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Neuropsychological and biochemical investigations in heterozygotes for phenylketonuria during ingestion of high dose aspartame (a sweetener containing phenylalanine)

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Abstract. Aspartame, a high intensity sweetener, is used extensively worldwide in over 5,000 products. Upon ingestion, aspartame is completely metabolized to two amino acids and methanol (approximately 50% phenylalanine, 40% aspartic acid, and 10% methanol). The effects of aspartame on cognitive function, electroencephalograms (EEGs) and biochemical parameters were evaluated in 48 adult (21 men, 27 women) heterozygotes for phenylketonuria (PKUH). PKUH subjects whose carrier status had been proven by DNA analysis ingested aspartame (either 15 or 45 mg/kg/day) and placebo for 12 weeks on each treatment using a randomized, double-blind, placebo-controlled, crossover study. A computerized battery of neuropsychological tests was administered at baseline weeks –2 and –1, and during treatment at weeks 6, 12, 18, and 24. Samples for plasma amino acids and urinary organic acids were also collected during these visits. EEGs were evaluated by conventional and spectral analysis at baseline week –1 and treatment weeks 12 and 24. The results of the neuropsychological tests demonstrated that aspartame had no effect on cognitive function. Plasma phenylalanine significantly increased, within the normal range for PKUH, at 1 and 3 h following the morning dose of aspartame in the group receiving the 45 mg/kg per day dose only. There were no significant differences in the conventional or spectral EEG analyses, urinary organic acid concentrations, and adverse experiences when aspartame was compared with placebo. This study reaffirms the safety of aspartame in PKUH and refutes the speculation that aspartame affects cognitive performance, EEGs, and urinary organic acids.

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Introduction

Aspartame has been demonstrated to be safe for the general population (Janssen et al. 1988; Butchko et al. 1989). However, since aspartame is completely metabolized to its components (approximately 50% phenylalanine, 40% aspartic acid, and 10% methanol), questions have been raised as to whether the plasma phenylalanine (phe) of aspartame is harmful to the approximately 2% of the general population who are heterozygous for phenylketonuria.

Heterozygotes for PKU (PKUH) have approximately half the normal ability to convert phe to tyrosine (tyr; Bartholomé et al. 1975; Grimm et al. 1977). This biochemical "susceptibility" led to the speculation that PKUH may have a genetic disadvantage and, to some extent, may suffer from biochemical imbalances observed only in homozygotes for PKU (Ford et al. 1977). The theory behind these speculations is based on phe sharing a common transport system with the other large neutral amino acids (LNAA: isoleucine, leucine, valine, tyrosine, tryptophan, and methionine) for entry into the brain (Fernstrom 1990). Because this system is saturated at normal plasma LNAA concentrations, the LNAAs compete for entry into the brain. It has been suggested that aspartame consumption may selectively increase plasma phe, and thus increase brain levels, by increasing the molar ratio of plasma phe concentration to the sum of the plasma concentration of the other LNAA (phe/LNAA). This potential competitive advantage for phe to enter the brain following aspartame consumption has been suggested to affect brain function, i.e., behavior, cognition, and the EEG, by altering brain neurotransmitter concentrations (Elsas et al. 1988). Matalon et al. (1988) claimed that PKUH receiving high doses of aspartame (100 mg/kg of body weight per day) may have plasma phe levels and urinary metabolites in the same

Table 1. Outline of study

	Pre-treatment			Treatment period I				Treatment period II		
Weeks	–2	–1	1–5	6	7–11	12	13–17	18	19–23	24
Medical history	×									
Physical exam	×									×
Hematology	×					×				×
Urine analysis	×					×				×
Urine pregnancy test[a]	×									
Blood chemistries	×					×				×
EEG		×				×				×
SVAT battery	×	×		×		×		×		×
Plasma amino acids	×	×		×		×		×		×
Urine organic acids	×	×		×		×		×		×
DNA analysis	×									
Test article administration			◄-----1-----►				◄--------2--------►			
Weekly phone call[b]			◄----------►							

[a] Females of childbearing potential
[b] To determine compliance or problems with dosage regimen

range as PKU patients requiring dietary treatment. They speculated that under these conditions consumers of aspartame may be at risk for brain damage.

