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Original Research Article

Cognition after a 4-week high phenylalanine intake in adults with phenylketonuria – a randomized controlled trial

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A B S T R A C T

Background: Phenylketonuria (PKU) is an autosomal recessive metabolic disorder characterized by increased phenylalanine (Phe) concentrations in the blood and brain. Despite wide agreement on treatment during childhood, recommendations for adults are still controversial.
Objective: To assess the impact of a 4-week increase in Phe intake (simulating normal dietary Phe consumption) on cognition, mood, and depression in early-treated adults with PKU in a double-blind, randomized controlled trial (RCT).
Methods: In a single-site crossover trial, 30 adult patients with classical PKU diagnosed at birth were recruited. All patients underwent a 4-week period of oral Phe administration (1500–3000 mg Phe/d) and a 4-week placebo period in a randomly assigned order with age, sex, and place of usual medical care as stratification factors. Analyses were based on the intention-to-treat (ITT) and per protocol (PP) approach to claim noninferiority (noninferiority margin –4%), with working memory accuracy as the primary endpoint and additional cognitive domains, mood, and depression as secondary endpoints.
Results: For the primary endpoint, a 4-week increase of Phe intake was noninferior to placebo with respect to working memory accuracy in both the ITT [point estimate 0.49; lower limit 95% confidence interval (CI): −1.99] and the PP analysis (point estimate −1.22; lower limit 95% CI: −2.60). Secondary outcomes (working memory reaction time, manual dexterity, mood, and depression) did not significantly differ between the Phe and placebo period, except for sustained attention (point estimate 31.0; lower limit 95% CI: 9.0). Adverse events were more frequent during the Phe than during the placebo period (95% CI: 1.03, 2.28, $P = 0.037$).
Conclusions: In early-treated adult patients with PKU, a 4-week high Phe intake was noninferior to continuing Phe restriction regarding working memory accuracy, and secondary outcomes did not differ except for sustained attention. Longer-term RCTs are required to determine whether low Phe levels need to be maintained throughout different periods of adulthood.
This trial was registered at the clinicaltrials.gov as NCT03788343.

Keywords: phenylketonuria, PKU, phenylalanine, metabolic control, suspension of phenylalanine restriction, cognitive performance, mood, depression

Introduction

Phenylketonuria (PKU) is an autosomal recessive metabolic disorder that occurs with a prevalence varying worldwide, with an average of ~1/10,000 newborns [1]. PKU is characterized by a deficiency of the enzyme phenylalanine (Phe) hydroxylase [2]. Impaired conversion of the amino acid Phe into tyrosine leads to increased concentrations of Phe in the blood and brain. If untreated during childhood, PKU has severe and irreversible neurologic and cognitive sequelae, such as mental retardation, behavioral and psychiatric problems, tremor, and epilepsy [2]. Early-initiated dietary protein restriction combined with Phe-free amino acid supplementation successfully prevents the

Abbreviations: AE, Adverse events; CI, confidence interval; D-KEFS, Delis–Kaplan Executive Function Test; ITT, intention-to-treat; IQ, intelligence quotient; IQR, interquartile range; mo, months; Phe, phenylalanine; PICO study, Phenylalanine and its impact on cognition; PKU, Phenylketonuria; POMS, Profile of Mood States; PP, per protocol; RCT, randomized controlled trial; TAP, Test of Attentional Performance.

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development of severe clinical manifestations [1]. However, despite early and continuous treatment, adult patients exhibit slight cognitive and psychosocial alterations [1,3,4].

Although there is general agreement on treatment during pregnancy and childhood, guidelines on target Phe concentrations during adulthood differ across countries. American guidelines recommend maintaining Phe concentrations <360 μmol/L, whereas European guidelines suggest a target Phe concentration of 120–600 μmol/L [5,6]. Many adult patients report difficulty adhering to the dietary recommendations, which leads to higher Phe concentrations than recommended [7]. Furthermore, a lifelong Phe-restricted diet can impose psychological, financial, and social burdens, which call into question the benefit of current treatment recommendations. As only a minority of patients are treated with sapropterin (Kuvan) or pegvaliase (Palynziq), a Phe-restricted diet remains the only treatment option for most early-treated adults with PKU.

