# Behavioural effects of phenylalanine-free amino acid tablet supplementation in patients with untreated Phenylketonuria

by

H. Serap Kalkano lu[1], Kirsten K. Ahring[2], Lisbeth Birk Møller[2], Ingrid Mikkelsen[2], Anne Romstad[2], Hans C. Lou[2], and Flemming Güttler[2 (*)]

[1] *Department of Paediatrics, Nutrition & Metabolism Unit, Hacettepe University, Ankara, Turkey*
[2] *The John F. Kennedy Institute, Glostrup, Denmark*

(*) To whom correspondence should be addressed

Flemming Güttler, M.D., Ph.D.
Head of the Department of
Inherited Metabolic Diseases and
Molecular Genetics
The John F. Kennedy Institute GI.
Landevej 7
DK-2600 Glostrup
Denmark

Phone: + 4543 26 01 00
Fax: + 45 43 43 11 30
E-mail: flg@kennedy.dk

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**Abbreviations**

AA, amino acids

BBB, blood brain barrier

LNAA, long neutral amino acids

LATl, long neutral amino acid transporter1

PAH, phenylalanine hydroxylase

PKU, phenylketonuria

**Running title:** Amino acid tablets in untreated PKU

**Keywords:** Untreated PKU, behaviour, long neutral amino acid tablets, supplementation

**Abstract**

In countries that do not have an effective screening program, it is important to provide better life quality to untreated cases. Studies have suggested that previously untreated PKU patients may benefit from a low phenylalanine (phe) diet. The difficulty of dietary therapy in older children and adults, has led to search for alternative treatments. Since1985, the John F. Kennedy Institute has treated some of their young adults with liberalized diet supplemented with large neutral amino acids without phe but enriched in tyrosine and tryptophan (PreKUnil tablets). Our hypothesis is that brain phe levels may become near normal in spite of Hyperphenylalaninemia over 1000 mmol/l. We performed a prospective double blinded placebo-controlled cross over study providing PreKUnil tablets or placebo to 19 untreated PKU patients (age 38-82 years) with severe intellectual disability. The adaptive behaviour domains inc1uding motor skills, socialization, daily living skills and communication were measured at the initiation of the study and at the end of each six months. Additionally, behavioural characteristics such as, changes in functioning, hyperactivity, attention span, emotionality, frustration tolerance, and responsiveness were recorded on a monthly questionnaire basis by their caretakers. 13 out of the 19 subjects (68%) improved significantly on the PreKUnil tablets. Our study suggests that socialization, emotionality, frustration tolerance, and mood will ameliorate in previously untreated PKU patients supplemented with large neutral amino acids enriched in tyrosine and tryptophan.

## Introduction

Phenylketonuria (PKU) is one of the most common disorders of amino acid metabolism caused by deficiency of the liver enzyme, phenylalanine hydroxylase (P AH). The chronically high phenylalanine (phe) levels in untreated PKU cause severe mental retardation and complex neurological problems (1). Early diagnosis and treatment prevents mental retardation and associated problems (2). Treatment of PKU requires restoration of blood phe levels as near normal as possible, as early as possible, for as long as possible, perhaps for a lifetime. There is almost universal consensus among clinicians that "diet for life" is the best approach for treatment of the severe forms of PKU (3, 4).

The incidence of untreated PKU has almost been eliminated in countries with screening programmes. However, in countries that do not have an efficient screening program, it is important to try to provide a better quality of life for untreated cases of PKU. Most adults born before newborn screening or not included in neonatal screening programmes are mentally retarded and may have many neuropsychological problems associated with their high blood phe levels. Recent studies suggest that previously untreated PKU patients my benefit from a diet low in phe (5). In adults who are untreated, the major goal is to reduce psychological, behavioural and health problems. The difficulty of maintaining a low-phe diet in mentally retarded adolescents and adults has led to the search for alternative treatment strategies.

The large neutral amino acids (LNAA) including phe apparently compete with the same binding site on the carrier for transportation across the blood-brain barrier (BBB)(6, 7, and 8). The concentration of LNAA other than phe in the blood may thus possess a very important influence on how much phe is transported into the brain (8). High levels of tyrosine, tryptophan, leucine, isoleucine, valine, histidine, methionine, and threonine in the blood reduce the uptake of phe by simple competition and vice versa (9)[1]. Since 1985, the John F. Kennedy Institute has treated some of the young adults with PKU with a liberalized diet supplemented by large neutral amino acids without phe but enriched in tyrosine and tryptophan (PreKUnil tablets) (10). Our hypothesis is that during treatment with PreKUnil tablets brain phe levels might become near' normal even though blood phe level s were elevated. Based on this theory we conducted a prospective double blinded placebo controlled cross over study providing PreKUnil tablets to 19 subjects with severe intellectual disability arising from untreated PKU and highly elevated blood phe levels.

