

Cognitive performance in adolescent phenylketonuria patients on relaxed diet supplemented with .phenylalanine-free amino acid tablets versus strict dietary therapy

by

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Abbreviations:

CNS, central nervous system

CSF, cerebrospinal fluid

PAH, phenylalanine hydroxylase

5-HIAA, 5- hydroxyindoleacetic acid

HPA, Hyperphenylalaninemia

HVA, homovanillic acid

PKU, phenylketonuria

RT, reaction time

The phenylalanine-free amino acid tablets (PreKUnil) used in the present study was donated by the manufacturer PreKUlab A/S, 41 Revvej, DK-4220 Korsør.

ABSTRACT. *Objectives.* The aim of the present study was to examine whether a normal neuropsychological performance and well-being would be preserved in young adults with phenylketonuria (PKU) in whom a strict dietary treatment was replaced by a protein-restricted free diet supplemented with tablets containing the essential amino acids, in particular tyrosine and tryptophan, but not phenylalanine.

Methodology, design, and patients. Fifty-three adolescent PKU patients who had been on a strict diet from birth were repeatedly assessed with a battery of 12 novel neuropsychological tests developed and designed to measure changes in cognitive function long before changes might be revealed by a declining IQ. All patients were tested at 15 years and 15~ years of age. Then, 23 patients changed to the tablet diet while 30 patients continued the usual strict diet. The two groups were tested at 16, 17, and 18 years of age. The test battery was evaluated with a normal control group of 36 young adults. Test scores were standardized and converted to T -scores with the control group as reference. Mean differences in T -scores between successive measurements of the PKU groups were subjected to unvaried and multivariate analysis of variance.

Results. Cognitive performance in 23 young PKU adolescents on alternative treatment with amino acid tablets and a more liberalized diet was not significantly different from the performance of 30 PKU adolescents on conventional treatment with strict diet. The two groups were followed from 15~ to 18 years of age. During this course neuropsychological functions improved in both groups. Mean plasma phenylalanine level s increased by 30% in the group on amino acid tablets. A group of 18 patients on amino acid tablets have been followed for another 2 years or more and no decline in test performances has been observed. At 19 years of age the percentage going on to college or university was the same in the two groups and also similar to that found in the Danish population.

Conclusion. The less restricted diet supplemented with the amino acid tablets used in the present study can be regarded as a safe and effective alternative to conventional treatment in adolescents with PKU where the compliance on the strict diet is poor.

Keywords: Adolescent phenylketonuria; relaxed diet; phenylalanine-free amino acid tablets; neuropsychological tests

Running title: Amino acid tablets in adolescent PKU

INTRODUCTION

Institution of a phenylalanine-restricted diet within the first two weeks of life is effective in the prevention of mental and neurological deficits in patients with phenylketonuria PKU (1). The aim is usually to achieve plasma phenylalanine levels below 300 $\mu\text{mol/l}$ during early childhood. Thereafter a gradual relaxation of the diet is accepted. Studies from our institution and other centres have demonstrated that cessation of treatment in adolescents is followed by a decreased performance on tests of vigilance, such as continuous recording of reaction times (R T) (2). Simultaneously the patients show an impaired CNS synthesis of dopamine and serotonin as judged by concentrations of homovanillic acid (HV A) and 5-hydroxyindoleacetic acid (5-HIAA) in CSF (2) and the deficient neurotransmitter synthesis is regarded as important for the development of mental symptoms (3,4).

We have previously shown that dietary supplementation with tyrosine and tryptophan enhances CNS synthesis of dopamine and serotonin (5, 6) and improves performance (5) in PKU adolescents who have discontinued dietary therapy. Based on these observations we introduced a new therapeutic alternative with a much more acceptable moderate protein restricted diet combined with supplementation of the essential amino acids, except phenylalanine. We observed that the amino acid tablet diet at this age when the brain is supposed to be fully developed was well tolerated and that the general well being of the patients was improved due to disappearance of the symptoms normally accompanying diet discontinuation. Here we report the results obtained in a controlled neuropsychological follow-up study of 53 patients followed from age 15 to 18 years either on the less restricted diet supplemented with amino acid tablets or on the usual phenylalanine restricted diet with formula.

MATERIAL AND METHODS

Formal IQ tests were not suitable for the present study, as certain mental functions (the ability to concentrate and to learn and retain new material) may decline long before changes are revealed by a falling IQ. To meet this challenge a battery of neuropsychological tests was developed that is more sensitive to possible dysfunctions and thus offers the opportunity of preventing a possible irreversible brain damage by reinstating the low-phenylalanine diet in time.

