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REVIEW

Large neutral amino acids supplementation in phenylketonuric patients

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Summary Phenylketonuria is an inborn error of amino acid metabolism that results in severe mental retardation if not treated early and appropriately. The traditional treatment, consisting of a low-phenylalanine diet, is usually difficult to maintain throughout adolescence and adulthood, resulting in undesirable levels of blood phenylalanine and consequent neurotoxicity. The neurotoxicity of phenylalanine is enhanced by its transport mechanism across the blood-brain barrier, which has the highest affinity for phenylalanine compared with the other large neutral amino acids that share the same carrier. The supplementation of large neutral amino acids in phenylketonuric patients has been showing interesting results. Plasma phenylalanine levels can be reduced, which may guarantee important metabolic and clinical benefits to these patients. Although long-term studies are needed to determine the efficacy and safety of large neutral amino acids supplements, the present state of knowledge seems to recommend their prescription to all phenylketonuric adult patients who are non-compliant with the low-phenylalanine diet.

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Abbreviations

BBB	blood-brain barrier
BH_4	tetrahydrobiopterin
LNAA	large neutral amino acids
PAH	phenylalanine hydroxylase
Phe	phenylalanine
PKU	phenylketonuria

Introduction

Phenylketonuria (PKU; OMIM # 261600) is an inborn error of amino acid metabolism first described in 1934 (Centerwall and Centerwall 2000; Matalon 2001). Eleven years later, Penrose suggested that the chemical explanation for the disease was hyperphenylalaninaemia (Scriver and Kaufman 2001). The hepatic hydroxylation of phenylalanine (Phe) into tyrosine is catalysed by the enzyme phenylalanine hydroxylase (PAH; L-phenylalanine-4-monooxygenase), which uses tetrahydrobiopterin (BH_4; (6*R*)-L-erythro-5,6,7,8-tetrahydrobiopterin) as a cofactor (Scriver and Kaufman 2001). Hyperphenylalaninaemia can result from mutations in the gene encoding PAH, in the loci for one of the three enzymes in the pathway for BH_4 biosynthesis, or in the loci for 4α-carbinolamine dehydratase or dihydropteridine reductase (DHPR), the two enzymes responsible for the regeneration of BH_4 from the oxidized form (Scriver and Kaufman 2001). BH_4 is also important as a cofactor for the hydroxylation of

L-tryptophan and L-tyrosine, and for nitric oxide synthase activity (Walter et al 2006) (Fig. 1).

PAH deficiency may be classified according to plasmatic Phe concentrations when patients are ingesting a normal protein-containing diet (Walter et al 2006). Considering the neonatal screening values of Phe plasma levels and the Portuguese Newborn Screening Commission, the disease severity can be classified into hyperphenylalaninaemia (blood Phe between 3 and 6 mg/dl), mild PKU (blood Phe between 6 and 20 mg/dl) and classical PKU (blood Phe higher than 20 mg/dl) (Rocha et al 2007). Normal blood Phe levels lie between 0.6 and 2 mg/dl (Hanley 2004; Scriver and Kaufman 2001). The overall global prevalence in screened populations is approximately 1:12 000 (Walter et al 2006), with a prevalence of 1:10 914 in Portugal (Rocha et al 2007).

The clinical presentation of untreated patients shows a progressive and irreversible neurological impairment, with low IQ levels. The clinical signs in untreated patients also include a mousy odour, eczema, reduced pigmentation, reduced growth and microcephaly. In these patients, it is also probable to find behavioural problems such as hyperactivity, purposeless movements, stereotypy, aggressiveness, anxiety and social withdrawal (Walter et al 2006).

Treatment of PKU

Phe levels within fluid compartments in the body are a result of a balance between diet, endogenous catabolism of proteins and conversion of Phe to other metabolites (Scriver and Kaufman 2001). The main objective of the traditional and most efficacious strategy for PKU treatment (Bickel et al 1953) is to maintain blood Phe levels below 6 mg/dl until 12 years of age, and below 8 mg/dl from that age onwards (Rocha et al 2007). During the early years of life, dietary Phe restriction is successful but, as the children get older, the dietary adherence is poorer (Finkelson et al 2001; Walter et al 2002). In some centres, about 60% or more of adolescents and adults are not following the dietary treatment. Interruption of treatment results in blood Phe levels rising, with such undesirable consequences as poor school performance, impairment of executive function, loss of IQ and deterioration of white matter in the brain (Burgard et al 1997; Fisch et al 1995; Griffiths et al 1995; Pietz et al 1998; Ris et al 1994; Schmidt et al 1994; Thompson et al 1990). One of the main reasons for discontinuation of treatment may be the taste and the quantity of the amino acid mixtures that usually must be prescribed to these patients.

