol Aspects Med*. 2013;34(2):139-158.
8. O'Kane RL, Viña JR, Simpson I, Zaragozá R, Mokashi A, Hawkins RA. Cationic amino acid transport across the blood-brain barrier is mediated exclusively by system y+. *Am J Physiol Endocrinol Metab*. 2006;291(2):E412-E419.
9. Sauer SW, Opp S, Hoffmann GF, Koeller DM, Okun JG, Kölker S. Therapeutic modulation of cerebral L-lysine metabolism in a mouse model for glutaric aciduria type I. *Brain*. 2011;134(1):157-170.
10. Kölker S, Boy SPN, Heringer J, et al. Complementary dietary treatment using lysine-free, arginine-fortified amino acid supplements in glutaric aciduria type I—a decade of experience. *Mol Genet Metab*. 2012;107(1):72-80.
11. Al Teneiji A, Bruun TUJ, Cordeiro D, et al. Phenotype, biochemical features, genotype and treatment outcome of pyridoxine-dependent epilepsy. *Metab Brain Dis*. 2017;32(2):443-451.
12. Yuzyuk T, Thomas A, Viau K, et al. Effect of dietary lysine restriction and arginine supplementation in two patients with pyridoxine-dependent epilepsy. *Mol Genet Metab*. 2016;118(3):167-172.
13. Mahajnah M, Corderio D, Austin V, et al. A prospective case study of the safety and efficacy of lysine-restricted diet and arginine supplementation therapy in a patient with pyridoxine-dependent epilepsy caused by mutations in ALDH7A1. *Pediatr Neurol*. 2016;60:60-65.
14. Elango R, Ball RO, Pencharz PB. Indicator amino acid oxidation: concept and application. *J Nutr*. 2008;138(2):243-246.
15. Elango R, Rasmussen B, Madden K. Safety and tolerability of Leucine supplementation in elderly men. *J Nutr*. 2016;146(12):2630S-2634S.
16. Chapman KP, Elango R, Ball RO, Pencharz PB. Splanchnic first pass disappearance of threonine and lysine do not differ in healthy men in the fed state. *J Nutr*. 2013;143(3):290-294.
17. Boucher JL, Charret C, Coudray-Lucas C, Giboudeau J, Cynober L. Amino acid determination in biological fluids by automated ion-exchange chromatography: performance of Hitachi L-8500A. *Clin Chem*. 1997;43(8):1421-1428.
18. van Karnebeek CDM, Stockler-Ipsiroglu S, Jaggumantri S, et al. Lysine-restricted diet as adjunct therapy for pyridoxine-dependent epilepsy: the PDE consortium consensus recommendations. *JIMD Rep*. 2014;15:47-57.
19. Coci EG, Codutti L, Fink C, et al. Novel homozygous missense mutation in ALDH7A1 causes neonatal pyridoxine dependent epilepsy. *Mol Cell Probes*. 2017;32:18-23.
20. Mercimek-Mahmutoglu S, Cordeiro D, Cruz V, et al. Novel therapy for pyridoxine dependent epilepsy due to ALDH7A1 genetic defect: L-arginine supplementation alternative to lysine-restricted diet. *Eur J Paediatr Neurol*. 2014;18(6):741-746.
21. Häberle J, Boddaert N, Burlina A, et al. Suggested guidelines for the diagnosis and management of urea cycle disorders. *Orphanet J Rare Dis*. 2012;7(1):32.
22. Ganetzky RD, Falk MJ. 8-year retrospective analysis of intravenous arginine therapy for acute metabolic strokes in pediatric mitochondrial disease. *Mol Genet Metab*. 2018;123(3):301-308.
23. Cynober L, Bier DM, Kadowaki M, Morris SM, Elango R, Smriga M. Proposals for upper limits of safe intake for arginine and tryptophan in young adults and an upper limit of safe intake for Leucine in the elderly. *J Nutr*. 2016;146(12):2652S-2654S.
24. McNeal CJ, Meininger CJ, Wilborn CD, Tekwe CD, Wu G. Safety of dietary supplementation with arginine in adult humans. *Amino Acids*. 2018;50(9):1215-1229.
25. Rosenthal MD, Carrott PW, Patel J, Kiraly L, Martindale RG. Parenteral or enteral arginine supplementation safety and efficacy. *J Nutr*. 2016;146(12):2594S-2600S.
26. Institute of Medicine. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*. Washington, DC: The National Academies Press; 2005 https://doi.org/10.17226/10490.

How to cite this article: Schmidt Z, Murthy G, Ennis M, Stockler-Ipsiroglu S, Elango R. Impact of enteral arginine supplementation on lysine metabolism in humans: A proof-of-concept for lysine-related inborn errors of metabolism. *J Inherit Metab Dis*. 2020;1–8. https://doi.org/10.1002/jimd.12233