Description of the test battery

Table 1 shows the major cognitive functions measured with the test battery.

Verbal learning and memory

Paired Associative Learning (visual) with retention. The test material consists of 15 cards with a nonsense syllable on the front and a single consonant on the back. Front and back of the cards are shown to the subject at a rate of 5 seconds per card. After shuffling of the pack the fronts are shown again and the subject is required to name the associated consonants. The test is repeated after one hour. *Free recall:* The test material comprises six cards each with a list of 21 one-syllable nouns. The lists are read aloud at the rate of one word per second, and after each reading the subject repeats the words he recalls. *Digit span, forward and backward:* The subject is required to repeat three-to-nine-digit numbers in forward and reversed order. The test is discontinued when the subject fails on two numbers of equal length.

Visual perception and short term memory

Square pattern: The test comprises 20 patterns. Each pattern is shown for two seconds, and the subject is required to identify the pattern among four possibilities. The test measures the ability to perceive a visual pattern at a glance and recognize it immediately afterwards. *Square pattern, rotated:* The test material is identical to the above, but the subject is allowed to scan the stimulus picture for 10 seconds, before the response picture is presented upside down. The test measures the ability to manipulate a visual pattern in memory, and owing to the longer stimulus period it is less sensitive to short lapses of attention.

Visuo-motor functions

The following three tests measure the rate of complex psychomotoric functioning. They have all been shown to be sensitive to initial symptoms of dementia in adults. *Trail A:* This is a so-called

tracking test. The subject is required, under time pressure, to connect the numbers 1 to 25 placed in random order on a piece of paper. *Trail B*: The requirements are essentially similar to those of Trail A, except that the subject must alternate between numeric and alphabetic series. The score in trail tests is the number of seconds required to finish the task. *SDMT (Symbol Digit Modalities Test)*: The subject is required to code symbols according to a digit key. The score is the total of correct coding within a time limit of 90 seconds. Simple visuo-motoric functioning is measured by the *Continuous visual reaction time*: The subject is seated in front of a computer. At random intervals a white square is shown on the screen, and the subject is required to make the square disappear as fast as possible by pressing the space bar. Total reaction time is recorded in centiseconds and the scores for the 50 percentile, the difference between percentiles 90 and 10, and total reaction time is computed. The test measures hand-eye coordination and the ability to sustain focused attention. It takes about 90 minutes to administer the test battery in its entirety. Eight parallel versions of the battery were developed to minimize retest effects.

Since 1969 differential diagnosis and treatment of Hyperphenylalaninemia in Denmark has been centralized to the John F. Kennedy Institute. Screening is performed at day 5-7 of life and neonates with Hyperphenylalaninemia, their parents and siblings are referred to the John F. Kennedy Institute. Differential diagnosis is carried out by means of mutation analyses (7) and a phenylalanine restricted diet is introduced to keep blood phenylalanine levels below 300 $\mu\text{mol/l}$. The family stays at the institute for 2-3 weeks. The phenylalanine restricted dietary treatment is monitored by weekly determinations of blood phenylalanine during the first two years of life. This interval is gradually increased up to a month. PKU patients (joined by their parents) are called in for a medical check-up and dietary counselling every third month in the first 2 years of life, then twice a year and after 8 years of age, once a year.

At 15½ years of age the PKU patients are proposed two alternatives: They may continue the low-phenylalanine diet or they may adopt a less restricted diet (no dairy products) that is supplemented with amino acid tablets (PreKUnil, PreKUlab A/S). The third possibility, no treatment at all, is not suggested. Usually, the choice of diet is left to the patient, but in cases where compliance with a strict diet has been particularly poor' .relaxed we advise the young adult to choose the tablet diet, rather than continuing a strict diet that is ignored most of the time.

Experimental design.

At 15 years of age all patients were tested with one of the parallel sets in the test battery in order to familiarize them with a new type of test. Six months later all patients were tested again with another of the parallel sets and this test result was regarded as the base reference for the following

tests. Then 23 patients were allocated to the less restricted diet plus amino acid tablets (group 2) and compared to 30 patients who continued the usual phenylalanine restricted diet supplemented with a formula (group 1). Regardless of treatment all patients were tested with one of the parallel sets of neuropsychological tests 6 months later (16 years of age) and then once a year for another two years. An overview of the experimental design together with background information for the groups is given in Table 2. A control group of 36 normal pupils at the same age as the PKU group were tested twice with an interval of 6 months.