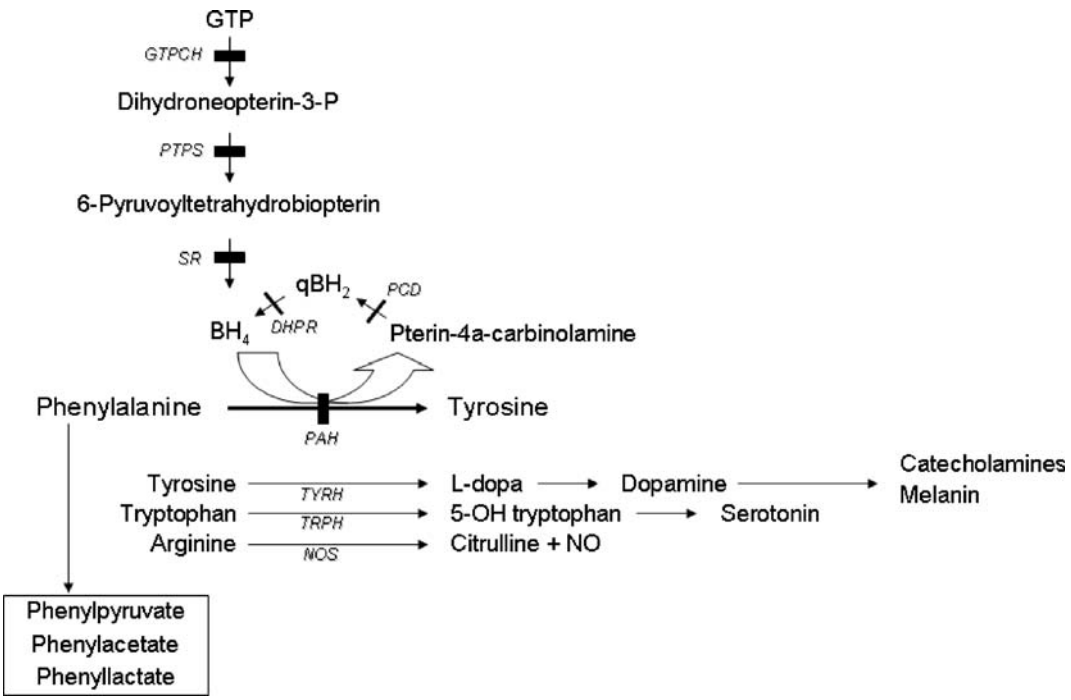

Fig. 1 Phenylalanine metabolism. BH_2, dihydrobiopterin; BH_4, tetrahydrobiopterin; *DHPR*, dihydropteridine reductase; GTP, guanosine triphosphate; *GTPCH*, guanosine triphosphate cyclohydrolase; NO, nitric oxide; *NOS*, nitric oxide synthase; P, phosphate; PAH, phenylalanine hydroxylase; PCD, pterin-4a-carbinolamine dehydratase; *PTPS*, pyruvoyl-tetrahydrobiopterin synthase; SR, sepiapterin reductase; *TRPH*, tryptophan hydroxylase; *TYRH*, tyrosine hydroxylase. The enzyme deficiencies are represented as solid bars across the arrows. Adapted from Walter et al (2006)

As desirable metabolic control is not easily achieved in these patients, the need for different strategies of treatment is imperative. In a minority of patients, PKU is due to a deficiency in one of the enzymes involved in the synthesis or recycling of its BH_4 cofactor. These patients must thus be treated with an oral administration of BH_4 in order to normalize their blood Phe levels (Scriver and Kaufman 2001). Since 1999 (Kure et al 1999), the number of reports of BH_4 responsiveness in PAH-deficient PKU patients has been increasing, expanding important knowledge for the elaboration of guidelines to evaluate its responsiveness and use in PKU treatment (Levy et al 2007). In Portugal, we hope that it will be soon possible to test and treat at least some PAH-deficient patients with sapropterin dihydrochloride (synthetic BH_4), allowing them to have a more liberal diet, apparently with very limited secondary effects (Burlina and Blau 2009; Lee et al 2008). Another strategy that has recently appeared in the field of nutritional treatment of PKU is the use of glycomacropeptide in the production of special foods and beverages (Ney et al 2009), which apparently has promising benefits regarding nutritional status in PKU (van Calcar et al 2009). All these strategies are of particular importance for the future treatment of PKU patients because, at the moment, other therapeutic strategies, such as gene therapy or treatment with Phe ammonia-lyase (a non-mammalian enzyme that degrades Phe), are not yet available (Sarkissian et al 2009).

Transport of Phe across the blood-brain barrier

Although PAH deficiency occurs at the hepatic level, the clinical effects of hyperphenylalaninaemia are on brain development and function (Scriver and Kaufman 2001). The blood-brain barrier (BBB) is formed by the brain capillary endothelial cells (Hawkins et al 2006). This barrier is made through the peculiar arrangement of these endothelial cells, characterized by a great number of tight junctions between them (Brightman and Reese 1969); thus, the BBB blocks the paracellular movement and also prevents the exchange of intrinsic proteins and lipids between the luminal (blood-facing) and abluminal (brain-facing) domains (van Meer and Simons 1986; van Meer et al 1986). Transport of large neutral amino acids (LNAA; Phe, leucine, tyrosine, tryptophan, threonine, isoleucine, valine, methionine and histidine) across the BBB is mediated by the large neutral amino acid transporter 1 (LAT1) (Hawkins et al 2006). LAT1 (SLC7A5) was first cloned in 1998 (Kanai et al 1998; Mastroberardino et al 1998). It consists of 507 amino acids with a molecular weight of ~55 kDa (Kanai et al 1998; Mastroberardino et al 1998), and is an amino acid exchanger with 1:1 stoichiometry (del Amo et al 2008). This facilitative transport system exists on both the luminal and abluminal membrane of brain endothelial cells (Sanchez del Pino et al 1992, 1995) and is sodium-independent (Smith 2000). Considering the competition between LNAA for LAT1 and the different affinity of this transporter for different LNAA, blood ratios of these LNAA and the individual affinity of LAT1 for each of them determine their net uptake from blood into the brain. Phe has the highest affinity for the system, with a K_m (half-saturation concentration in the absence of competitors) of 11 μmol/L (Smith 2000). Thus, high plasma Phe concentrations impairs uptake of the other LNAA (the branched-chain amino acids, tyrosine and tryptophan) into the brain (Scriver and Kaufman 2001). On the other hand, brain efflux of some small neutral amino acids (alanine, serine and cysteine) (Ohtsuki and Terasaki 2007) and also of LNAA seems to occur by a sodium-dependent mechanism with energy consumption (O'Kane et al 2004; O'Kane and Hawkins 2003). Therefore, the brain has access to all the neutral amino acids through the facilitative system LAT1 and has the ability to adjust the composition of its extracellular fluid by a sodium-dependent system (Hawkins et al 2006; Ohtsuki and Terasaki 2007).