Some association studies showed that higher concurrent Phe concentrations during adulthood might be linked with decreased cognitive performance in around 25% of the cognitive domains assessed [3, 8–16]. Different cognitive domains assessed with various cognitive tasks have been included in these association studies, such as executive functions [3,10,12,13,16], processing speed [13], working memory accuracy [9], verbal memory, and visual memory [10]. A recent meta-analysis across 757 early-treated adults with PKU suggests that impairments might occur in reasoning, visual speed measures, sustained attention, visuomotor control, and cognitive flexibility [4]. Effect sizes were larger with speed than accuracy measures and with visuospatial than verbal stimuli [4]. However, there is a lack of double-blind, randomized controlled studies in early-treated adults with PKU to prove the benefit of continuing the dietary restrictions. Only 1 randomized controlled trial with limited power assessing the impact of Phe on cognition in 9 early-treated adults suggested an adverse effect of a 4-week intervention with Phe on sustained attention and mood. However, no effects of high Phe were observed on other cognitive domains, such as reaction time, visuospatial processing, working memory accuracy, inhibition, cognitive flexibility, and visuomotor control [14].

The present noninferiority study examined the impact of a 4-week increase of Phe intake (simulating normal dietary Phe consumption) on cognition, mood, and depression in early-treated adults with classical PKU in a double-blind, randomized, crossover, controlled trial. The hypothesis was that a 4-week Phe load would not have a negative impact on the accuracy of working memory, other cognitive domains, mood, or depression scores.

Methods

Trial design

The PICO study (Phenylalanine and its Impact on COgnition) was a monocentric, randomized, double-blind, placebo-controlled, crossover, noninferiority trial. The detailed study protocol has been published previously [17]. Ethical approval was obtained from the local Ethics Committee in Bern, Switzerland. The trial complied with the Declaration of Helsinki and Good Clinical Practice guidelines and was registered with clinicaltrials.gov (NCT03788343). The study ran between 10 July 2019 (first patient in) and 17 June 2022 (last patient out). The first intervention started on 19 August 2019, and the last ended on 18 May 2022. During the COVID-19 pandemic, the study was interrupted for 9 weeks (March–May 2020).

Patients

Patients fulfilling all of the following inclusion criteria were eligible for the study: PKU diagnosed after a positive newborn screening, intervention with a Phe-restricted diet starting within the first 30 days of life, aged ≥18 years, capable of following the study procedure. Patients with any one of the following exclusion criteria were excluded: patients who had not been following a Phe-restricted diet during the 6 months before the study or who were not willing to continue the diet, Phe concentration > 1600 μmol/L in the 6 months before the study, concomitant disease states suspected to substantially affect primary or secondary outcomes (e.g., untreated vitamin B12 deficiency), drug or alcohol abuse, change in medications likely to substantially interfere with cognitive performance (i.e., antipsychotics, opioids, benzodiazepines, other sedatives), known or suspected hypersensitivity or allergy to one of the ingredients of the placebo, females who are pregnant or intend to get pregnant during the course of the study, lactation, female participants of childbearing potential (between menarche and menopause without hysterectomy or bilateral ovarectomy), not using or not willing to continue using a highly efficient method of contraception (Pearl index <1) for the entire study duration, known or suspected noncompliance, inability of the participant to follow the study procedures according to the medical history and an interview with the study physician at recruitment (e.g., language problems, psychological disorders, dementia), participation in another study with an investigational drug within the 30 d preceding and during the present study, previous enrollment in the current study, conditions interfering with MRI (e.g. magnetic particles in the skull or brain, cardiac pacemakers, deep brain stimulators, cochlear implants).

Patients were recruited from 7 metabolic outpatient clinics in Bern, Zurich, Lausanne, and Basel, Hamburg and Ulm, and Innsbruck. All study appointments took place at the Department of Diabetes, Endocrinology, Nutritional Medicine, and Metabolism, Inselspital, University Hospital Bern, Bern, Switzerland. Participants provided written informed consent before enrollment.

Travel costs for all 4 study visits were reimbursed, and a 1-time compensation of CHF 100 was given to symbolically remunerate the time and effort participants invested in the study. In addition, patients received a personalized written report on their individual cognitive performance after the unblinding.

Trial procedure

Participants were asked to follow their usual Phe-restricted diet and take their Phe-free amino acid supplements throughout the study. The intervention consisted of a 4-week period of oral Phe administration, simulating a controlled, temporary discontinuation of the patients' Phe restriction and elevating Phe to levels expected in an off-diet situation. For the controlled Phe-restricted situation, a placebo (pregelatinized corn starch, Lycatab C) was given to patients in the same manner, using a crossover design. Both Phe and placebo were administered in capsules. Patients received 1500–3000 mg Phe/day according to their sex and weight, or the corresponding dose of placebo [17].