Aspartame is currently being consumed by over 200 million consumers worldwide, approximately 2% of whom are PKUH. This prompted us to conduct our study. Our objective was to determine aspartame's effect, at two dose levels over several months, on cognitive function, EEGs, and biochemical parameters in PKUH.

Materials and methods

Study population. A total of 49 adult phenylketonuric heterozygotes (27 females and 22 males) aged 18 to 54 years, who were known to our research group, were enrolled in the study. Exclusion criteria were: (1) subjects with PKU; (2) concomitant illness and medication that would interfere with results; (3) the taking of investigational medication within 30 days prior to study; and (4) positive pregnancy test. Twenty-four subjects (9 men and 15 women) were randomized to receive 45 mg/kg aspartame per day and 25 subjects (13 men and 12 women) 15 mg/kg aspartame per day. One subject was dropped from the study prior to test article ingestion because of an inability to complete the neuropsychological tests successfully. All 48 subjects who received the test article completed the study. These subjects had a mean (± SD) age of 37 ± 1.77 years and 36.7 ± 1.94 years in the 15 and 45 mg/kg aspartame per day groups, respectively. The mean (± SD) body weight of the subjects was 67.5 ± 3.96 kg for women and 78.9 ± 2.28 kg for men. All subjects provided written informed consent to participate in the study. The study protocol was approved by the University of Heidelberg Institutional Review Board.

Subjects received aspartame, either 15 or 45 mg/kg per day and placebo in a randomized, double-blind, placebo-controlled, crossover design. Opaque-white capsules containing either 300 mg of aspartame or microcrystalline cellulose (placebo) were used. The capsules were ingested in three doses at 7 a.m., 1 p.m., and 7 p.m. for 12 weeks on each treatment (Table 1). Subjects were first randomized to 15 or 45 mg/kg per day sub studies of 24 subjects each, and then randomized to receive aspartame-placebo or placebo-aspartame according to a computerized randomization schedule prepared prior to initiation of the study.

Times of blood sampling for the biochemical investigations, the cognitive test battery, and EEG measurements are outlined in Table 1. Subjects ingested one-third of the daily intake of test capsules immediately before a standardized breakfast taken in the hospital. At weeks 6 and 12 of treatment, a blood sample for amino acids and blood chemistry was drawn approximately 1 h after the ingestion of the morning capsules, immediately before the initiation of the cognitive test battery. Another blood sample for amino acid determination was obtained upon the completion of the cognitive test about 2 h later. Together with the EEG recording the entire test session required about 4 h.

Carrier status of subjects was determined by DNA restriction fragment length polymorphisms (RFLPs) of the phe hydroxylase gene (Lichter-Konecki et al. 1988) and mutation analysis as described by Lidsky et al. (1985). Predicted residual activity for the PKUH was estimated as described by Okano et al. (1991).

Routine hematology, urine pregnancy test (for women of childbearing age), fasting blood chemistry, and urine analysis (including microscopic exam) were performed as shown (Table 1). Plasma amino acids were quantified by an automatic cation exchange column chromatography analyzer (BIOCAL 2000), except for tryptophan (trp), which was analyzed by HPLC (Qureshi et al. 1984). Plasma amino acid profiles were determined at baseline weeks –2 and –1 and at treatment weeks 6, 12, 18, and 24 (Table 1). In addition to the amino acids, the following amino acid ratios were determined: Phe/LNAA, Trp/LNAA, Tyr/LNAA, and Phe/Tyr. Urine organic acids were determined in the first urine in the morning at intervals shown (Table 1). The organic acids related to phe (phenylacetic, phenylpyruvic, phenyllactic, *o*-hydroxyphenylacetic, *m*-hydroxyphenylacetic, and mandelic) and tyrosine (*p*-hydroxyphenylpyruvic, *p*-hydroxyphenylacetic, and *p*-hydroxyphenyllactic) were analyzed. Organic acids in urine were determined using automatic gas chromatography/mass spectrometry. Samples were prepared according to the method of Tanaka et al. (1980), with a modification using a mass-selective detector (HP 59970 C) in the quantification, as described by Hoffmann et al. (1989).

