



# LARGE NEUTRAL AMINOACID SUPPLEMENTATION IMPROVES EXECUTIVE FUNCTIONS IN DIET NON-COMPLIANT PHENYLKETONURIA ADOLESCENTS

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## INTRODUCTION

Dietary restriction of phenylalanine (Phe) is the cornerstone of treatment in phenylketonuria (PKU), but maintenance of good biochemical control can be difficult even in young patients. Adolescents and young adults generally do not comply with recommendations for the monitoring and control of Phe concentrations. In the recent years, with the recognition that IQ is not the only aspect of brain function influenced in PKU, and that early and continuously treated PKU (ETPKU) individuals experience significant neurocognitive sequelae, much effort is being made to develop more acceptable treatment modalities for all ages. Sapropterin dihydrochloride (BH4, tetrahydrobiopterin) can be used for only a subset of patients. Large neutral amino acid (LNAA) supplementation is an alternative adjuvant treatment, especially for sapropterin non-responsive and diet non-compliant PKU individuals. LNAAs decrease the brain Phe concentration by competing with Phe for shared amino acid transporters to cross the blood-brain barrier. We designed a prospective study to evaluate the effect of LNAA supplementation on executive functions (EF) of diet non-compliant PKU adolescents we also aimed to assess EF by both laboratory measures and questionnaires assessing day-to-day EF, to further understand their possible relationship.

## MATERIAL AND METHOD

### Subjects

PKU children between age 12-18 years, for whom LNAA supplementation will be introduced were invited to join the study. EF assessments were performed twice for each individual, at baseline and at 6 months of LNAA supplementation. Day-to-day EF was assessed with the Behavior Rating Inventory of Executive Function (BRIEF) parent and teacher forms. Wisconsin Card Sorting Test (WCST) and Stroop Test were introduced as laboratory measures. Blood phenylalanine, tyrosine levels and phenylalanine/tyrosine ratios were assessed as part of routine follow-up.

### Neuropsychological tests

**The Behavior Rating Inventory of Executive Function (BRIEF):** It is purported to be a measure of EF that is more ecologically valid than most other laboratory tasks. This inventory is a standardized informant report designed to assess EF within the context of an individual's day-to-day environment. It yields nine nonoverlapping clinical scale scores reflecting different aspects of EF, including inhibitory control, self-monitoring, planning and organization, emotional control, and working memory. In addition, two broad indices (Behavioral Regulation Index and Metacognition Index) and an overall index reflecting EF in general (Global Executive Composite) are computed.

**Stroop task:** Color words are presented in conflicting hues (e.g., the word blue printed in red ink). Participants must inhibit the prepotent tendency to read the word and instead name the color of the stimulus. Performance in this inhibitory condition is often compared with that in one or more control conditions (e.g., reading color words printed in black ink). Ability to inhibit interferences in a timely fashion, and that in addition has been widely used in functional brain imaging studies.

**Wisconsin Card Sorting Test:** It is commonly used to assess shifting during this test, through trial and error, participants must deduce the rule (e.g., based on color, shape, or number) by which a set of stimulus cards is sorted. As the task progresses, the correct sorting rule periodically changes without warning, requiring that the participant shift sets and re-determine the correct sorting rule. Difficulties in shifting often manifest as perseveration, or a tendency to continue sorting based on a previous rule despite feedback indicating that this rule is no longer the correct basis for sorting.

**LNAA supplementation and diet:** NeoPhe® tablets or powders are used as LNAA supplementation (0.4 g/kg/day LNAA). Recommended daily natural protein intake was 0.6 g/kg/day consuming together with LNAAs. Blood phe, tyr, phe/tyr levels were routinely checked in the follow-up.

## RESULTS

Twenty-three patients, 7 girls and 16 boys, were included in the study. Their ages at PKU diagnosis were ranging from 3 days to 17 months, IQ levels were ranging between 50-118 (performed previously with WISC-R). Their mean blood Phe levels in the last two years was 20.0±3.3 mg/dl. Their mean blood Phe levels in the first three 3 years of life was 7.6±2.5 m/dl which was negatively correlated with their IQ levels. With LNAA supplementation, the decrease in blood Phe levels and Phe/tyrosine ratios were median 8% (p=0.089) and 27.8% (p=0.01) respectively.

Behaviour Regulatory Index demonstrated mean 20.1% and 17.9%, Metacognition Index demonstrated 25.5% and 19.5% and Global Executive Composite demonstrated 23.7% and 19.0% improvement, with LNAA supplementation on parent and teacher assessments respectively (Table 1). Parents reported the best improvement on initiate, planning/organizing, monitoring subscales, while improvement of emotional control was more prominent according to teachers (Table 1). Total errors, Perseverative responses and errors have decreased, conceptual level responses have increased with LNAA supplementation when tested by WCST (Table 2). There was not any statistically significant change with LNAA on consecutive Stroop tasks (Table 3). There was not any correlation between BRIEF, WCST, Stroop test scores and blood Phe, Tyr, Phe/Tyr levels (spearman correlation; data not shown).