Randomization procedure

Eligible patients were randomly allocated (1:1) to either the Phe–-placebo group (4-week intervention with Phe followed by a 4-week intervention with placebo) or the placebo–Phe group (4-week intervention with placebo followed by a 4-week intervention with Phe) with a 4-week washout period between the 2 interventions. Computer-generated central randomization (programmed in Stata, Version 15

SE [18]) was used to assign participants to the 2 groups, with age, sex, and place of usual medical care (i.e., study center site Bern or elsewhere) as stratification factors. Because of the small sample size and the large number of strata, blocks of size 2 were used to ensure optimal balance. The randomization list was prepared by an independent statistician at the Clinical Trial Unit, Bern, Switzerland, and was imported into REDCap (Vanderbilt University [19]). The list was transferred to the Laboratorium of Dr. G. Bichsel AG, where the Phe and placebo capsules were produced. Participants were enrolled by the study staff and randomized after assessment according to the inclusion and exclusion criteria. The entire study team and participants were blinded to the randomization result until after the initial analysis.

Four study appointments in weeks 0, 4, 8, and 12 took place before (weeks 0 and 8) and after (weeks 4 and 12) the Phe and placebo intervention periods. A 4-week washout period was scheduled between weeks 4 and 8 to prevent carry-over effects. Each study appointment comprised blood sampling after an 8–12-h overnight fast, MRI acquisition (not reported here), and a neuropsychologic assessment.

Trial outcomes

All cognitive outcomes are presented in the study protocol [17] and Table 2. The selection of cognitive domains for the primary and secondary outcomes was based on the results of previous research with early-treated adults with PKU available at the time of the study setup. Previous studies presented *1*) particular vulnerability of working memory accuracy (*n*-back task) to an off-diet condition [20], *2*) a relationship between poor performance on working memory tasks and high concurrent Phe levels [9,21], *3*) significantly more errors in working memory accuracy in patients with early-treated PKU than controls [9], and *4*) altered performance in processing speed, executive functions, attention, and motor skills in adult patients with early-treated PKU [4,9,13–15,21].