Cognitive function was evaluated with a computerized cognitive test battery (SVAT; de Sonneville et al. 1988, 1993). The neuropsychological test battery consisted of six computerized tests and one non-computerized test for short-term memory. The subjects were required to respond to visual stimuli presented on a computer screen by pressing a key on a two-button response panel. The tests were always presented by the same neuropsychologist and in the same order for all test sessions. The battery included: (1)

Table 2. Plasma amino acid concentrations (μmol/l) and plasma ratio determinations

	15 mg/kg per day Mean ± SD			45 mg/kg per day Mean ± SD		
	Aspartame	Placebo	*P* value	Aspartame	Placebo	*P* value
Phenylalanine						
1 h post-dose	95.4 ± 21.01	95.6 ± 30.48	0.97	116.9 ± 29.48	90.4 ± 21.23	<0.01
3 h post-dose	94.1 ± 26.38	95.4 ± 30.15	0.84	112.5 ± 40.30	88.7 ± 22.36	<0.01
Phenylalanine/LNAA[a]						
1 h post-dose	0.166 ± 0.023	0.154 ± 0.030	0.06	0.207 ± 0.034	0.162 ± 0.026	<0.01
3 h post-dose	0.168 ± 0.027	0.162 ± 0.033	0.32	0.207 ± 0.039	0.158 ± 0.022	<0.01
Tyrosine/LNAA[a]						
1 h post-dose	0.107 ± 0.022	0.106 ± 0.025	0.75	0.107 ± 0.021	0.106 ± 0.019	0.75
3 h post-dose	0.095 ± 0.039	0.087 ± 0.030	0.59	0.097 ± 0.052	0.094 ± 0.036	0.59

[a] LNAA, Large neutral amino acids (isoleucine, leucine, valine, tyrosine, tryptophan, methionine)

a simple reaction time task for the measurement of baseline speed (reference level); (2) divided attention tasks: a series of binary choice target detection tasks, following the additive factor model (Sternberg 1969), which probes the following processing stages (task manipulation between brackets): encoding, i.e., processing of the stimulus at the physical code level (stimulus quality), memory search (size of target set or load); decision (stimulus type, i.e., target versus non-target); response organization (stimulus-response compatibility); (3) a focused attention task; (4) a sustained attention task, including vigilance; and (5) a short-term memory task (forward and backward auditory digit span).

Standard EEGs (10/20 system, HF 70 Hz, TC 0.3 s, bipolar and monopolar montage against common reference with subjects lying relaxed with eyes closed) were recorded at the times shown in Table 1 using a 17 channel Nihon-Kohden apparatus. Six leads of the monopolar records (F3, F4, C3, C4, 01, 02) and a time code signal were stored on magnetic tape (Johne and Reilhofer PCM apparatus) in digital format (500 Hz analog-digital rate and 12-bit amplitude resolution). Clinical evaluation of EEGs was performed independently by two neurologists to determine whether differences existed between treatments. From each monopolar record of the EEGs, 10 sections of approximately 8 s duration, free of artifacts and paroxysms, and in the alert eyes-closed state, were chosen visually (Benninger et al. 1984, 1985). The fast Fourier transformation was performed to yield the power in the following frequency ranges: delta (1.0–3.5 Hz), theta (> 3.5–7.5 Hz), alpha (> 7.5–12.5 Hz), beta 1 (> 12.5–18.0 Hz), beta 2 (> 18.0–25 Hz). Mean power frequency (MPF), mean alpha-theta frequency (MAT; restricted to the 6.0–12.5 Hz band), and half-power frequency (HPF) were computed. Spectral parameters were also calculated using the method of Epstein et al. (1989).

Statistical evaluation. Quantitative variables, including error rate and response time variables from the SVAT tests, EEG spectral data, organic acids in urine, and amino acid levels in plasma, were subjected to analyses of variance (ANOVA) appropriate for a crossover study. No adjustment was made for the large number of statistical tests. Statistical significance is assumed at *P*-values of 0.05. All pairwise tests are two-tailed. Statistical tests for within subject differences in the clinical EEG evaluations and the occurrence of adverse events were done by the exact form of McNemar's test (Fleiss 1981).

