Phenylketonuria: impact and implications

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CHAPTER

Magnetoencephalography in adults with phenylketonuria: A placebo-controlled crossover trial into the effect of elevated phenylalanine levels

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ABSCTET

Introduction
Phenylketonuria (PKU) is an inborn error of metabolism caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH). Consequently, phenylalanine (Phe) accumulates and causes neurocognitive damage. Therapy with a stringent protein restricted diet, can prevent these severe sequelae. Nowadays there is debate on the necessity of the strict dietary control in adult patients. In the present study magnetoencephalography (MEG) was used to study the effect of elevated plasma Phe levels on brain activity in PKU patients.

Methods
The pattern of the MEG power spectrum was studied in patients participating in a placebo-controlled crossover study in early and continuously treated adults with PKU, of which the results on executive functioning and mood have been published previously (Ten Hoedt 2010). Nine adults with PKU underwent two 4-week supplementation periods: one with Phe, mimicking normal dietary intake and one with a placebo. MEG registries were performed in an eyes closed and an eyes open, resting state condition, at the end of every study period. Relative spectral power was calculated in delta, theta, alpha, beta and gamma frequency bands.

Results
Phe-loading did not result in a difference in the power of the five frequency bands of the MEG, compared to placebo. Furthermore there was no difference in the relative power analyzed for the 10 different cortical areas between the Phe-loading study period and the placebo study period.

Conclusions
High plasma Phe levels do not induce power spectrum changes. Possibly elevated Phe levels have an effect on functional interactions between brain regions. Therefore research on the functional connectivity in the MEG recordings of these patients is warranted.
INTRODUCTION

Phenylketonuria (PKU; MIM 261600) is an autosomal recessive inherited inborn error of amino acid metabolism caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH; EC 1.14.16.1), which converts phenylalanine (Phe) into tyrosine. The deficient activity of the enzyme results in an elevated Phe level, which is neurotoxic. If untreated, PKU leads to progressive intellectual disability and neurological impairment (1). These sequelae can be prevented almost entirely by detection through newborn screening followed by the initiation of a dietary treatment, consisting of a limited Phe intake by a protein-restricted diet and supplementation of all amino acids but Phe.

There is increasing controversy about the necessity of the strict dietary control in adulthood and there is a wide variation in dietary guidelines for adults (2). Dietary discontinuation in adulthood might not cause a decrease in IQ (3), but executive function deficits, associated with high Phe levels, have been reported (4;5). The prefrontal cortex plays a major role in the executive functioning and dopamine is crucial for the function of this part of the cortex. Since tyrosine is a precursor of the essential neurotransmitter dopamine, it is hypothesized that dopamine deficiency due to a shortage of tyrosine could be the mechanism underlying executive function deficits in patients with PKU (6). Dopaminergic neurons projecting on the prefrontal cortex are highly sensitive to even small decreases in tyrosine (7). Another hypothesis for dopamine depletion in the prefrontal cortex, is hypomyelinisation resulting in down-regulation of the neurotransmitter production (8).

In PKU patients various studies have been performed to measure the brain activity and to study structural changes of the brain in order to unravel possible mechanisms of the neuropathology associated with PKU. Magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (MRS) studies reported diffuse white matter abnormalities which are correlated with lifetime and concurrent Phe levels (9;10) and brain Phe (11).

Computerized electroencephalogram (EEG) spectral studies in children and adults with PKU, demonstrated that elevated Phe levels results in a reduced mean frequency of the EEG power spectrum (12;13).

A potentially suitable method to measure brain activity in PKU patients is magnetoencephalography (MEG). MEG measures the brain's magnetic fields originating from neuronal currents (14). Advantages of MEG above conventional EEG recordings
are that MEG is hardly affected by the skull, a reference electrode is not required and that measurements from a large number of sensors are more easily provided. Therefore MEG generates a more accurate recording of ongoing brain activity (15).

In order to investigate the effects of elevated plasma Phe levels on resting state brain activity, we performed MEG registries in patients participating in the double-blind placebo-controlled crossover Phe-loading study in early and continuously treated adults with PKU, of which the results on executive functioning and mood have been published previously (16). We hypothesized that elevated Phe levels might induce slowing of the frequency pattern of brain activity. Because executive function deficits of the prefrontal cortex have been reported to be associated with elevated Phe levels (17), a more prominent effect might be seen in the frontal areas.