Consequences of PKU for the transport across the BBB

Brain Phe concentration results mainly from its transport across the BBB (McKean 1972), explicable by the greater affinity of the LNAA carrier for Phe than for the other LNAA (Pietz et al 1999). The primary consequence of increased blood Phe is increased brain Phe. The second consequence, resulting from the competition that exists between Phe and all the other LNAA for transport at the BBB level (LAT1-mediated), is a disturbance in brain uptake of the other LNAA (mainly the branched-chain amino acids tyrosine and tryptophan) (Scriver and Kaufman 2001). Brain availability of these amino acids can be seriously disturbed by a modest supraphysiological plasma Phe concentration (in the range of 200–500 μmol/L to 3.3–8.3 mg/dl) (Pardridge 1998). Indeed, assessment of BBB permeability to Phe and leucine using a double indicating method at different plasma Phe concentrations in normal volunteers and PKU patients clearly shows a reduction in the net uptake of LNAA in PKU patients (Knudsen et al 1995). The imbalance caused by excess

plasmatic Phe will affect brain tryptophan availability (Fernstrom and Wurtman 1972) with a concomitant reduction in brain serotonin synthesis (Burlina et al 2000; Surtees and Blau 2000), as well as brain tyrosine availability, which certainly results in reduced dopamine, noradrenaline and adrenaline biosynthesis (Puglisi-Allegra et al 2000). As already indicated, BH_4 is the essential cofactor of Phe, tyrosine and tryptophan monooxygenases (Surtees and Blau 2000). Thus, despite the normal bioavailability of BH_4 in patients with classical PKU, the increased brain concentrations of Phe will promote a competitive inhibition of brain tyrosine and tryptophan monooxygenases, resulting in a diminished production of catecholamines and serotonin, respectively (Surtees and Blau 2000). An additional consequence of the impaired LNAA transport across the BBB is perturbed protein synthesis that has been observed in animal models (Binek-Singer and Johnson 1982; McKean et al 1968). Moreover, high brain Phe concentrations also cause a reduction in brain weight, cell number and deoxyribonucleic acid levels, possibly as a consequence of some secondary undernutrition due to the unavailability of total essential amino acids at the brain level (Hoeksma et al 2009; Huether et al 1983; Surtees and Blau 2000). Finally, the abnormalities in myelinization that worsen the clinical picture of PKU patients appear to be a combination of increased myelin turnover and decreased myelin production, both appearing secondary to an increase in brain Phe concentrations (Surtees and Blau 2000). All these factors contribute to the pathophysiology of the neurological impairment seen in PKU, especially in non-treated patients or in those who were late-diagnosed. In summary, it seems that a high brain Phe concentration is not the only factor responsible for the brain dysfunction seen in PKU patients (van Spronsen et al 2009).

Interestingly, the impact of plasma Phe concentrations on brain Phe content is not the same in all patients with PKU. There is individual variability/susceptibility related to the transport of LNAA across the BBB that is not clearly explained by the genotype (Pardridge 1998). This finding helps to explain why, despite having significantly elevated plasma Phe levels, some patients show normal or low brain Phe concentrations and normal executive functions (Weglage et al 2001, 2002). Intellectual outcome seems to be more closely related to brain Phe levels than to plasma Phe levels (Brumm et al 2004; Koch and Guttler 2000; Koch et al 2000; Moats et al 2000, 2003; Moller et al 2000). With this in mind, and considering that we should always emphasize the treatment of the patient rather than of the plasma Phe level, it is crucial to maintain an open window for new perspectives in the treatment of PKU patients.

LNAA supplementation in the treatment of PKU patients

The role of LNAA and aspects related to their transport into the brain in PKU patients has been an issue since 1953, with the work of Christensen, who proposed that high blood concentrations of Phe could interfere with the transport of the other LNAA into the brain (Christensen 1953). However, it was only 30 years later that branched-chain amino acid supplementation (with valine, isoleucine and leucine) was shown to result in a near 21% reduction of cerebrospinal fluid-serum ratio of Phe, with no reported amino acid imbalances (Berry et al 1982). A few years later, a LNAA therapy for adult PKU patients non-compliant with the Phe-restricted diet was proposed for the first time. This study was developed at the John F. Kennedy Institute in Denmark, and apparently had a good outcome (Lou et al 1994). One year later, Dotremont and colleagues (Dotremont et al 1995) published a study of LNAA supplementation, for one month, in four PKU patients. A daily supplement of 0.8 g of LNAA (lysine-free) per kg body weight, together with a low protein diet (0.6 g/kg per day), led to a negative nitrogen balance due to lysine deficiency. It was concluded that the formulation was not adequate to treat PKU patients (Dotremont et al 1995). As mentioned already, it should not be forgotten that the main objective of dietary treatment of PKU patients is the prevention of neurological damage. Thus, the previous studies were important but could not clearly show any advantage in terms of reduction of brain Phe concentrations. The possibility of detecting brain Phe concentrations by proton magnetic resonance spectroscopy (^{1}H MRS) was of crucial importance, considering the need to find a way to quantify the real efficacy of LNAA mixtures (Kreis et al 1995; Novotny et al 1995). With the possibility of measuring the brain influx of Phe by ^{1}H MRS (Pietz et al 1995), it was finally confirmed that LNAA have the ability to block Phe transport into the brain (Pietz et al 1999). The work of Pietz and colleagues involved giving an oral purified L-Phe dose (100 mg/kg body weight), with or without LNAA, and measuring the influx of Phe into the brain (Pietz et al 1999). The LNAA mixture was composed of 150 mg/kg body weight of each of the following amino acids: valine, methionine, isoleucine, leucine, tyrosine, histidine and tryptophan. Despite the increased plasma Phe concentrations observed in response to the Phe challenge, cerebral Phe concentrations remained unchanged, or even decreased, during the concomitant LNAA mixture administration. Although, as the authors argued,

