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RESEARCH REPORT

Large neutral amino acids in daily practice

Kirsten Kiær Ahring

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Abstract At the Kennedy Centre for Phenylketonuria, Denmark, large neutral amino acids (LNAAs) are being used to treat adult and adolescent patients who are nonadherent to dietary treatment for phenylketonuria (PKU). At the start of treatment, a patient must undergo dietary analysis and regular blood sampling to measure plasma amino acid (AA) concentrations. The aim of this analysis and treatment is that the patient receives 25–30% of the daily protein requirement from LNAA supplementation and the remaining 70–75% from natural, low-phenylalanine proteins (although some patients have difficulties in maintaining this level of protein intake). Patients are therefore able to follow a more "normal" diet than those adhering to a PKU diet with AA supplementation (in which only 20% of the daily protein requirement is provided from the diet and 80% from AA supplementation). LNAAs have also been used to treat older patients with untreated/late-diagnosed PKU who show profound intellectual, psychological, and behavioral impairments. Treatment with LNAAs has been shown to improve measures of concentration and awareness of external stimuli in some of these patients and thus enhance their socialization, emotionality, frustration tolerance, and mood.

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K. K. Ahring (✉)
Kennedy Institute, Centre for PKU,
Gl. Landevej 7,
2600 Glostrup, Denmark
e-mail: kka@kennedy.dk

Abbreviations

AA	Amino acid
LNAA	Large neutral amino acid
OMIM	Online Mendelian Inheritance in Man (database)
Phe	Phenylalanine
PKU	Phenylketonuria
t.i.d	Three times a day

Introduction

The Kennedy Centre for Phenylketonuria, Denmark, has a strong tradition of research into phenylketonuria (PKU; OMIM 262600) and has always placed patient care at the forefront of that research. This includes early investigations into the benefits of treatment with large neutral amino acids (LNAAs) (Andersen and Avins 1976), the effects of tyrosine (Tyr) and/or tryptophan supplementation (Lou 1985; Lou et al. 1987), and the effects of dietary discontinuation on brain neurotransmitter levels (Güttler and Lou 1986). L–phenylalanine (L-Phe) is an LNAA that enters and exits parenchymal cells via an L–type sodium (Na^+)-independent carrier used by other LNAAs (Scriver 2007). This transporter facilitates the passage of L-Phe into the brain through the blood–brain barrier. The rationale for use of LNAAs is that these molecules compete with Phe for transporters across the blood–brain barrier, and therefore, large concentrations of the other LNAAs in the blood reduce the uptake of Phe into the brain (Smith et al. 1987). In particular, Tyr and tryptophan are transported into the brain, restoring levels of monoamine neurotransmitters and improving performance on neuropsychological tests (Güttler and Lou 1986). In addition

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to LNAAs blocking the influx of excess Phe into the brain in PKU, LNAAs may also promote protein synthesis (Pietz et al. 1999).

Management of PKU patients involves placing them on a low-Phe diet that restricts the intake of high-protein foods. The main goal of treatment is to maintain blood Phe concentration within safe limits (120–360 μmol/L from 0–3 years of age in Denmark; Giovannini et al. 2007) in order to improve neuropsychological performance, prevent neurologic deterioration, and reverse key biochemical abnormalities (Scriver and Kaufman 2001). However, low-Phe food and amino acid (AA) supplements should be used in combination with a low-Phe diet to fulfil nutritional requirements. The safety and efficacy of LNAA therapy for PKU in a diet containing defined quantities of Phe and LNAA supplements was shown to reduce ambient hyperphenylalaninemia (Matalon et al. 2006) by 50%. These findings suggest that supplementary therapy with LNAAs might improve treatment outcomes for PKU. Tablets and powders comprising LNAAs enriched with Tyr and tryptophan are available for treating PKU.

Use of LNAAs in daily practice

At the Kennedy Centre, a wide variety of patients are treated for PKU, ranging from babies and children to adolescents and adults and including pregnant women, as well as patients with untreated/late-diagnosed PKU. A total of 354 patients with early-diagnosed PKU and 50 with late-diagnosed PKU are currently being treated. At the centre, LNAAs are not offered to children or pregnant women but are considered appropriate for treating adults over the age of 18 years, adolescents with poor dietary adherence, and those with untreated/late diagnosed PKU. The centre has experience with the long-term use of PreKUnil® LNAA tablets (Korsør, Denmark) and the short-term use of NeoPhe® LNAA powder (Korsør, Denmark). Of the known 151 adult patients (>18 years old), 80 have chosen LNAAs as an alternative to a low-protein diet, 37 follow the diet, and 34 are out of contact.

Transferring the patient's treatment from a low-protein diet with AA supplementation to LNAA treatment is relatively straightforward. The patient attends the clinic for an initial dietary interview and analysis. At the start of LNAA treatment, weekly blood samples are taken to assess Phe and Tyr levels. Phe levels up to 1,500 μmol/l are allowed. This weekly sampling period can be increased to every 3 weeks after some time, and once patients have been stabilized on LNAA therapy, they are followed up every 3 months at either clinic visits or by telephone. Patients are all tested during childhood and early adult hood by a neuropsychologist as follows: Bayleys test at age 1, Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R) at age 4½, Wechsler Intelligence Scale for Children-III (WISC-III) at 6,10, and 14 years, and Wechsler Adult Intelligence Scale III (WAIS-III) at age 22. There is no difference between testing of LNAA users and PKU patients on the diet. All patients on LNAA are tested from the beginning of treatment on a regular basis. However, results have not been published.