[1] 9, data to be published

**Patients and Methods**

The project was initiated in March 2001, offering the PreKUnil treatment to all late diagnosed PKU patients in Denmark. We approached all the people with untreated PKU in "The Intellectual Disability Health Services" in Denmark. The majorities had severe or profound intellectual disability and were unable to give consent. Therefore, it was necessary to discuss the potential impact of the treatment with health carers, family members and others involved in the care of the patients to seek consent from each of these parties. Out of 60 late-diagnosed patients, 19 were participating (Table 1).

There were seven females and 12 males. Patients varied in age from 38 to 82 years. Their mutations were ana1ysed on both alleles and 16 of them were c1assified as suffering from classical severe PKU. In two samples (Pt no. land 16) the mutations could not be detected and one of the patients was not eager to give us a blood specimen (Pt no. 3). There were no changes in their diet and their phe levels were approximately the same during the project. The patients were divided into two groups and each group was treated with either tablets or placebo for a six months period and the next six months received the opposite treatment. Caretakers were advised to divide the daily dosage of amino acid (PreKUnil, Denmark) tablets into three and give them along with a mixed meal in order to obtain optimal benefits of the amino acid composition. All patients were visited and tested psychologically prior to the study, and six and 12 months after the start of the project.

There are many scales for testing behaviours of mentally retarded patients, but for .several reasons we have found them unsuitable for this study. Some of the scales are very Ion g, some are not well designed for the measurement of behavioural problems of severely or profoundly retarded patients, and some of them were containing many irrelevant items. A simple scale was needed to asses the effects of tablets on these cases. So, we developed a scale based on the "Vineland Adaptive Behaviour Scale". This checklist is composed of simple and relatively concrete descriptive items that could be completed by nurses and other institutional staff members. The scale was useful for this study, as it was simple and still sufficiently long to obtain an acceptable reliability of sub domains. It contained all psychometric characteristics allowing us to make a meaningful conc1usion (Tab1e 2).

The adaptive behaviour domains (motor skills, socialization, daily living skills and communication) and their sub domains were measured to register the effects of the tablets at the beginning of the project and at the end of each six months (Table 2). Neurological observations inc1uded degree of seizures, tremor, and self injury. Additionally, some other behavioural characteristic of the patients such as changes in functioning, hyperactivity, attention span, emotionality, frustration; tolerance and responsiveness were recorded by their caretakers on a monthly basis on questionnaire scales. At the

beginning of the study and every six months, patients were evaluated and caretakers were interviewed by two of us (HSK & KKA).

**Results**

At the end of the study, 13 out of the 19 subjects (68%) benefited from the amino acid tablets. According to our behaviour scale the most important changes were their increased awareness and their less aggressive and more social behaviour. At the end of the study 9 out of 19 (47%) could remain eye contact and 8 out of 19 (42%) had developed a meaningful smile and 10 of them (53%) were les s aggressive and four were less anxious. Screaming and shouting were decreased. The non-parametric analyses by a Wilcoxon rank some test revealed significant improvement in the groups when on amino acid (AA) (PreKUnil) tablets compared to placebo (Table 3).

The most significant detail that the caretakers and the nurses noted on their questionnaires at the end of the study was the patient increased awareness about things happening in their surroundings (14/19, 74%) . A few of the patients (3/19) became more aggressive and anxious, probably because they were not used to communicate with the surroundings. The establishing of an "eye contact" (9/19, 47%), was another important and common finding (Table 4). Because of these developments, the caretakers were more motivated. Even though our study group was made of mostly self-oriented patients un-eager to participate in group activities, at the end of the study it was observed that during their AA treatment period 3 of the patients were participating in group works. Rather dramatically, one of the patients was participating in a song by making musical murmurs. There were no significant differences among the two groups as far as following rules and being polite was concerned.

Four unique patients helped us in our evaluation objectiveness. Two of them were siblings (Pt no. 17 and 18) living in the same house and the other two (Pt no. 5 and 6) were ones living in the same institution and always being looked after by the same nurses and caretaker groups. One of the siblings showed during the AA treatment period much more social adaptance and joy, was interpretending himself efficiently, but when we changed the tablets into placebo it was observed that he became nervous, anxious and introvert. Oppositely, the other patients (pt. no 5 and 6) showed nearly the same positive development while taking placebo tablets. Even if we could observe no significant changes in behaviour in our visits, the caretakers stated that while they were taking placebo tablets their screaming and shoutings decreased, and they became calmer. But as an objective observation there were no change in the number of epileptic seizures. In our study no significant changes were found in gross and fine motor functions in the two groups (Table 5).