there could be an increased efflux of brain Phe in the presence of the LNAA mixture, the most plausible mechanism involved was certainly a block in Phe brain influx caused by the LNAA mixture. The same work raised again the question whether young adult patients off diet might profit from the continuing intake of amino acid mixtures. Indeed, in most countries, including Portugal, the concept of "diet for life" is adopted (Rocha et al 2007).

Considering that in the work of Pietz and colleagues (Pietz et al 1999) only adult male PKU patients were included, and that its results were found after an initial challenge with Phe, it was important to see whether LNAA supplementation could also be of interest for patients on a normal diet pattern. In six patients (four female and two male) on a relaxed diet (average protein intake ranging from 0.6 to 1.0 g/kg per day), the ingestion of LNAA tablets (PreKUnil, Nilab, Denmark), at 0.4 g/kg of body weight, resulted in slightly lower blood Phe concentrations after one and six months of therapy, and in a gradual decline in brain Phe concentrations. On the other hand, an increase on blood tyrosine and tryptophan levels was observed (Koch et al 2003). The PreKUnil tablets used were comprised L-forms of tyrosine, tryptophan, methionine, isoleucine, threonine, valine, leucine, histidine and arginine (Koch et al 2003). Similarly to the results presented by Pietz and colleagues (1999), the increased blood LNAA concentrations (mainly tyrosine and tryptophan) possibly had an inhibitory effect of brain Phe influx, shown by the severe reduction in brain Phe concentrations over the six months of the study (Koch et al 2003). The authors of this study added another positive benefit from the better brain amino acid profile achieved. They suggested that the higher blood tyrosine and tryptophan concentrations could reduce the risk of depression in PKU patients, usually attributed to diminished dopamine and serotonin brain levels (Blows 2000; Guttler and Lou 1986; Puglisi-Allegra et al 2000).

Although the benefits for brain Phe concentrations were clear, blood Phe levels seem not to be particularly influenced by the ingestion of LNAA mixtures. Accordingly, a new formulation with a different amino acid pattern (NeoPhe, Prekulab, Korsor, Denmark) was tested in 11 patients with PKU (Matalon et al 2006). These new tablets had all the amino acids present in preKUnil, but with changes in their concentrations, plus added lysine (Matalon et al 2006). The inclusion of lysine, while retaining arginine, seems to be important considering that these two amino acids use the same carrier at the gastrointestinal level (Hidalgo and Borchardt 1990). The daily administration of LNAA supplements (from 0.5 to 1 g/kg body weight) resulted in a 52–55% average reduction of plasma Phe concentrations, probably as a result of the competition between Phe and the other LNAA for absorption at the gastrointestinal level (Matalon et al 2006). However, the reduction in plasma Phe concentrations could also be the result of a higher degree of amino acid anabolism, or could result from the fact that the lower amount of natural protein available allowed a better competitive effect of LNAA. Nevertheless, these results emphasized the importance of LNAA supplements also for controlling blood Phe concentrations, even though that study did not measure its effects on brain Phe levels (Matalon et al 2006).

Even with these encouraging results (Matalon et al 2006), the need for results from a double-blind placebo control trial was unequivocal. Patients from six centres were recruited in order to test the effect of LNAA supplementation (taken daily at 0.5 g/kg body weight) on blood Phe levels. In all of the 20 patients studied, blood Phe concentrations dropped significantly while on LNAA supplements. The average blood Phe reduction with LNAA supplements was of 39%, which was considered statistically significant, when compared with the decline of about 5.4% of the placebo trial (Matalon et al 2007).

Considering the beneficial effect of LNAA supplementation on plasma Phe levels, there was a great wish to find positive correlations between blood and brain Phe concentrations. However, and similarly to previous works (Brumm et al 2004; Koch and Guttler 2000; Koch et al 2000; Moats et al 2000, 2003; Moller et al 2000), there was no positive correlation between blood and brain Phe levels when plasma Phe concentration was below 1200 μmol/L (20 mg/dl), in a very recent report (Schindeler et al 2007). Once again, the results confirmed the interest in LNAA supplementation in the regulation of plasma Phe levels, most probably because of the competitive absorption of LNAA at the intestinal mucosa (Broer 2008). The competitive intestinal absorption of LNAA was verified using both rabbit and mouse intestinal tissue (Karasov et al 1986; Munck and Munck 1994; Stevens et al 1982) and human intestinal epithelial Caco-2 cells, where Phe and other LNAA were shown to compete for transport from the apical to the basolateral membrane (Berger et al 2000).