METHODS

Patients
Patients were recruited via treating metabolic physicians in the Academic Medical Center Amsterdam (AMC), the University Medical Centers of Maastricht, Nijmegen, and Groningen (The Netherlands), and the Centrum Pinocchio, Diepenbeek (Belgium). Eligible for inclusion were PKU patients aged ≥ 18 years, who were diagnosed by newborn screening and continuously treated. Another inclusion criterion was that in the year prior to the start of the study the mean plasma Phe level had to be < 1100 µmol/L. Exclusion criteria were mental or legal incapacitation, drug or alcohol misuse, a diagnosis of neurological disease, psychiatric illness, lack of fluency in Dutch and inability to comply with study procedures. For females, additional exclusion criteria were pregnancy or the wish to become pregnant within a year of the start of the study. Patients signed an institutionally approved informed consent prior to enrolment. Approval for this study was granted by the Ethical Committee of the AMC, Amsterdam. The trial was registered with The Netherlands National Trial Register (NTR # 1056) before recruiting of patients.

Procedure
Throughout the study, patients had to continue their prescribed low Phe diet and amino acid supplementation. In order to determine the patients Phe intake, daily natural protein consumption was evaluated by a dietician through a 3-day dietary record prior to the start of the study. The study design was double-blind and cross-over: patients were
randomly assigned to group 1 or group 2, starting with consumption of either capsules containing a placebo or with capsules containing Phe. After patients completed 4 weeks, they crossed over to the alternative arm. Throughout the period in which the patients consumed the Phe-containing capsules, the received amount of Phe was individually calculated, taking into account the patient’s dietary Phe intake. The purpose was to reach the total Phe intake likely to be consumed by a healthy adult of the same sex and weight and thus mimicking the condition of being fully ‘off diet’. The protein intake of healthy Dutch adults is higher in men than in women. Average Phe intakes aimed at were: 4,500 mg/day for male patients between 60 and ≤ 95 kg, 4,000 mg/day < 60 kg, 5,000 mg/day > 95 kg, 3,000 mg/day for female patients between 50 and ≤ 85 kg, 2,500 mg/day < 50 kg, and 3,500 mg/day > 85 kg. The capsules were identical in appearance for placebo and Phe. Patients had the choice to swallow the capsules whole or to open the capsules and add the content to their amino acid mixture. Patients took 2-8 capsules a day (average 5 capsules), divided over 3 doses. Patients were asked to record the intake of the capsules in a diary, in order to monitor compliance with the capsules. Between the two study periods there was a washout period of at least 4 weeks in which patients consumed their usual PKU diet and amino acid formulas.

**Randomization and masking**

A randomization coding list was rendered by an independent data manager. A non-deterministic version of the minimization method (18) was used to balance treatment arms. No stratification factors were used. The list, containing numbers linked to the patients by the study physician, and allocated treatment assignments, was sent to the pharmaceutical company which used it to prepare the capsules. All patients and investigators were masked to treatment assignment. The capsules were prepared and coded by Nutricia Liverpool (Liverpool, UK) and distributed to the patients by the study physician (A.H.) at the start of each study period. Capsules were packed in identical tubs, labelled with patient number, phase number, and a three letter code and bar code indicating placebo or Phe. In order to prevent identification and comparison by the patients, three different three letter codes were used for both Phe- and placebo-containing capsules.
Biochemical monitoring
During the two 4-week study periods and during the week before each study period, Phe levels in bloodspots were measured twice weekly. Phe levels were measured using tandem mass spectrometry (standard neutral loss method). Results were not disclosed to the investigators until data were ready for analysis.

IQ
IQ was estimated using a 4-subtests short form of the Wechsler Adult Intelligence Scale-Revised (WAIS-III) (19) consisting of vocabulary, picture completion, block design and similarities.

Magnetoencephalography
The MEG recordings were performed at the last day of every study period. Patients were situated in a magnetically shielded room. MEG registration was performed in two no-task conditions: an eyes closed resting state condition of 5 minutes (EC), followed by an eyes open resting state condition of 5 minutes (EO). Magnetic fields were recorded while subjects were placed inside a magnetically shielded room (Vacuumschmelze GmbH, Germany) using a 151 channel whole-head MEG system (CTF systems Inc., Canada). A third order software gradient (Vrba, 1996) was used with a recording passband of 0.25 – 125 Hz. Spatiotemporal signal space separation (tSSS) (20) was performed on all MEG recordings in order to suppress interference of signals originating from distant and nearby source like magnetized wires behind the teeth. Four artefact free trials of 6.55 seconds in duration (4,096 samples) of each condition (EC and EO) were selected for further analysis. The selection was performed by a single investigator who was blinded (A.H.). Results of the selected trials were averaged for each subject. MEG recordings were filtered with a band pass of 0.5-48 Hz. For the 2 study periods the difference in relative band power was computed in the following frequency bands: 0.5-4 Hz (delta band), 4-8 Hz (theta band), 8-13 Hz (alpha band), 13-30 Hz (beta band) and 30-48 Hz (gamma band). Furthermore the difference in relative power was computed for the ten different cortical areas (left and right side of the following regions: frontal, central, occipital, temporal and parietal), between placebo and Phe-loading.