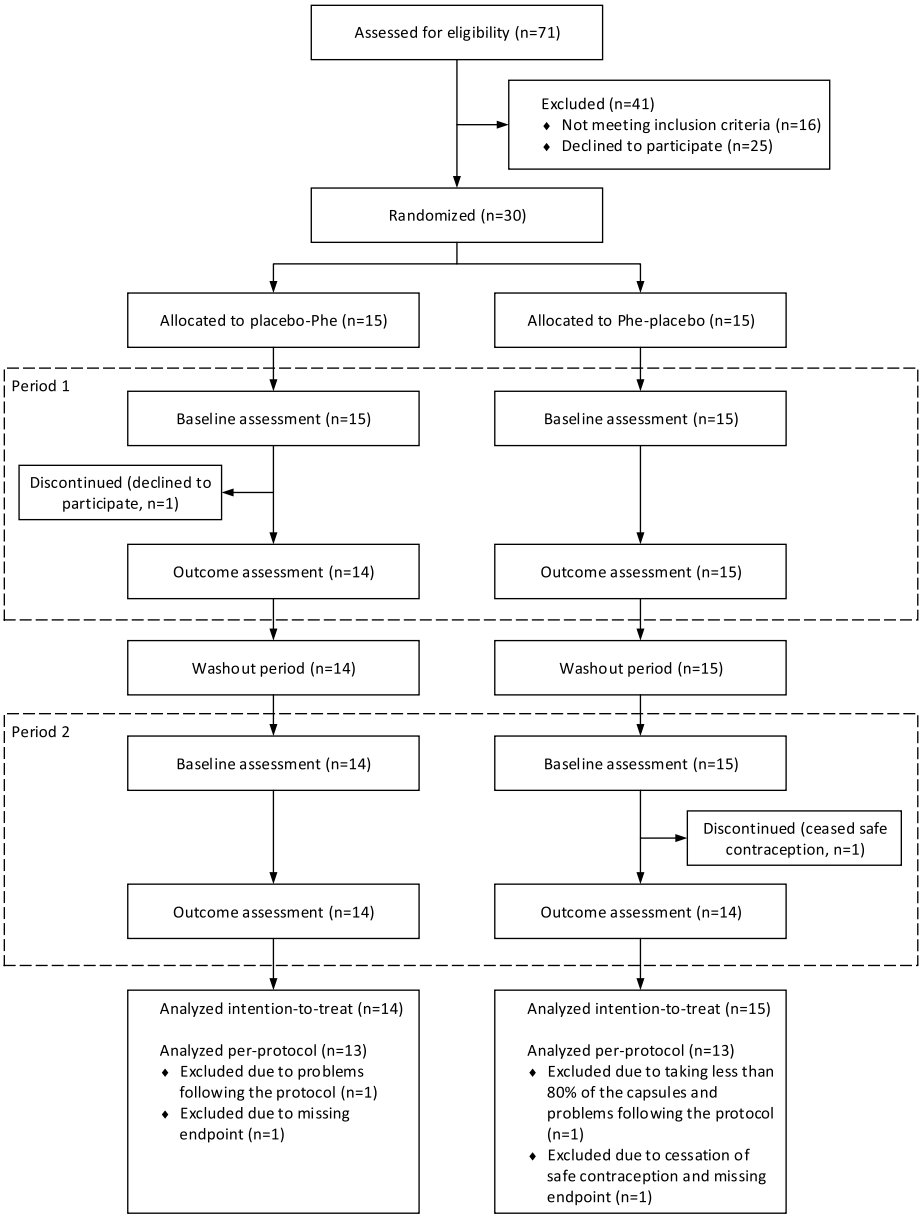

FIGURE 1. Screening, randomization, and follow-up of the participants. One patient of the placebo–Phe group performed only the baseline assessment and then declined to start the intervention. Therefore, he was not included in either the ITT or the PP analysis. ITT, intention-to-treat; PP, per protocol.

Consequently, the primary endpoint was working memory accuracy, assessed with the computerized visual *n*-back task [Test of Attentional Performance (TAP)] [22], known to be of particular vulnerability during an on/off-diet condition in patients with PKU [20]. In this task, a sequence of numbers is presented, and participants are asked to decide whether the number matches the 1 or 2 numbers before.

Key secondary endpoints included working memory reaction time assessed with the computerized visual *n*-back task (milliseconds, TAP) [22]; sustained attention assessed with a computerized attention task (standard deviation of reaction time in milliseconds, TAP) [22]; manual dexterity assessed with the analog Pegboard task (assembly subtest, Purdue Pegboard) [23]; mood [Profile of Mood States (POMS)] [24], and decrease (Beck Depression Inventory) [25], both assessed with a paper–pencil questionnaire. Inhibition and cognitive flexibility were assessed using the third and fourth conditions of the color-word interference test of the Delis–Kaplan Executive Function System (D-KEFS, [26]), respectively. However, these data are not reported because of concerns regarding their reliability raised by one of the peer reviewers.

Intelligence quotient (IQ) was treated as a background variable and was estimated with the short form of the WAIS-IV using the 4 subtests matrix reasoning, symbol search, vocabulary, and arithmetic [27,28].

All cognitive tests were performed in the morning between 9 and 12 am after a low-protein breakfast (including coffee in case of regular coffee consumption). All cognitive assessments were conducted after the MRI examination.

Plasma Phe levels were measured on a Biochrom 30 (Saturn and Venus) amino acid analyzer by high-performance ion-exchange liquid chromatography with post-column photometric detection of ninhydrin-derivatized amino acids. Adverse events (AEs) and study compliance were assessed during the study appointments and weekly phone calls. To assess adherence to medication, partially used and unused capsules were collected at each follow-up visit to the study site. Any changes in the participants' well-being were noted as AE and were evaluated according to their grade and relationship with the intervention according to the ICH E2A guidelines [29].

Statistical analysis

The sample size estimate was derived using Stata Version 15 SE [18], and based on a paired means test of the primary endpoint (working memory accuracy, *n*-back task, and test–retest reliability 0.67) [22]. Statistical analyses were performed using R (Version 4.2.1) [30]. The noninferiority margin was set at −4% based on the SD of the performance of healthy controls [31]. According to the Gaussian normal distribution, a performance change of one standard deviation represents a significant deviation from the average performance of a reference group. In some cognitive domains, such as working memory accuracy, a 1 standard deviation difference may result in noticeable difficulties in performing complex activities. The clinical impact of a 1 standard deviation difference can vary depending on the individual's baseline performance or age. Therefore, we include the baseline performance as a fixed effect in our statistical model. Assuming a difference of 0, a standard deviation of 8% [20], a power of 80%, and a 1-sided alpha level of 0.05, 26 participants are required to show noninferiority. To allow for drop-outs, the recruitment target was set at 30 participants. A sample size reassessment after 50% of patients had been recruited suggested a required sample size of 21 patients.