There were no improvements in feeding themselves without support and on bathroom education on AA tablets. In addition, no significant changes in the everyday habits were observed (Tab le 6). Among all the patients just one of them (pt. no. 16) helped the caretakers preparing the dinner table while taking AA tablets.

**Discussion**

Untreated PKU usually results in progressive mental retardation and a pronounced decrease in IQ scores to less than 50. The deterioration is directly related to phenylalanine levels (II). The pathogenesis of impaired cognitive development and neurophysiological functions in PKU is complex in nature, but there is an emerging consensus that phe itself, at elevated concentrations, is the harmful molecule (12). In untreated PKU the cells achieve net intracellular accumulation of phe due to trams membrane fluxes of phe in the somatic cells, mediated by the AA carrier (13). Several neutral amino acids interact with membrane transport systems other than the LATI to achieve conservative uptake into parenchymal cells. Phe does not interfere with this process (14). These same amino acids, however, leave parenchymal cells on LA TI, and this flux is blocked when intracellular phe is elevated (15). Accordingly, amino acids sharing the carrier are likely to be sequestered in parenchymal tissues in the presence of Hyperphenylalaninemia. The corresponding transport relationships are different in the brain. Here an excess of phe impedes cellular influx rather than efflux of these amino acids (16). Thus, the presumed net effect of Hyperphenylalaninemia on the inter-organ traffic of amino acids, such as the essential branched-chain group, tryptophan and tyrosine, will be to deprive the brain and to sequester them in the parenchymal tissues.

Newborn screening for PKU in Denmark began in 1965 and was centralized at the John F. Kennedy Institute from 1967. In the beginning 80's the first group of well-treated teenagers started to face social and psychological problems associated with maintaining the diet and some of them were not able to follow strict diet. Since 1985, the John F. Kennedy Institute has treated some of their young adults age s IS years and older with PreKUnil tablets and a liberalized diet (submitted). The basic concept of the pills was inspired by the work of Anderson (6). The recognition that phenylalanine and other large neutral amino acids share common receptor sites on a blood brain barrier transport system led to the hypothesis that administration of other large neutral amino acids to patients with elevated plasma phenylalanine concentrations might reduce the amount of phenylalanine reaching the brain and prevent some of its toxic effects on the central nervous system (11). Several studies have shown that a regimen of large neutral amino acids may help individuals unable to maintain low serum phenylalanine levels (7, 8).

This study suggests that supplementation of phenylalanine-free amino acid tablets enriched in tyrosine and tryptophan can be considered as an offer for treatment of individuals with previously untreated PKU, to improve their quality of life and at the same time reduce the cost of their care (less time consuming, less drugs for epilepsy, depression, headaches etc).

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**Table 1.** Clinical characteristics of the patients included in the study

| Patient No | Age | Sex | Mutation | Phe Levels (mmol/l) |
|---|---|---|---|---|
| 1 | 49 | M | | 1383 (849-1684) |
| 2 | 82 | M | IVS12nt lg>a/? | 1270 (1020- 1684) |
| 3 | 50 | F | | |
| 4 | 72 | M | R408W / R408W | 1726 (1418-2120) |
| 5 | 55 | F | IVSlnt Ig>a /? | 1628 (1413- 1868) |
| 6 | 48 | M | R408W / R158Q | 852 (302-1387) |
| 7 | 38 | M | IVS12nt Ig>alIVSl2nt Ig>a | 1656 (1320- 2100) |
| 8 | 75 | M | R408W / ? | 1393 (1201- 1554) |
| 9 | 77 | F | IVS12nt Ig>a/? | 1265 (1132- 1374) |
| 10 | 53 | M | IVS12nt Ig>a / IVS12nt Ig>a | 1391 (1176- 1710) |
| 11 | 49 | M | IVS12nt Ig>alIVSl2nt Ig>a | 1567 (1383- 1821) |
| 12 | 40 | F | IVS12nt Ig>a / IVS12nt Ig>a | 1312 (1090- 1760) |
| 13 | 43 | F | IVSI2ntlg>a/IVSI2ntlg>a | 1344 (1175- 1675) |
| 14 | 50 | F | E280K / V245E | 1378 (1025- 1851) |
| 15 | 45 | F | IVSI2ntlg>a/IVSI2nt Ig>a | 1339 |
| 16 | 61 | M | | 1331 (1192- 1436) |
| 17 | 53 | M | IVS 12nt 1 g>a / R408W | 1545 (1202- 1884) |
| 18 | 51 | M | IVS12nt Ig>a / R408W | 1530(1286-1792) |
| 19 | 51 | M | IVS 12nt Ig>a/ IVS 12nt Ig>a | 1667 (1307- 2054) |