**Table 1.** Mean Behavior Rating Inventory of Executive Function (BRIEF) Score changes from baseline to sixth month of LNAA supplementation

BRIEF scales	Mean (SD) change* (%)	
	Parent (n=23)	Teacher (n=21)
<b>BRI Scale Scores</b>		
Inhibit	14 (11)	16.4 (11.1)
Shift	23.3 (12.7)	14.2 (12.2)
Emotional control	20.7 (14)	22.7 (21.1)
<b>MI Scale Scores</b>		
Initiate	26.6 (14)	17.8 (16.2)
Working memory	23.2 (12.2)	18.3 (15.8)
Plan/organize	26.1 (10.2)	19.8 (23.4)
Organization of materials	18.6 (12.2)	20.6 (20.2)
Monitor	27.6 (12.4)	18.7 (16.5)
<b>Composite scores</b>		
Behavioral Regulation Index	20.1 (9.8)	17.9 (15.2)
Metacognition Index	25.5 (7.9)	19.5 (15.8)
Global Executive Composite	23.7 (7.7)	14.1 (14.5)

\*p<0.0001 for all subscales based on one-sample t-test.

Abbreviation: BRIEF=Behavior Rating Inventory of Executive Function; SD=standard deviation.

**Table 2.** Wisconsin Card Sorting Test (WCST) scores before and after LNAA supplementation

WCST scales	Before LNAA*	After LNAA*	p**
Total no. of trials administered	123.7 (10.1)	118.5 (17.6)	0.093
Errors	60.6 (18.8)	45.0 (19.3)	<0.001
Correct response	63.1 (13.0)	73.5 (12.1)	0.002
No. of categories completed	3.6 (1.9)	4.2 (1.6)	0.091
Perseverative response	46.4 (27.2)	32.3 (21.8)	0.002
Perseverative errors	39.5 (20.9)	28.2 (16.5)	0.002
Non-perseverative errors	21.2 (10.5)	17.9 (8.4)	0.116
% Perseverative responses	31.4 (15.7)	22.8 (12.1)	0.002
Trials to complete 1st category	24.9 (23.8)	17.1 (12.1)	0.124
Conceptual level responses	46.9 (18.2)	61.0 (15.7)	0.001
% Conceptual level responses	38.8 (17.4)	53.3 (17.7)	<0.001

\*Mean (SD), \*\*Student t pair test

**Table 3.** Stroop task scores before and after LNAA supplementation

Stroop scales	Before LNAA*	After LNAA*	p**
RT, word neutral (black)	103 (27)	98 (27)	0.334
RT, word neutral (colored)	114 (32)	107 (28)	0.084
RT, shape color	174 (61)	153 (43)	0.06
RT, incongruent word	233 (84)	207 (79)	0.08
RT, incongruent color	337 (84)	302 (81)	0.038

\*Mean (SD); as millisecond, \*\*Student t pair test, RT: reading time

## DISCUSSION

Executive function refers to higher-order cognitive abilities that facilitate the flexible modification of thought and behavior in response to changing cognitive or environmental demands. EF encompasses abilities such as planning, organization, cognitive flexibility, inhibitory control, and working memory. These abilities are considered executive because they require the integration and processing of information across a range of cognitive domains, sensory modalities, and response modalities. The prefrontal cortex (PFC) and associated brain regions play a crucial role in EF. Early-treated PKU is associated with a slight decrease in intelligence, coupled with impairments in specific aspects of cognition. The cognitive deficits in early-treated PKU are best conceptualized as disorders of EF and the most consistent findings of PKU-related EF impairment were in executive working memory and prepotent response inhibition.

The extent to which performance on EF measures administered in the laboratory correspond with EF in day-to-day life remains unclear. Indeed, numerous studies involving individuals with and without brain dysfunction have reported only modest correlations between performance on laboratory tests of EF and the extent of EF problems in the real world. BRIEF is a measure of EF that is more ecologically valid than most other laboratory tasks, designed to assess EF within the context of an individual's day-to-day environment.

In their MRS and neuropsychological study Schindeler et al. has shown that LNAA supplementation had a specific impact on executive functions particularly in verbal generativity and flexibility. Possible LNAA treatment targets include reduction of brain Phe concentrations, reduction of blood Phe concentrations, augmentation of cerebral neurotransmitter synthesis, and/or elevation of brain non-Phe LNAA concentrations. Pietz et al. has shown that LNAA prevent Phe from crossing the blood brain barrier and dentering the brain, thereby normalizing brain function. They also showed that ETPKU adults, EEG spectral analysis revealed acutely disturbed brain activity but with concurrent LNAA supplementation, Phe influx was completely blocked and there was no slowing of EEG activity.

We have shown that, LNAA supplementation improves EF impairments and this improvement was more evident in daily life. Apart from the objective scores obtained BRIEF and WCST, teachers, parents and even patients themselves emphasized many subjective improvements with LNAA supplementation. We also observed that PKU adolescents has become more social and extrovert, more willing to express themselves. What should be noted is that these EF improvements were independent with their ages, blood Phe levels or IQ levels. Every individual has shown at least some improvement from his/her baseline EF with LNAA supplementation.

This is the first prospective study, evaluating the effect of long term LNAA supplementation on EF, in both daily-life (BRIEF) and laboratory (WCST and Stroop). As our hypothesis was based on the fact that PKU individuals have EF impairments, a control group was not included in the study. Although our sample size is small, our results are promising, and further randomized control studies are needed to support positive effects of LNAA supplementation on executive functions.

## References

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