Data analyses. Each test was standardized with the control group as reference group and all test scores were transformed to T-scores with a mean of 50 % and a SD of 10. All results are presented as T-scores. A parallel version of the battery was used at each trial. The statistical differences in T-scores between the base reference results (second trial) and the results of the third, fourth, and fifth trial, were evaluated by t-tests. Furthermore, a composite score for each trial consisting of the mean T-score for all test results was calculated. The composite scores for the five trials were subjected to repeated measurements MANOV A. Retest effects and group differences in changes were analyzed by unvaried and multivariate analysis of variants.

RESULTS

Table 3 lists mean IQ (WISC) values for the PKU patients at age 6, 10, and 14 years. The PKU group apparently scores a little above 100. However, the test was standardized in Denmark in the 1960ties with a mean of 100 and later studies have shown that the mean has increased. It is noteworthy that 41 % of the 10 year old PKU patients score in the upper end of the IQ scale (Table 4). The PKU patients scored below the normal control group on almost every test in the new neuropsychological test battery and the total PKU group was inferior "to the controls on 6 of 13 tests. This finding indicates that the test battery is more sensitive to deficits in cognitive functions than an IQ test. Both the PKU group and the normal control group did better on most tests at the second testing (Table 2) and no significant group differences were found (data not shown).

The means in T -scores for the testing when both groups were still on conventional phenylalanine-restricted diet at 15~ years of age (2. testing, the reference test) are presented to the left in Table 5. Just after the 2. testing 23 young adults changed to a relaxed diet supplemented with phenylalanine-free amino acid tablets (group 2) and 30 PKU adolescents continued the conventional strict dietary treatment with phenylalanine-free formula (group 1). The mean differences in T -scores between a 3., 4., and 5. testing (at age 16, 17, and 18 years) and the 2. testing is shown to the right in Table 5. Neither the unvaried nor the multivariate analysis of variance showed any significant differences between the groups. Neuropsychological functions improved to the same extend in both groups. The performance was slightly higher in most functions in group 1 as compared to group 2, but these differences were demonstrated already at the 1st and 2nd test, where all patients were still on the traditional phenylalanine-restricted diet. These differences were maintained through time and are not statistically significant. The group on amino acid tablets comprised a number of individuals for whom compliance to the strict diet had been particularly poor. At age 14 years the mean IQ (WISC-R) was 104 (later group 1) and 102 (later group 2). Mean plasma phenylalanine levels increased by approximately 30% in group 2.

Finally, a note should be made on 18 patients who have been on the relaxed diet supplemented with amino acid tablets for another two years or more and who were not included in the statistical analysis because they started on this treatment before the final test battery was selected. This smaller group of patients has also been followed closely with the neuropsychological testing and so far we have seen no signs of decline in test performances. Furthermore, the percentage of this group of patients going on to college or university was comparable with that found in the Danish population.

DISCUSSION

Thirty-five years of experience with early (<3 weeks postnatally) well controlled dietary treatment (blood phenylalanine levels 2-5 times normal) maintained for the first 15 years of life have revealed normal cognitive development, normal neuropsychological test performances and usually a normal psychiatric status and social adjustment in subjects with PKU (8,9,10). Studies on early treated subjects with good dietary control in childhood suggest that the risk of intellectual deterioration declines after the first decade, the inference being that from 10 years of age onwards the nervous system may be sufficiently mature to withstand the neurotoxic influence of persistent Hyperphenylalaninemia (mild PKU) on IQ (8,11,12,13).

A review of 21 published articles on neuropsychological performance of adolescents and young adults with classic PKU off diet who were treated early and fairly strictly revealed deficits in abstract reason, both in conceptual and Visuo-spatial areas. Some PKU individuals displayed deficits when required to integrate information (14). Studies of young adults off diet (15, 16) suggest that higher level problem solving, Le. "executive functions", was most noticeably affected. Individuals with PKU were not significantly different from controls on simple reaction time tests (tests of visual motor speed), but they tended to make more errors and to slow down to a greater extent as the complexity of the task increased (for review see 14). In addition, they showed a greater variation in their response times, indicating a deficit in sustained attention (17, 18, 19). Soft neurological signs (tremor, unusual brisk tendon reflexes) have been observed in more than 30% of early treated, well controlled young adults who have been off diet for some years (20, 21, 22, 23). In addition to the neuropsychological effects, it has been demonstrated that as phenylalanine concentrations increase, the water content of white matter increases, an effect which begins to become visible on magnetic resonance imaging (MRI) when concentrations increase above 600 $\mu\text{mol/l}$ (24, 25,26).