Statistical analysis
MEG data, obtained at the end of each study period, were pooled across groups per condition (placebo, Phe). For the relative band power, repeated measures analysis
of variance (ANOVA) was performed, with experimental condition (placebo versus Phe-loading), eyes state (EO or EC) and cortical areas used as within-subject factors. For the relative power of the brain regions, repeated measures analysis of variance (ANOVA) was performed, with experimental condition (placebo versus Phe-loading), eyes state (EO or EC) and frequency band (delta, theta, alpha, beta and gamma) were used as within-subject factors.

RESULTS

Patients
129 patients were eligible for inclusion in the study, of which 12 patients agreed to participate. One patient was excluded before the start of the study because of a co-existing illness which might affect cognitive abilities. Patients were randomly allocated to one of the study arms: either Phe-loading – placebo or placebo – Phe-loading. One patient withdrew before the start of the investigations because of pressure at work and one patient withdrew after the first neuropsychological test because his partner feared elevated-Phe-related negative changes in his behaviour. All of the remaining 9 patients completed the study. Table 1 shows the patients’ baseline characteristics.

IQ
The mean IQ, estimated from the 4-subtest short form of the Wechsler Adult Intelligence Scale-Revised, were within normal range (M = 97.22, range 83 -117).

Phe levels
Mean blood Phe value was 1,271 µmol/L (SD ± 90) in the Phe-loading phase and 657µmol/L (SD ± 80) in the placebo phase. This was significantly different (Wilcoxon signed-ranks test \( p=0.008 \)).

Magnetoencephalography
Statistical analysis of the relative band power showed that Phe-loading did not result in a difference in the power of the 5 frequency bands, compared to placebo. Furthermore there was no difference in the relative power analyzed for the 10 different cortical areas between Phe-loading and placebo.
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DISCUSSION

This is the first study investigating the effects of elevated Phe levels on the brain activity in early treated adults with PKU, measured with MEG. We demonstrated that no significant effect of elevated Phe levels can be established on the relative power in the frequency bands and cortical areas. Since hyperphenylalaninemia in PKU patients has predominantly an effect on the brain function and development, it was expected that MEG disturbances could be found.

Executive function deficits, associated with high Phe levels have been reported in PKU patients (17). The outcome of the neuropsychological examination in our previously published study did indeed reveal impairment in sustained attention during elevated Phe levels (16). Possibly dopamine depletion, either due to a deficiency of its precursor tyrosine (6) or due to hypomyelinisation (8) plays an important role in the development of the executive function deficits. Previously, it was hypothesized that neurotransmitter deficiency in PKU patients due to high Phe levels, might influence brain activity. Electroencephalography (EEG) studies have been performed to examine this effect and it was demonstrated that elevated Phe levels influence the frequency distribution of EEG background activity in early treated children and adults with PKU. Phe-loading resulted in a shift in the dominant peak of the background activity from the alpha band to the lower frequency spectrum as well as an increase in slow wave activity in the theta and delta band (12;13). Krause et al (21) demonstrated a slowing in mean power frequency during high Phe levels, with reversal of the effect on lowering the plasma Phe levels. This indicates an increase in the power concentration of the slow wave frequencies, which might be associated with decreased alertness (22).

MEG is another and more accurate method to measure the electrical field produced by brain activity. Slowing of the MEG background activity was demonstrated in studies involving Parkinson patients (23;24). In Parkinsons disease the deficit of dopamine in the striatum is a prominent neuropathological feature, resulting in an impaired outflow from the basal ganglia to the prefrontal cortex. Dopamine suppletion has no reversing effect on the slowing of the background activity (23). Functional interactions between different brain regions, functional connectivity, have been studied as well in Parkinson patients. In contrast with the spectral changes, functional connectivity in these patients responds to dopamine suppletion (25). The conclusion of these studies might be that dopamine has an effect on functional interactions between brain regions, but does not affect power (15). This might be a
possible explanation why the MEG recordings in PKU patients with elevated Phe levels do not show any disturbance; the possibly decreased brain dopamine levels might only affect functional connectivity in PKU patients as well.

We recognize that a limitation of the present study is the small number of participating patients. The small number of patients may have prevented unfavourable effects of Phe-loading reaching significance. Furthermore the registrations did contain noise artefacts (hence the need for tSSS), which might have influenced the data.

In conclusion, high Phe levels did not have an effect on power frequency spectrum of brain activity in PKU patients, measured with MEG. Since there might be an effect on functional interactions between brain regions, analysis of the functional connectivity in the MEG recordings of these patients is warranted.

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REFERENCES