Results

DNA analysis of the phe hydroxylase gene confirmed PKUH in all 48 subjects studied. In 31 individuals the underlying mutation was identified, and in the other subjects the carrier status was confirmed by determination of the DNA RFLPs and determination of the DNA haplotype. No difference in the plasma phe concentrations was found in PKUH bearing a mutation in the phe hydroxylase gene, which results in a predicted residual activity (PRA) of > 50% of normal versus PKUH with a PRA of 50%.

There were no significant differences in mild adverse effects (e.g., headache) when aspartame was compared with placebo. No severe side effects were observed. Regular telephone calls and capsule counting revealed excellent compliance (mean > 96%, range 88–102%).

There was a significant rise in plasma phe for both blood drawings in the high-dose group (45 mg/kg per day) in contrast to the low dose group (15 mg/kg per day), the same was true for the ratios of phe/tyr and the ratio of phe/LNAA (Table 2). No significant effects of treatment were found for any of the other amino acids, including aspartate.

There was no increase in excretion of organic acids in urine even in the high dose group. Only *o*-hydroxyphenylacetic, *m*-hydroxyphenylacetic, *P*-hydroxyphenylacetic, and *p*-hydroxyphenyllactic acids were detected consistently. The other organic acids (e.g., phenylpyruvic acid) were found only on rare occasions and, when present, most were found only in trace amounts (< 2 mmol/mol creatinine). Their scarcity limited the statistical analyses of these organic acids.

No statistically or clinically significant treatment differences were observed with the EEGs, and there were no statistically significant treatment differences for any of the EEG spectral parameters. Specifically, there were no statistically significant differences in the MAT (Fig. 1), MPF (Fig. 1), or the HPF when aspartame was compared with placebo, regardless of the method used for computation of spectral values.

Overall, statistical analysis of the neuropsychological data resulted in 230 significance tests of treatment, only 8 of which (3%) revealed significant differences. This small number of significant results is less than might be expected by chance. Specifically, there were no statistically significant treatment effects among the error rate vari-

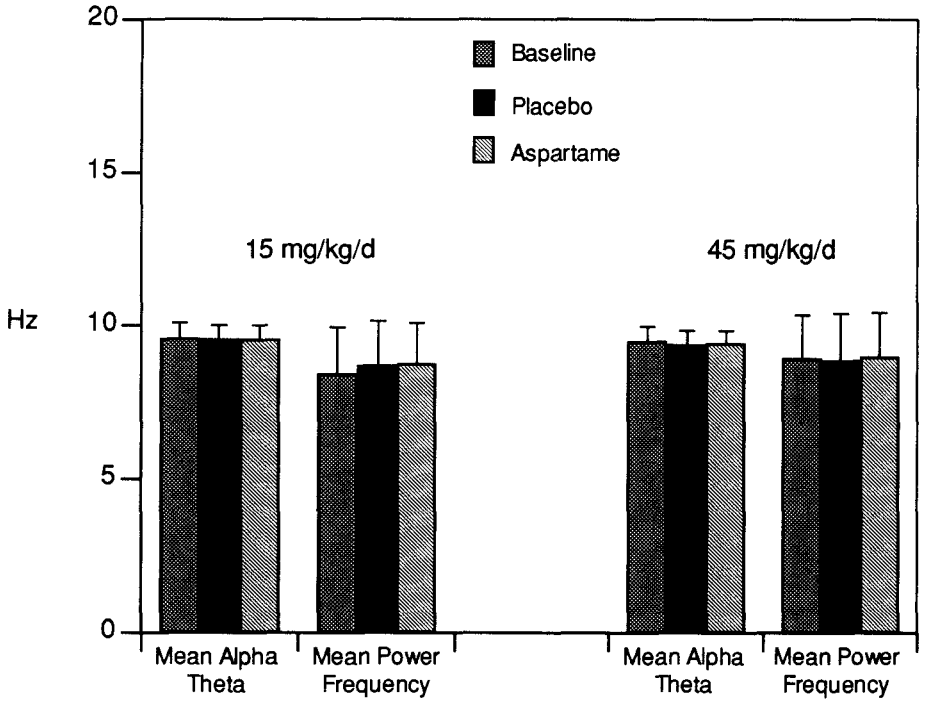

Fig. 1. MAT and MPF (mean with a SD *error bar*) of the EEG at baseline (week −1) and following ingestion of aspartame and placebo doses of 15 and 45 mg/kg per day at weeks 12 and 24