A quite recent follow-up study on patients who were treated 20-35 years ago have revealed that the subjects who maintained a phenylalanine-restricted diet reported fewer problems than the diet discontinuers, who had an increased rate of eczema, asthma, mental disorders, headache, hyperactivity, and hypo activity. Psychological data showed that lower intellectual and achievement test scores were associated with dietary discontinuation and with higher childhood and adult blood phenylalanine levels. Abnormal MRI's were associated with higher brain phenylalanine levels (4).

Concentrations in the cerebrospinal fluid of the neurotransmitters, dopamine and serotonin are

reduced in untreated Hyperphenylalaninemia (2, 27, 28, 29). Possible mechanisms for the impaired neurotransmitter synthesis include decreased transport of precursor amino acids across the blood-brain barrier into the neuronal cell and inhibition of tyrosine and tryptophan hydroxylases (3). Deficiencies in these neurotransmitters seem to play a role in behavioural disturbances associated with discontinuation of dietary therapy in PKU (30, 31, 32, 33, 34). We found that dietary supplementation with tyrosine and tryptophan in PKU patients who had terminated the diet increased dopamine and serotonin synthesis (5, 6) and improved performance on behavioural tests (5, 25, 35).

The neutral amino acids may be transported across the blood-brain barrier by a common carrier (36). The branched chain amino acids (leucine, isoleucine, and valine) have in fact been reported to reduce phenylalanine concentrations in the cerebrospinal fluid in PKU patients (37, 38, 39). Dietary supplementation with branched chain and other neutral amino acids produced improvement in neurologic, cognitive and behavioural measures (40, 41). It is noteworthy that normal brain phenylalanine levels were found in adult untreated PKU patients with normal IQ in spite of high blood phenylalanine levels (42, 43). When blood levels of the amino acids that compete with phenylalanine for transport across the blood-brain barrier were increased by extensive oral administration to PKU patients, it was possible to lower brain phenylalanine and to prevent neurotoxic effects (disturbed EEG) caused by an oral phenylalanine challenge (44).

The disadvantage of treating adults with PKU is the difficult regimen that needs constant monitoring and support. The advantages of continuing treatment are that the treatment reduces key biochemical abnormalities, reduces the risk of impaired dopamine and serotonin synthesis, reduces the risk of a probable impaired myelin turnover, improves neuropsychological performance, and may improve MRI changes (8). Confronted with these advantages and disadvantages in continuing treatment probably throughout life it is understandable that young people with PKU are faced with a very difficult decision whether to continue or to discontinue treatment. Many PKU centres are of the opinion that current knowledge makes it difficult to justify a policy of deliberately terminating treatment. Exceptions include subjects with mild PKU whose blood phenylalanine concentrations remain below 900 $\mu\text{mol/l}$ on a normal diet. Exceptions also include the group of subjects whose nutritional or emotional well-being is clearly being damaged as a result of attempting treatment, and those who wish to stop treatment despite having appraised the information now available. Indeed, whatever final conclusion emerges on the risks (or otherwise) of stopping diet, the individual patient's view is likely to have an important place in forming an opinion in each instance (8). Records on adolescents with PKU who have decided to stop a low phenylalanine diet often

reveal that they often have brought themselves on a very protein restricted diet, i.e. 0.5 g/kg/body weight in order to avoid headache and hangover-like symptoms. Such a very protein restricted diet is dangerous.

For these young adults with PKU an alternative treatment would be highly appreciated. The amino acid tablets used in the present study are developed on systematically scientific studies (44) and based on experimental results indicating that they may neutralize the negative effect of stopping a low-phenylalanine diet (5,34,35,45). Thus, a free diet, low in protein and supplemented with the present amino acid tablets, and with vitamins, minerals, and trace elements should be safe, as the tablets contain the essential amino acids, which may be ingested in too low amounts on a low protein diet, and high amounts of tyrosine and tryptophan which may counteract the impaired synthesis of neurotransmitters. In addition, the tablets contain branched chain amino acids which reduce phenylalanine transport into brain tissue in patients with PKU.

In conclusion, the present study provides evidence that the biochemical and behavioural effects following diet discontinuation in classical PKU are prevented when a protein restricted free diet is supplemented with the present phenylalanine-free amino acid tablets, and with vitamins, minerals, and trace elements (cf.32).

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