An individual's recommended daily protein intake is 1 g/kg of ideal body weight. It is recommended that LNAAs comprise 25–30% of a patient's daily protein requirement and natural protein from food to make up the remaining 70–75%. If it is not possible for patients to take in enough natural protein with low Phe levels to meet their daily requirement, then using an AA supplement can fulfil part of the daily protein requirement. In comparison, patients adhering to a low-protein diet with AA supplementation will receive 80% of their protein from these AAs and 20% from natural protein in the diet. All patients receive the same mixture of LNAA. The minimum dosage of LNAA is ten tablets taken with main meals three times a day (t.i.d.) for patients who weigh 50–60 kg. The number of tablets prescribed increases as patient weight increases, up to 15 tablets t.i.d for patients weighing >90 kg. Most patients have no problems swallowing the amount of tablets. No side effects have been reported. However, a few patients with classic PKU have returned to a regular low-protein diet and AA supplement because they in general felt better on the diet. The composition of LNAA tablets and powder are shown in Table 1.

Table 1 Composition of large neutral amino acid (LNAA) tablets and powder used at the Kennedy Centre in Denmark

	Tablets (100g tablets)	Powder (100g)
Energy (kJ)	1146	1318
Protein equivalent (g)	55.8	60.0
Amino acids (g)	68.0	73.5
Carbohydrate (g)	14.1	18.7
Fat (g)	1.4	0
Vitamin B_6 (mg)	6.3	7.35
Vitamin B_{12} (μg)	11.3	13.2
Biotin (mg)	N/A	0.14
Folic acid (μg)	N/A	0.95

Product information taken from http://www.prekulab.com accessed prior to Jan 2009; composition information may have changed since date of access

N/A not available

Practical use of LNAAs: case 1

This 20-year-old woman had classic PKU, with plasma Phe levels between 120–1500 μmol/L, and weighed 55 kg. As she was studying to become a nurse and training for a marathon, she preferred LNAA treatment, which allowed her freedom in lifestyle. Analysis of her diet revealed that her total protein intake was 43.8 g, of which 25.8 g (59%) was from food and 18 g (41%) from LNAAs. Thus, her daily protein intake was only 80% of the recommended intake for her weight and was made up of a larger proportion of LNAAs than recommended. To rectify this protein deficit, she increased her intake of natural proteins to 34.4 g (largely by increasing her daily intake of meat) and used daily AA supplementation in addition to LNAAs. This increased her daily total protein intake to 69.4 g, which was more than the recommended daily requirement. In this way she was able to meet her daily protein requirements without restricting her diet or activities.

Practical use of LNAAs: case 2

This 22-year-old man had mild PKU, with plasma Phe levels in the range of 120–1,500 μmol/L. He had an active lifestyle and a high level of daily activity from his job as a lifeguard and from sporting activities, but he had gained 10 kg (increasing from 69–79 kg) between clinic visits. He enjoyed eating meat and had a daily protein intake of 166.9 g, of which 18 g (11%) was from LNAAs and 148.9 g (89%) from food. This ratio of natural protein to LNAA intake was too high, and his dose of LNAA was too low as a result of his weight gain. He was also following a diet that was too high in meat and other natural proteins (e.g., regular milk) and too low in fruit and vegetables. Adjustment of LNAA dosage and changes to his diet, including restriction of meat intake and replacing regular milk with low-protein milk, produced beneficial changes in his protein intake. After these changes, the patient was eating a total of 110.5 g protein each day, of which 19.5% (18%) was from LNAAs and 91 g (82%) was from food.

Untreated/late-diagnosed PKU patients

Patients with previously untreated and/or late-diagnosed PKU can also benefit from a low-Phe diet. For these individuals, the main therapeutic goals are improvement in psychological well-being, behavioral difficulties, and health. However, because of the difficulties associated with dietary restriction in these patients, alternatives to low-protein diets have been sought. In 1976, screening in Denmark revealed 110 individuals with late-diagnosed PKU (Güttler and Wamberg 1977). About 50 of these patients are still alive, aged between 43 and 75 years, but geographical distance to the clinic and difficulties in drinking AA supplements and adhering to low-protein diets have been barriers to their effective treatment. To date, only 20 of these patients are receiving LNAA treatment.

In a double-blind crossover study conducted in 2000–2001, 19 patients (12 men and seven women) with late-diagnosed or untreated PKU were given LNAAs for 6 months and placebo for 6 months (Kalkanoglu et al. 2005). All patients had severe intellectual disability and were aged between 38 and 85 years. They received their usual diet in addition to LNAAs or placebo based on body weight. Seven of 16 patients (44%) showed significantly improved concentration during treatment with LNAAs, and ten of the 16 (63%) were more aware of external stimuli. The main areas of improvement shown by these patients were in socialization, emotionality, frustration tolerance, and mood. However, five of the 19 patients showed no improvement while taking LNAAs, and no significant improvements were seen in their motor or daily-living skills.

Conclusions

LNAAs are easy for patients to use and allow them to eat a somewhat free diet (consuming 70–75% of their daily protein requirement as natural proteins). In comparison with a low-protein diet, LNAAs present greater convenience for patients in maintaining a social life, and normal activities such as school, work, sports, and holidays, and consequently are more likely to be associated with patient adherence than are low-protein diets. One disadvantage for some patients is the requirement to obtain 70–75% of daily protein from natural food, as some find it difficult to maintain this level of low-Phe protein intake and have to eat more protein-rich food. Future research is required to determine the long-term benefits and consequences of LNAA use in comparison with a low-protein diet and AA supplementation. In order to maintain commitment to patients with PKU receiving the best possible treatment, a realistic approach to the “diet for life” is required, in addition to learning from other clinicians’ experiences and retaining an open mind when considering alternative products or approaches to treating patients with this condition.

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