It is important to appreciate that, although some of the present results are encouraging, at the moment LNAA cannot be used during pregnancy. This strategy, therefore, does not apply to maternal PKU cases.

Finally, it should be mentioned that all the work done in the field of LNAA supplementation in PKU

(Table 1) may also be of special interest in the treatment of other inherited metabolic diseases (e.g. maple syrup urine disease, tyrosinaemia and homocysteinuria) (Matalon et al 2006).

Conclusions

Although treatment of PKU patients with LNAA supplements has shown interesting results, this strategy still raises some controversial questions. At the moment, it is recommended only for non-compliant adult PKU patients who are not following the dietary treatment. In these patients, the potential reduction of plasma Phe levels may be of particular importance. However, for individuals already complying with the diet and amino acid mixtures, LNAA supplementation is of limited value. In the meantime, the future utility of LNAA treatment must be considered, mainly because the concept of "diet for life" is adopted by several treatment centres, and because compliance of patients seems acceptable, even when considering the daily number of LNAA tablets prescribed. On the other hand, the most promising alternative strategy for treatment of PKU, which is the use of BH_4, has been tested successively, although without the ability to normalize blood Phe levels in all patients. We think that, at present, more

Table 1 The reported effects of LNAA supplements in patients with PKU

Authors/location	Patients Number (*n*) and ages	Study design	Results
Dotremont et al (1995)	Patients with PKU *n*=4	One-month study using a LNAA supplement formula without lysine on a dosage of 0.8 g/kg per day, with patients maintaining a low-protein diet (0.6 g/kg per day)	The formulation used was considered inadequate given the negative nitrogen balance observed
Pietz et al (1999) Germany and Switzerland	5 PKU patients with moderate phenotype and 1 PKU patient with severe phenotype *n*=6 (6 male) Ages: 26–30 years	Cross-over study with an initial challenge with Phe (100 mg/kg) followed by a LNAA mixture composed of 150 mg/kg of each amino acid; brain PHE concentration was measured by ^{1}H MRS	LNAA supplementation blocked PHE influx into the brain and there was no slowing of EEG activity
Koch et al (2003) USA	Classical PKU *n*=6 (4 female; 2 male) Ages: 20–34 years	All the subjects were treated with 0.4 g/kg per day of a LNAA supplement (PreKUnil, Nilab, Denmark) while on an increased natural protein diet	No change in blood Phe concentrations but brain PHE concentrations gradually decreased during the study (6 months)
Matalon et al (2006) Russia, Ukraine, USA and Denmark	Patients with PKU *n*=11 (7 female; 4 male) Ages: old enough to swallow pills	Open-label study with 8 patients (mean age 20.5 years) receiving 0.5 g/kg per day and 3 patients (mean age 16.5 years) receiving 1.0 g/kg per day of LNAA mixture (NeoPhe, Prekulab, Korsor, Denmark) while maintaining the same diet as usual prior to enrolling in the trial	Blood PHE concentrations significantly decreased by 50% after one week in all the patients after NeoPhe; with treatment discontinuation, blood Phe levels returned to pre-trial levels
Matalon et al (2007) USA, Italy, Brazil, Ukraine, Russia, Denmark	All classical PKU patients except one *n*=20 (12 female; 8 male) Ages: 11–32 years	Double-blind placebo control study using a dosage of 0.5 g/kg per day of LNAA mixture (NeoPhe, Prekulab, Korsor, Denmark) while maintaining the same diet as usual prior to enrolling in the trial	Blood Phe concentration dropped significantly (39%) in the 20 patients after one week of treatment with NeoPhe
Schindeler et al (2007) Australia	Classical PKU *n*=16 (9 female, 7 male) Ages: 11–45 years	Prospective double-blind cross-over study using a LNAA powder mix at the dosage of 250 mg/kg per day	Higher plasma Phe levels were found when LNAA intake was lowest. Absence of a positive correlation between plasma and brain Phe when plasma Phe level was under 1200 μmol/L. LNAA supplementation alone with a positive effect on executive functions.

long-term studies, evaluating efficacy and safety, are necessary in order to generalize LNAA therapy as a recommendation for adolescents and adults with PKU.

References

Berger V, Larondelle Y, Trouet A, Schneider YJ (2000) Transport mechanisms of the large neutral amino acid L-phenylalanine in the human intestinal epithelial caco-2 cell line. *J Nutr* **130**: 2780–2788

Berry HK, Bofinger MK, Hunt MM, Phillips PJ, Guilfoile MB (1982) Reduction of cerebrospinal fluid phenylalanine after oral administration of valine, isoleucine, and leucine. *Pediatr Res* **16**: 751–755. doi:10.1203/00006450-198209000-00009

Bickel H, Gerrard J, Hickmans EM (1953) Influence of phenylalanine intake on phenylketonuria. *Lancet* **262**: 812–813. doi:10.1016/S0140-6736(53)90473-5

Binek-Singer P, Johnson TC (1982) The effects of chronic hyperphenylalaninaemia on mouse brain protein synthesis can be prevented by other amino acids. *Biochem J* **206**: 407–414

Blows WT (2000) Neurotransmitters of the brain: serotonin, noradrenaline (norepinephrine), and dopamine. *J Neurosci Nurs* **32**: 234–238

Brightman MW, Reese TS (1969) Junctions between intimately apposed cell membranes in the vertebrate brain. *J Cell Biol* **40**: 648–677. doi:10.1083/jcb.40.3.648

