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The effects of large neutral amino acid supplements in PKU: An MRS and neuropsychological study

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Abstract

Objective: To determine the effects of large neutral amino acid (LNAA) supplements on brain and plasma phenylalanine (Phe) levels and other metabolites in early treated subjects with classical phenylketonuria (PKU), and to investigate the relationship between these metabolites and neuropsychological performance.

Methods: This was a prospective, double blind, cross over study consisting of four two-week phases with a 4 week washout period. Sixteen subjects (7 males), with classical PKU were recruited into the study and completed all 4 phases. Each phase consisted of either the LNAA supplement or placebo, and either the patient's usual medical product or not. Subjects were instructed to follow their usual Phe restricted diet, maintain energy intake and complete a 3-day food record during each phase. At the end of each phase, brain Phe and other metabolites were measured by proton magnetic resonance spectroscopy (MRS), and plasma amino acids quantified. A detailed neuropsychological assessment was performed on the same day as the MRS and plasma collection.

Results: There was no correlation between plasma and brain Phe, but few of the plasma Phe readings were over 1200 μmol/L. Plasma Phe decreased with LNAA supplementation when patients were not taking their medical formula. LNAA supplementation had a specific impact on executive functions particularly in verbal generativity and cognitive flexibility. Measures of attention were better on medical product, with or without LNAA supplements.

Conclusions: LNAA supplementation was associated with a trend to a lowering of plasma Phe levels. LNAA supplementation had a specific impact on executive functions particularly in verbal generativity and flexibility. For individuals already complying with diet and PKU medical product, additional supplementation with LNAA is of limited value. LNAA supplementation may be of benefit to those unable to comply with PKU medical product by reducing plasma Phe, perhaps by competing with Phe at the level of transport across the gut.

Keywords: Phenylketonuria; Brain–plasma barrier; Magnetic resonance spectroscopy; Large neutral amino acids; Amino acid transport

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Introduction

Phenylalanine is a known neurotoxin and untreated individuals with phenylketonuria (PKU)[2] with high plasma phenylalanine (Phe) levels usually develop severe intellectual disability [1]. In contrast, general cognitive ability is within the normal range in individuals with PKU treated with a low Phe diet from the neonatal period [2]. However, studies have shown that even early-treated individuals with PKU may develop subtle cognitive abnormalities. Neuropsychological studies suggest a specific cognitive profile in which reduced attention, reaction time and executive functioning are most commonly observed [3,4]. While intellectual impairment is thought to be irreversible, it has been suggested that the specific deficits observed in early-treated PKU may be reversible. Indeed, there is evidence suggesting that neuropsychological test performance is more closely related to concurrent plasma Phe levels than to long-term dietary control, and that dietary interventions that induce relatively small changes in plasma Phe levels can improve neuropsychological performance as Phe levels decrease [5,6]. Behaviour, in particular levels of depression and anxiety, has also been found to differ between early treated PKU and unaffected peers [7].

A puzzling finding is that a small proportion of untreated individuals with PKU have preserved cognitive abilities despite having significantly elevated plasma Phe levels [8–10]. This has led to the speculation that there may be individual differences in the permeability of the central nervous system to Phe, which could have a protective effect on brain function. Findings from international studies using proton magnetic resonance spectroscopy (MRS) to measure *in vivo* levels of certain metabolites in the brain have supported this hypothesis; individuals may have similar plasma Phe levels yet their brain Phe levels may be very different. Intellectual outcome was more closely related to brain Phe levels than plasma Phe levels [11,12].

To date there appear to have been no published investigations of the relationship between brain Phe concentrations and performance on tests of the specific cognitive or behavioural functions that are typically found to be impaired in persons with phenylketonuria. It has been proposed that supplementation of the Phe restricted diet with large neutral amino acids (LNAA) might be a successful adjunct to treatment, may have a beneficial effect on mood and cognition [13–15], and may offer a neuroprotective effect by competing with Phe for transportation through the plasma–brain barrier [8,12,13].

In this study, we have attempted to evaluate the potential role that LNAA added to the amino acid products used for PKU might play in the regulation of Phe transport across the brain–plasma barrier, and have examined the relationship between LNAA supplementation and cognitive and affective outcome under four different therapeutic combinations.

Methods

Subjects

Sixteen subjects with early treated classical PKU (plasma levels at some stage >1000 μmol/L) were recruited into the study (7 males, 9 females, median age 24 y 9 m range 11 y 8 m–45y 1 m)), all currently on diet and medical products for PKU. All subjects had been treated at The Children's Hospital, Westmead (CHW), and were recruited via letter invitation or telephone contact. Informed consent was obtained for all subjects, and the study was approved by The Children's Hospital at Westmead Ethics Committee.

Clinical protocol

This study was a prospective, double blind, cross over study. Subjects completed four phases as outlined below. Subjects were required to stay on each phase for 14 days with a 4 week minimum washout period between phases.

The four phases of the study were as follows:

Phase 1: medical product/active phase. Subjects took their usual medical product, usual Phe restricted diet and the LNAA (active) tablets.

Phase 2: medical product/placebo phase. Subjects took their usual medical product, usual Phe restricted diet and placebo tablets. This phase is equivalent to usual treatment.

Phase 3: no medical product/active. Subjects did not take their usual medical product, but took LNAA (active) tablets and maintained their usual Phe restricted diet and energy intake.

Phase 4: no medical product/placebo. Subjects did not take their usual medical product, took placebo tablets and maintained their usual Phe restricted diet and energy intake.

LNAA dosage was 250 mg/kg/day, based on actual weight or ideal weight if the subject was overweight. The subjects took three equal daily doses consumed with breakfast, lunch and dinner. The composition of the 400 mg LNAA tablets is shown in Table 1. Left-over tablets were collected at the end of each phase and counted, in order to assess compliance.

Placebo tablets were compounded to match the appearance of the active tablets. Both the LNAA and placebo tablets contained menthol to mask the LNAA taste. Subjects and investigators were blinded as to the order of the phases, which were randomly allocated by a Hospital

Table 1
Composition of LNAA powder mix (amounts shown are gram per 100 g of LNAA powder)

Amino acid	g/100 g
L-Histidine	15.11
L-Isoleucine	7.53
L-Leucine	7.53
L-Lysine	7.53
L-Methionine	15.11
L-Threonine	7.53
L-Tryptophan	15.11
L-Tyrosine	15.11
L-Valine	7.53

[2] *Abbreviations used:* LNAA, large neutral amino acids; PKU, phenylketonuria; Phe, phenylalanine; Tyr, tyrosine; MRS, magnetic resonance spectroscopy; TR, repetition time; TE, echo time; TSE, turbo spin echo; NAA, *N*-acetylaspartate; Cho, composite peak arising from glycerophosphocholine, phosphocholine with a small contribution from free choline; Cre, composite peak arising from the *N*-methyl resonances of creatine and phosphocreatine.