In line with the CONSORT statement for noninferiority trials, the primary and secondary endpoints were analyzed based on the intention-to-treat (ITT) and per protocol (PP) approach [32]. For the primary endpoint, noninferiority was claimed if the lower 1-sided 95% confidence interval (CI) limit lay above the noninferiority margin of −4% in both analyses. The primary endpoint was assessed using a mixed-effects model with the 4-week values (working memory accuracy) as an outcome and fixed effects for the intervention period (Phe or placebo). According to suggestions from the European Medicines Agency [33)] and van Breukelen (2006) [34], baseline value (working memory accuracy), period, and randomization stratification indicators were used as fixed effects, and a random intercept was applied on participant ID. One-sided 95% Wald CIs were constructed. All secondary endpoints were analyzed using the same approach as above, reporting point estimates and 2-sided 95% Wald CIs. The number of AE was analyzed using a generalized linear mixed-effects model with a Poisson distribution, with intervention (Phe or placebo), period, and stratification variables as fixed effects and a random intercept for participant ID.

The progress of the study was monitored regularly by the clinical trial unit at the University of Bern, Switzerland. The trial was registered at clinicaltrials.gov (NCT03788343), kofam.ch (SNCTP000003117), and the International Clinical Trials Registry Platform of the WHO (status of the study: closed).

Results

Participants

Of the 71 patients screened, 30 were randomized, and 29 completed the study (Figure 1). The ITT analysis included 14 patients in the placebo–Phe group and 15 in the Phe–placebo group. The PP analysis included 13 patients in the placebo–Phe group and 13 in the Phe–-placebo group. Patients' ages ranged between 19 and 48 y. In the PP analyses, 1 participant was excluded because of low compliance (<80% of capsules taken during the intervention periods), and 1 participant was excluded after the third study visit because of unwillingness to follow safe contraception.

TABLE 1
Demographic and clinical characteristics at baseline (ITT population)

	Overall ($N = 29$)	Phe–Placebo ($N = 15$)	Placebo–Phe ($N = 14$)
Age (y)			
Median	36	35	36
Interquartile range	27–38	29–38	22–42
Range	19–48	21–48	19–48
Sex, *n* (%)			
Female	13 (45)	7 (47)	6 (43)
Male	16 (55)	8 (53)	8 (57)
Education, *n* (%)			
High school	1 (3)	0 (0)	1 (7)
College/Apprenticeship	24 (83)	13 (87)	11 (79)
Graduate school	4 (14)	2 (13)	2 (14)
Intelligence quotient (IQ)			
Median	97	98	96
Interquartile range	90–107	90–106	90–108
Range	61–115	80–115	61–115
Plasma Phe (μmol/L)			
Median	749	733	750
Interquartile range	593–959	598–942	572–953
Range	380–1208	466–1084	380–1208

IQ was assessed with the short form of the WAIS-IV using the 4 subtests matrix reasoning, symbol search, vocabulary, and arithmetic [27].
Abbreviations: ITT, intention-to-treat analysis; PP, per protocol; Phe, phenylalanine.

TABLE 2
Changes of the primary and secondary endpoints within the Phe and placebo periods[1]

	ITT					PP				
	N	Placebo Mean (SD)	Phe Mean (SD)	Point Estimate (95% CI)	*P* value	*N*	Placebo Mean (SD)	Phe Mean (SD)	Point estimate (95% CI)	*P* value
Primary endpoint										
Working memory accuracy[2]	57	−0.5 (7.2)	0.0 (4.0)	0.49 (–2.5, 3.5)	0.75	52	0.8 (3.1)	–0.3 (4.1)	–1.2 (–2.9, 0.46)	0.15
Secondary endpoints										
Working memory reaction time[3]	57	–11 (152)	61 (170)	72 (−0.14, 144)	0.050	52	−9 (156)	67 (177)	76 (−2.9, 154)	0.058
Sustained attention[4]	56	−19 (33)	13 (50)	31 (9.0, 53)	0.007	51	−20 (33)	17 (43)	32 (12, 53)	0.005
Manual dexterity[5]	57	2 (3)	1 (5)	−1.2 (−3.4, 0.91)	0.25	52	2 (3)	0 (5)	−1.6 (−3.7, 0.58)	0.15
Anxiety[6]	57	0 (4)	1 (9)	0.19 (−3.7, 4.0)	0.92	52	0 (4)	1 (10)	0.25 (−4.0, 4.5)	0.91
Vigor[6]	57	−1 (7)	−4 (6)	−2.8 (−6.2, 0.68)	0.11	52	−2 (7)	−5 (5)	−2.8 (−6.4, 0.83)	0.13
Fatigue[6]	57	0 (7)	3 (8)	3.2 (−0.76, 7.1)	0.11	52	0 (7)	4 (8)	3.3 (−1.0, 7.7)	0.13
Anger[6]	57	1 (5)	2 (6)	0.77 (−1.7, 3.3)	0.53	52	1 (5)	2 (6)	0.75 (−2.0, 3.5)	0.58
Depression[7]	57	1 (3)	2 (4)	1.0 (−1.0, 3.0)	0.32	52	1 (3)	2 (5)	1.2 (−0.94, 3.4)	0.26