**Table 2.** Checklist for assessment of behavioural changes in severely retarded cases, adapted by Vineland Adaptive Behaviour Scale

| **SOCIALIZATION** | | |
|---|---|---|
| Interpersonal Relationship | Play and Leisure Time | Coping Skills |
| Looking other people in the eye<br>Meaningful smile<br>Agressivity<br>Anxiety | Hitting buttons and musical keyboards<br>Participating in group activities<br>Playing with toys<br>Rubbing soft toys | Screaming<br>Shouting<br>Following rules<br>Beginning politeness |
| **MOTOR SKILLS** | | |
| Gross | Fine | |
| Walking<br>Running<br>Jumping<br>Sitting without support<br>Climbing | Putting cubes into cups<br>Making tower with cubes<br>Playing with balls | |
| **COMMUNICA TION** | | |
| Receptive<br>Awareness of external stimuli<br>Concentrating<br>Turns eyes and head towards sound<br>Follows instructions<br>Listening and attending | Expressive<br>Smiling<br>Laughing<br>Humming of tunes<br>Talking | Written<br>Identifying letters and words<br>Beginning to read<br>Beginning to write |
| **DAILY LIVING SKILLS** | | |
| Personal<br>Eating without support<br>Bathroom education<br>Using diaper<br>Cooperation of self-care | Domestic<br>Preparing food and table<br>Stealing food from others plate<br>Preparing bed | Community<br>Using telephone<br>Understanding hot and cold<br>Understanding money |

**Table 3.** Changes in patients' behaviour and social skills following treatment with amino acid tablets

| **SOCIALIZA TION** | Number of patients presenting improvement on AA tablets[3] | P value (Wilcoxon test)[2] |
|---|---|---|
| **Interpersonal Relationship** | | |
| Looking other people in the eye | 9/19 | P: 0.00 1 |
| Meaningful smile | 8/19 | P: 0.002 |
| Less aggressive | 10/19 | P: 0.003 |
| Less anxious | 4/19 | P: 0.001 |
| **Play and Leisure Time** | | |
| Hitting buttons and musical keyboards | 3/19 | P: 0.007 |
| Participating in group activities | 3/19 | P: 0.007 |
| Playing with toys | 0/19 | P: 1 |
| Rubbing soft toys | 0/19 | P: 1 |
| **Coping Skills** | | |
| Less screaming | 6/19 | P: 0.024 |
| Less shouting | 4/19 | P: 0.008 |
| Following rules | 0/19 | P: 1 |
| Beginning politeness | 0/19 | P: 1 |

[2] Wilcoxon Rank Sum Test
[3] AA = amino acid

**Table 4.** Changes in patients' behaviour and communication habits during AA tablets treatment

| **COMMUNICATION** | Number of patients presenting improvement on AA tablets | P value (Wilcoxon test) |
|---|---|---|
| **Receptive** | | |
| Awareness of external stimuli | 14/19 | P: 0.003 |
| Concentrating | 5/19 | P: 0.034 |
| Turns eyes and head towards sound | 0/19 | P: 1 |
| Follows instructions | 0/19 | P: 1 |
| Listening and attending | 5/19 | P: 0.046 |
| **Expressive** | | |
| Smiling | 10/19 | P: 0.034 |
| Laughing | 11/19 | P: 0.02 |
| Humming of tunes | 2/19 | P: 0.317 |
| Talking | 0/19 | P: 1 |
| Self Injury | 4/19 | P: 0.008 |

**Table 5**. Changes in patients' behaviour and communication habits during AA labels

| **MOTOR SKILLS** | Number of patients presenting improvement on AA tablets |
|---|---|
| **Gross** | |
| Walking | 0/19 |
| Running | 0/19 |
| Jumping | 0/19 |
| Sitting without support | 0/19 |
| Climbing | 0/19 |
| **Fine** | |
| Putting cubes into cups | 1/19 |
| Making tower with cubes | 0/19 |
| Playing with balls | 1/19 |

**Table 6**. Changes in patients' daily living skills during AA tablets treatment

| **DAILY LIVING SKILLS** | Number of patients presenting improvement on AA tablets |
|---|---|
| **Personal** | |
| Eating without support | 0/19 |
| Bathroom education | 0/19 |
| Using diaper | 0/19 |
| Cooperation of self-care | 0/19 |
| **Domestic** | |
| **Preparing food and table** | 1/19 |
| Stealing food from others plate | 0/19 |
| Preparing bed | 0/19 |
| **Community** | |
| Using telephone | 0/19 |
| Understanding hot and cold | 0/19 |
| Understanding money | 0/19 |