Broer S (2008) Amino acid transport across mammalian intestinal and renal epithelia. *Physiol Rev* **88**: 249–286. doi:10.1152/physrev.00018.2006

Brumm VL, Azen C, Moats RA, et al (2004) Neuropsychological outcome of subjects participating in the PKU adult collaborative study: a preliminary review. *J Inherit Metab Dis* **27**: 549–566. doi:10.1023/B:BOLI.0000042985.02049.ff

Burgard P, Rey F, Rupp A, Abadie V, Rey J (1997) Neuropsychologic functions of early treated patients with phenylketonuria, on and off diet: results of a cross-national and cross-sectional study. *Pediatr Res* **41**: 368–374. doi:10.1203/00006450-199703000-00011

Burlina A, Blau N (2009) Effect of BH(4) supplementation on phenylalanine tolerance. *J Inherit Metab Dis* **32**: 40–45. doi:10.1007/s10545-008-0947-1

Burlina AB, Bonafe L, Ferrari V, Suppiej A, Zacchello F, Burlina AP (2000) Measurement of neurotransmitter metabolites in the cerebrospinal fluid of phenylketonuric patients under dietary treatment. *J Inherit Metab Dis* **23**: 313–316. doi:10.1023/A:1005694122277

Centerwall SA, Centerwall WR (2000) The discovery of phenylketonuria: the story of a young couple, two retarded children, and a scientist. *Pediatrics* **105**: 89–103. doi:10.1542/peds.105.1.89

Christensen HN (1953) Metabolism of amino acids and proteins. *Annu Rev Biochem* **22**: 233–260. doi:10.1146/annurev.bi.22.070153.001313

del Amo EM, Urtti A, Yliperttula M (2008) Pharmacokinetic role of L-type amino acid transporters LAT1 and LAT2. *Eur J Pharm Sci* **35**: 161–174. doi:10.1016/j.ejps.2008.06.015

Dotremont H, Francois B, Diels M, Gillis P (1995) Nutritional value of essential amino acids in the treatment of adults with phenylketonuria. *J Inherit Metab Dis* **18**: 127–130. doi:10.1007/BF00711746

Fernstrom JD, Wurtman RJ (1972) Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science* **178**: 414–416. doi:10.1126/science.178.4059.414

Finkelson L, Bailey I, Waisbren SE (2001) PKU adults and their return to diet: predicting diet continuation and maintenance. *J Inherit Metab Dis* **24**: 515–516. doi:10.1023/A:1010546000617

Fisch RO, Chang PN, Weisberg S, Guldberg P, Guttler F, Tsai MY (1995) Phenylketonuric patients decades after diet. *J Inherit Metab Dis* **18**: 347–353. doi:10.1007/BF00710427

Griffiths P, Paterson L, Harvie A (1995) Neuropsychological effects of subsequent exposure to phenylalanine in adolescents and young adults with early-treated phenylketonuria. *J Intellect Disabil Res* **39**(Pt 5): 365–372

Guttler F, Lou H (1986) Dietary problems of phenylketonuria: effect on CNS transmitters and their possible role in behaviour and neuropsychological function. *J Inherit Metab Dis* **9**(Supplement 2): 169–177. doi:10.1007/BF01799701

Hanley WB (2004) Adult phenylketonuria. *Am J Med* **117**: 590–595. doi:10.1016/j.amjmed.2004.03.042

Hawkins RA, O'Kane RL, Simpson IA, Vina JR (2006) Structure of the blood-brain barrier and its role in the transport of amino acids. *J Nutr* **136**: 218S–226S

Hidalgo IJ, Borchardt RT (1990) Transport of a large neutral amino acid (phenylalanine) in a human intestinal epithelial cell line: Caco-2. *Biochim Biophys Acta* **1028**: 25–30. doi:10.1016/0005-2736(90)90261-L

Hoeksma M, Reijngoud DJ, Pruim J, de Valk HW, Paans AM, van Spronsen FJ (2009) Phenylketonuria: high plasma phenylalanine decreases cerebral protein synthesis. *Mol Genet Metab* **96**(4): 177–182. Epub 2009 Feb 6

Huether G, Neuhoff V, Kaus R (1983) Brain development in experimental hyperphenylalaninaemia: disturbed proliferation and reduced cell numbers in the cerebellum. *Neuropediatrics* **14**: 12–19. doi:10.1055/s-2008-1059547

Kanai Y, Segawa H, Miyamoto K, Uchino H, Takeda E, Endou H (1998) Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). *J Biol Chem* **273**: 23629–23632. doi:10.1074/jbc.273.37.23629

Karasov W, Solberg D, Carter S, et al (1986) Uptake pathways for amino acids in mouse intestine. *Am J Physiol* **251**: G501–508

Knudsen GM, Hasselbalch S, Toft PB, Christensen E, Paulson OB, Lou H (1995) Blood-brain barrier transport of amino acids in healthy controls and in patients with phenylketonuria. *J Inherit Metab Dis* **18**: 653–664. doi:10.1007/BF02436753

Koch R, Guttler F (2000) Benefits of mutation analysis and examination of brain phenylalanine levels in the management of phenylketonuria. *Pediatrics* **106**: 1136. doi:10.1542/peds.106.5.1136

Koch R, Moats R, Guttler F, Guldberg P, Nelson M Jr (2000) Blood-brain phenylalanine relationships in persons with phenylketonuria. *Pediatrics* **106**: 1093–1096. doi:10.1542/peds.106.5.1093