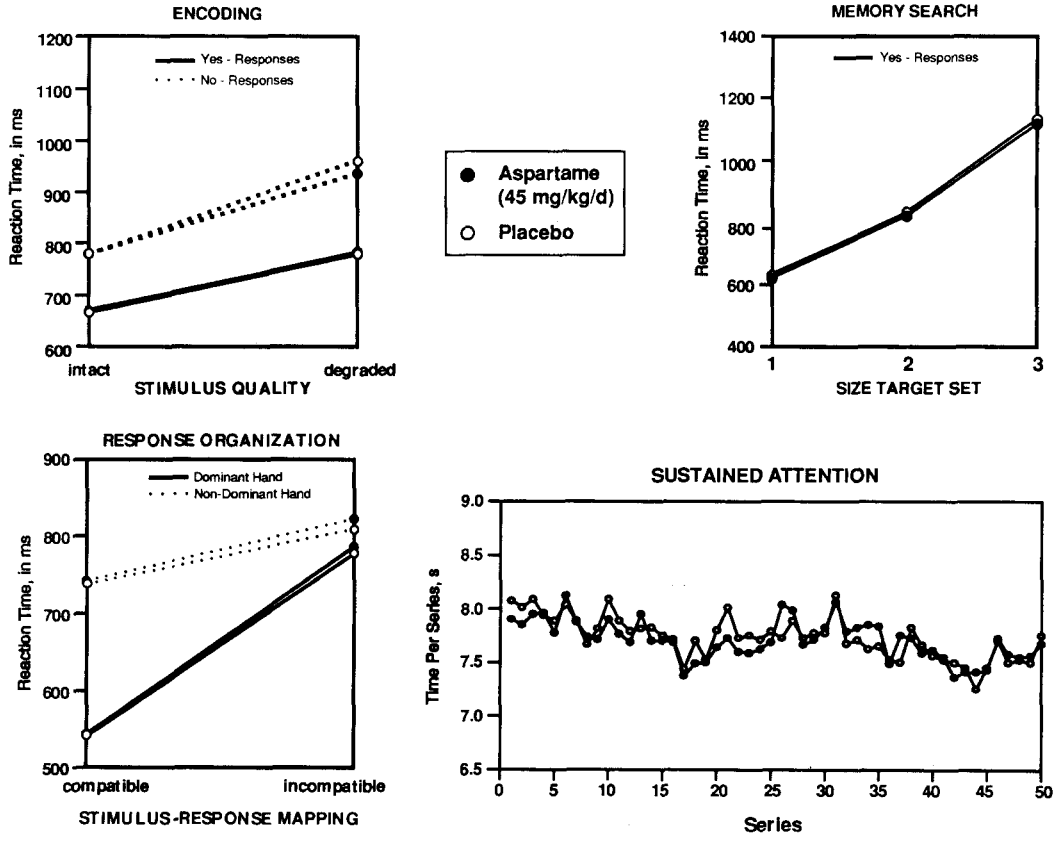

Fig. 2. Performance results of divided and sustained attention tasks for the 45 mg/kg per day substudy for aspartame and placebo. Mean response times are plotted as a function of treatment and task manipulation. Illustrations show mean reaction time of responses to the encoding task, memory search task, response organization task, and the sustained attention task

ables. Furthermore, all the significant results were found in the low-dose group, which further supports a lack of treatment effect.

There was no treatment effect and no interactions of treatment with task manipulation in the task battery were seen (Fig. 2). Response time increases with stimulus degradation (Fig. 2), but this effect is the same for both treatments. This demonstrates that aspartame does not affect the encoding stage of processing. Reaction time increases linearly with memory load, but this effect is the same with both treatments (Fig. 2), confirming that aspartame does not affect memory search. Incompatible stimulus-response mapping results in an increase in reaction time, and the effect is largest for the dominant hand (Fig. 2). This effect is the same with both treatments, confirming that aspartame does not affect response organization. The fluctuation in speed of performance with the time-on task, a measure of sustained attention, is the same in both treatments (Fig. 2), demonstrating that aspartame does not impair sustained attention capacity. The results of the other tasks (not shown) also provide no evidence for an unfavorable aspartame effect with regard to baseline speed, focused attention, and short-term memory capacity. In conclusion, the results confirm that aspartame has no effects on cognitive performance.

Discussion

The purpose of this study was to evaluate whether even very small imbalances of phe (derived from aspartame) in relation to LNAA have any effect on cognitive function and brain electrical wave activity in PKUH. It has been speculated that an additional phe load given to a PKUH could increase phe in the brain and consequently inhibit catecholamine synthesis, thereby promoting interferences in cognitive function, or cause deleterious nervous system effects (Maher et al. 1987). However, the proposed theory that aspartame will selectively increase the phe/LNAA is not unique to aspartame. A similar fluctuation in amino acids occurs following normal dietary practices, e.g., a sucrose-sweetened beverage leads to a similar increase in Phe/LNAA similar to that following an aspartame-sweetened beverage (Burns et al. 1991).

A sensitive battery of cognitive tests (SVAT) was chosen to evaluate cognitive performance. A primary reason for selecting this battery was that it is sensitive to changes in phe concentrations in children (de Sonneville et al. 1990; Schmidt et al. 1992), and in adults (Pietz et al. 1993). It has been demonstrated in PKU patients that a higher phe concentration parallels a slower performance in the sustained attention and divided attention tasks (de Sonneville et al. 1990; Schmidt et al. 1992). In addition, this test battery demonstrated significant differences in task performance between dietary well-controlled PKU patients and normal controls, revealing attention deficits in several processing stages and an impaired sustained attention (Schmidt et al. 1992; Pietz et al. 1993).

Three cognitive variables showed a significant main effect of treatment only in the 15 mg/kg group, not in the high-dose group. As no significant increase in plasma phe concentrations were observed in this group, these few test variables were most probably significant by chance, which is not unexpected since such a large number of significance tests (230) were performed. The absence of any effect in the high-dose group provides further support for the lack of a significant treatment effect.

We did not observe any effect on brain wave activity or cognitive function with the additional phe intake via aspartame ingested for 12 weeks in 48 PKUH. This is in contrast to the report by Elsas and Trotter (1988), who investigated only 6 PKUH for only 2 weeks, and then suggested that plasma phe changes as low as 7 μmol/l decrease the MPF of the EEG. Although our study used a lower dose of phe [45 mg/kg aspartame, as against 100 mg/kg phe used by Elsas et al. (1988)], in the high-dose group we observed a significant change in plasma phe of about three-fold the change Elsas et al. (1988) thought to be necessary to provoke brain electrical wave changes.

Clinical experience of untreated patients with mild hyperphenylalaninemia (plasma phe levels between 250 and 600 μmol/l) shows no increased rate of seizures or intelligence deficits (Scriver et al. 1989). In a study involving patients with PKU, a subgroup of patients whose plasma phe concentrations remained below 600 μmol/l exhibited a performance level in the cognitive tests battery which was in no way different from the performance of controls (de Sonneville et al. 1990). In contrast, patients with PKU according to the quality of their dietary treatment clearly show differences from controls. Also, there was no evidence from our study that an additional aspartame load of 45 mg/kg in PKUH resulted in urine aromatic acids derived from phe and tyrosine that approached levels found in the classic untreated PKU (Matalon et al. 1988). In our study no difference between aromatic acid excretion in urine following placebo and aspartame was found.

In conclusion, this study reaffirms the safety of aspartame, or corresponding phe loads from any dietary source, in PKU heterozygotes. These results refute the speculation that aspartame affects cognitive performance, EEGs, or urine organic acids when ingested in doses up to 20 times the current level of aspartame consumption (Butchko et al. 1991).

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