Koch R, Moseley KD, Yano S, Nelson M Jr, Moats RA (2003) Large neutral amino acid therapy and phenylketonuria: a promising approach to treatment. *Mol Genet Metab* **79**: 110–113. doi:10.1016/S1096-7192(03)00078-7

Kreis R, Pietz J, Penzien J, Herschkowitz N, Boesch C (1995) Identification and quantitation of phenylalanine in the brain of patients with phenylketonuria by means of localized in vivo ^{1}H magnetic-resonance spectroscopy. *J Magn Reson B* **107**: 242–251. doi:10.1006/jmrb.1995.1084

Kure S, Hou DC, Ohura T, et al (1999) Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *J Pediatr* **135**: 375–378. doi:10.1016/S0022-3476(99)70138-1

Lee P, Treacy EP, Crombez E, et al (2008) Safety and efficacy of 22 weeks of treatment with sapropterin dihydrochloride in patients with phenylketonuria. *Am J Med Genet A* **146A**: 2851–2859. doi:10.1002/ajmg.a.32562

Levy H, Burton B, Cederbaum S, Scriver C (2007) Recommendations for evaluation of responsiveness to tetrahydrobiopterin (BH_4) in phenylketonuria and its use in treatment. *Mol Genet Metab* **92**: 287–291. doi:10.1016/j.ymgme.2007.09.017

Lou HC, Toft PB, Andresen J, et al (1994) Unchanged MRI of myelin in adolescents with PKU supplied with non-phe essential amino acids after dietary relaxation. *Acta Paediatr* **83**: 1312–1314. doi:10.1111/j.1651-2227.1994.tb13025.x

Mastroberardino L, Spindler B, Pfeiffer R, et al (1998) Amino-acid transport by heterodimers of 4F2hc/CD98 and members of a permease family. *Nature* **395**: 288–291. doi:10.1038/26246

Matalon KM (2001) Developments in phenylketonuria. *Top Clin Nutr* **16**: 41–50

Matalon R, Michals-Matalon K, Bhatia G, et al (2006) Large neutral amino acids in the treatment of phenylketonuria (PKU). *J Inherit Metab Dis* **29**: 732–738. doi:10.1007/s10545-006-0395-8

Matalon R, Michals-Matalon K, Bhatia G, et al (2007) Double blind placebo control trial of large neutral amino acids in treatment of PKU: effect on blood phenylalanine. *J Inherit Metab Dis* **30**: 153–158. doi:10.1007/s10545-007-0556-4

McKean CM (1972) The effects of high phenylalanine concentrations on serotonin and catecholamine metabolism in the human brain. *Brain Res* **47**: 469–476. doi:10.1016/0006-8993(72)90653-1

McKean CM, Boggs DE, Peterson NA (1968) The influence of high phenylalanine and tyrosine on the concentrations of essential amino acids in brain. *J Neurochem* **15**: 235–241. doi:10.1111/j.1471-4159.1968.tb06202.x

Moats RA, Koch R, Moseley K, et al (2000) Brain phenylalanine concentration in the management of adults with phenylketonuria. *J Inherit Metab Dis* **23**: 7–14. doi:10.1023/A:1005638627604

Moats RA, Moseley KD, Koch R, Nelson M Jr (2003) Brain phenylalanine concentrations in phenylketonuria: research and treatment of adults. *Pediatrics* **112**: 1575–1579

Moller HE, Ullrich K, Weglage J (2000) In vivo proton magnetic resonance spectroscopy in phenylketonuria. *Eur J Pediatr* **159** (Supplement 2): S121–125. doi:10.1007/PL00014374

Munck BG, Munck LK (1994) Phenylalanine transport in rabbit small intestine. *J Physiol* **480**(Pt 1): 99–107

Ney DM, Gleason ST, van Calcar SC, et al (2009) Nutritional management of PKU with glycomacropeptide from cheese whey. *J Inherit Metab Dis* **32**: 32–39. doi:10.1007/s10545-008-0952-4

Novotny EJ Jr, Avison MJ, Herschkowitz N, et al (1995) In vivo measurement of phenylalanine in human brain by proton nuclear magnetic resonance spectroscopy. *Pediatr Res* **37**: 244–249. doi:10.1203/00006450-199502000-00020

O'Kane RL, Hawkins RA (2003) Na^+-dependent transport of large neutral amino acids occurs at the abluminal membrane of the blood-brain barrier. *Am J Physiol Endocrinol Metab* **285**: E1167–1173

O'Kane RL, Vina JR, Simpson I, Hawkins RA (2004) Na^+-dependent neutral amino acid transporters A, ASC, and N of the blood-brain barrier: mechanisms for neutral amino acid removal. *Am J Physiol Endocrinol Metab* **287**: E622–629. doi:10.1152/ajpendo.00187.2004

Ohtsuki S, Terasaki T (2007) Contribution of carrier-mediated transport systems to the blood-brain barrier as a supporting and protecting interface for the brain; importance for CNS drug discovery and development. *Pharm Res* **24**: 1745–1758. doi:10.1007/s11095-007-9374-5

Pardridge WM (1998) Blood-brain barrier carrier-mediated transport and brain metabolism of amino acids. *Neurochem Res* **23**: 635–644. doi:10.1023/A:1022482604276

Pietz J, Kreis R, Boesch C, Penzien J, Rating D, Herschkowitz N (1995) The dynamics of brain concentrations of phenylalanine and its clinical significance in patients with phenylketonuria determined by in vivo ^1H magnetic resonance spectroscopy. *Pediatr Res* **38**: 657–663. doi:10.1203/00006450-199511000-00005

Pietz J, Dunckelmann R, Rupp A, et al (1998) Neurological outcome in adult patients with early-treated phenylketonuria. *Eur J Pediatr* **157**: 824–830. doi:10.1007/s004310050945

Pietz J, Kreis R, Rupp A, et al (1999) Large neutral amino acids block phenylalanine transport into brain tissue in patients with phenylketonuria. *J Clin Invest* **103**: 1169–1178. doi:10.1172/JCI5017

Puglisi-Allegra S, Cabib S, Pascucci T, Ventura R, Cali F, Romano V (2000) Dramatic brain aminergic deficit in a genetic mouse model of phenylketonuria. *Neuroreport* **11**: 1361–1364. doi:10.1097/00001756-200004270-00042

Ris MD, Williams SE, Hunt MM, Berry HK, Leslie N (1994) Early-treated phenylketonuria: adult neuropsychologic outcome. *J Pediatr* **124**: 388–392. doi:10.1016/S0022-3476(94)70360-4

Rocha JC, Vilarinho L, Cabral A, Vaz Osório R, de Almeida MF (2007) Consensus for the nutritional treatment of phenylketonuria. *Acta Pediatr Port* **38**: 44–54

Sanchez del Pino MM, Hawkins RA, Peterson DR (1992) Neutral amino acid transport by the blood-brain barrier. Membrane vesicle studies. *J Biol Chem* **267**: 25951–25957

Sanchez del Pino MM, Peterson DR, Hawkins RA (1995) Neutral amino acid transport characterization of isolated luminal and abluminal membranes of the blood-brain barrier. *J Biol Chem* **270**: 14913–14918. doi:10.1074/jbc.270.25.14913

Sarkissian CN, Gámez A, Scriver CR (2009) What we know that could influence future treatment of phenylketonuria. *J Inherit Metab Dis* **32**(1): 3–9. Epub 2008 Aug 3

Schindeler S, Ghosh-Jerath S, Thompson S, et al (2007) The effects of large neutral amino acid supplements in PKU: an MRS and neuropsychological study. *Mol Genet Metab* **91**: 48–54. doi:10.1016/j.ymgme.2007.02.002

Schmidt E, Rupp A, Burgard P, Pietz J, Weglage J, de Sonneville L (1994) Sustained attention in adult phenylketonuria: the influence of the concurrent phenylalanine-blood-level. *J Clin Exp Neuropsychol* **16**: 681–688. doi:10.1080/01688639408402681

Scriver CR, Kaufman S (2001) Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds; Childs B, Kinzler KW, Vogelstein B, assoc. eds. *The Metabolic and Molecular Bases of Inherited Disease*, 8th edn. New York: McGraw-Hill, 1667–1724

Smith QR (2000) Transport of glutamate and other amino acids at the blood-brain barrier. *J Nutr* **130**: 1016S–1022S

Stevens BR, Ross HJ, Wright EM (1982) Multiple transport pathways for neutral amino acids in rabbit jejunal brush border vesicles. *J Membr Biol* **66**: 213–225. doi:10.1007/BF01868496

Surtees R, Blau N (2000) The neurochemistry of phenylketonuria. *Eur J Pediatr*. **159**(Supplement 2): S109–113. doi:10.1007/PL00014370

Thompson AJ, Smith I, Brenton D, et al (1990) Neurological deterioration in young adults with phenylketonuria. *Lancet* **336**: 602–605. doi:10.1016/0140-6736(90)93401-A

van Calcar SC, MacLeod EL, Gleason ST, et al (2009) Improved nutritional management of phenylketonuria by using a diet containing glycomacropeptide compared with amino acids. *Am J Clin Nutr* **89**(4): 1068–1077. Epub 2009 Feb 25

van Meer G, Simons K (1986) The function of tight junctions in maintaining differences in lipid composition between the apical and the basolateral cell surface domains of MDCK cells. *EMBO J* **5**: 1455–1464

van Meer G, Gumbiner B, Simons K (1986) The tight junction does not allow lipid molecules to diffuse from one epithelial cell to the next. *Nature* **322**: 639–641. doi:10.1038/322639a0

van Spronsen FJ, Hoeksma M, Reijngoud DJ (2009) Brain dysfunction in phenylketonuria: is phenylalanine toxicity the only possible cause? *J Inherit Metab Dis* **32**: 46–51. doi:10.1007/s10545-008-0946-2

Walter JH, White FJ, Hall SK, et al (2002) How practical are recommendations for dietary control in phenylketonuria? *Lancet* **360**: 55–57. doi:10.1016/S0140-6736(02)09334-0

Walter JH, Lee PJ, Burgard P (2006) Hyperphenylalaninaemia. In Fernandes J, Saudubray J-M, van den Berghe G, Walter JH, eds. *Inborn Metabolic Diseases*. Heidelberg: Springer, 221–232

Weglage J, Wiedermann D, Denecke J, et al (2001) Individual blood-brain barrier phenylalanine transport determines clinical outcome in phenylketonuria. *Ann Neurol* **50**: 463–467. doi:10.1002/ana.1226

Weglage J, Wiedermann D, Denecke J, et al (2002) Individual blood-brain barrier phenylalanine transport in siblings with classical phenylketonuria. *J Inherit Metab Dis* **25**: 431–436. doi:10.1023/A:1021234730512