Abbreviations: CI, confidence interval; ITT, intention-to-treat; PP, per protocol; Phe, phenylalanine; POMS, Profile of Mood States.
[1] Means are changes before and after the placebo or Phe period for the primary and secondary endpoints for the ITT analysis, point estimates, and 2-sided 95% Wald CIs,
[2] Percentage,
[3] milliseconds,
[4] Standard deviation of reaction time, milliseconds (summary for correct responses),
[5] Assembly subtest,
[6] Score of Profile of Mood States (POMS; anxiety, vigor, fatigue, and anger),
[7] Beck Depression Inventory (BDI-II) score.

Baseline demographic and clinical characteristics were well balanced between the 2 intervention groups (Table 1). The median plasma Phe levels were 733 μmol/L for the Phe–placebo group and 750 μmol/L for the placebo–Phe group.

Primary endpoint

In the ITT analysis, the point estimate (adjusted difference) of working memory accuracy between Phe and placebo was 0.49 (lower limit 95% CI: −1.99). In the PP analysis, the point estimate was −1.22 (lower limit 95% CI: −2.60) (Figure 2). Hence, the noninferiority margin of −4% was not crossed in either analysis, demonstrating that Phe was noninferior to placebo with respect to working memory accuracy in both, the ITT and the PP analyses.

Secondary endpoints

Working memory reaction time, manual dexterity, mood (anxiety, vigor, fatigue, and anger), and depression did not differ statistically significantly between the Phe and placebo periods (Table 1, Figure 3). Sustained attention significantly differed between the intervention periods. Results were comparable for the ITT and PP analyses (Table 2).

Phe levels

After the Phe period, median Phe levels had increased by 623 μmol/L [interquartile range (IQR) 497–749] with no significant difference between the randomization groups: Phe–placebo (median Phe after the Phe period 1455 μmol/L, IQR: 1228–1746) and placebo–Phe (median Phe after the Phe period 1457 μmol/L, IQR: 1373–1698) (Figure 4).

AE

A total of 129 AEs were reported by 26 participants (Supplemental Table 1, [35]). The most common AE were tiredness (12%), mood changes (11%), difficulties with concentration (9%) or memory (8%), and flu-like symptoms (7%). Most (84.5%) of AE were classified as mild, 14% as moderate, and 1% as severe (described in detail below). Half (50%) of AE were classified as possibly related to the

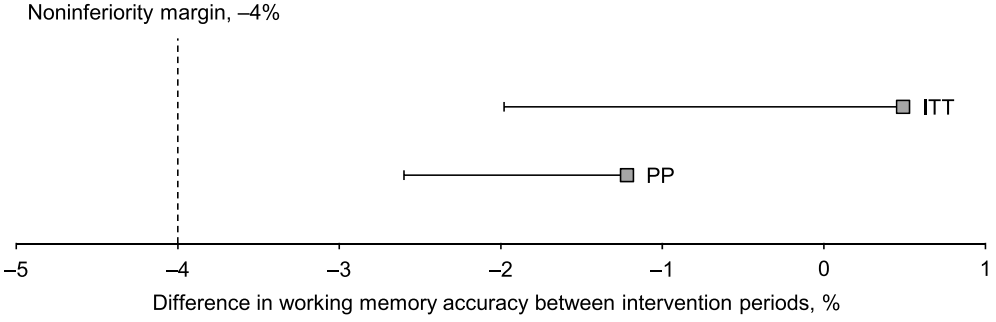

FIGURE 2. Point estimates and one-sided confidence intervals of the difference in working memory accuracy between the Phe period and the placebo period. The lower limit of the confidence interval should exceed −4% in the ITT and PP analysis to claim noninferiority. ITT intention-to-treat; PP, per protocol.

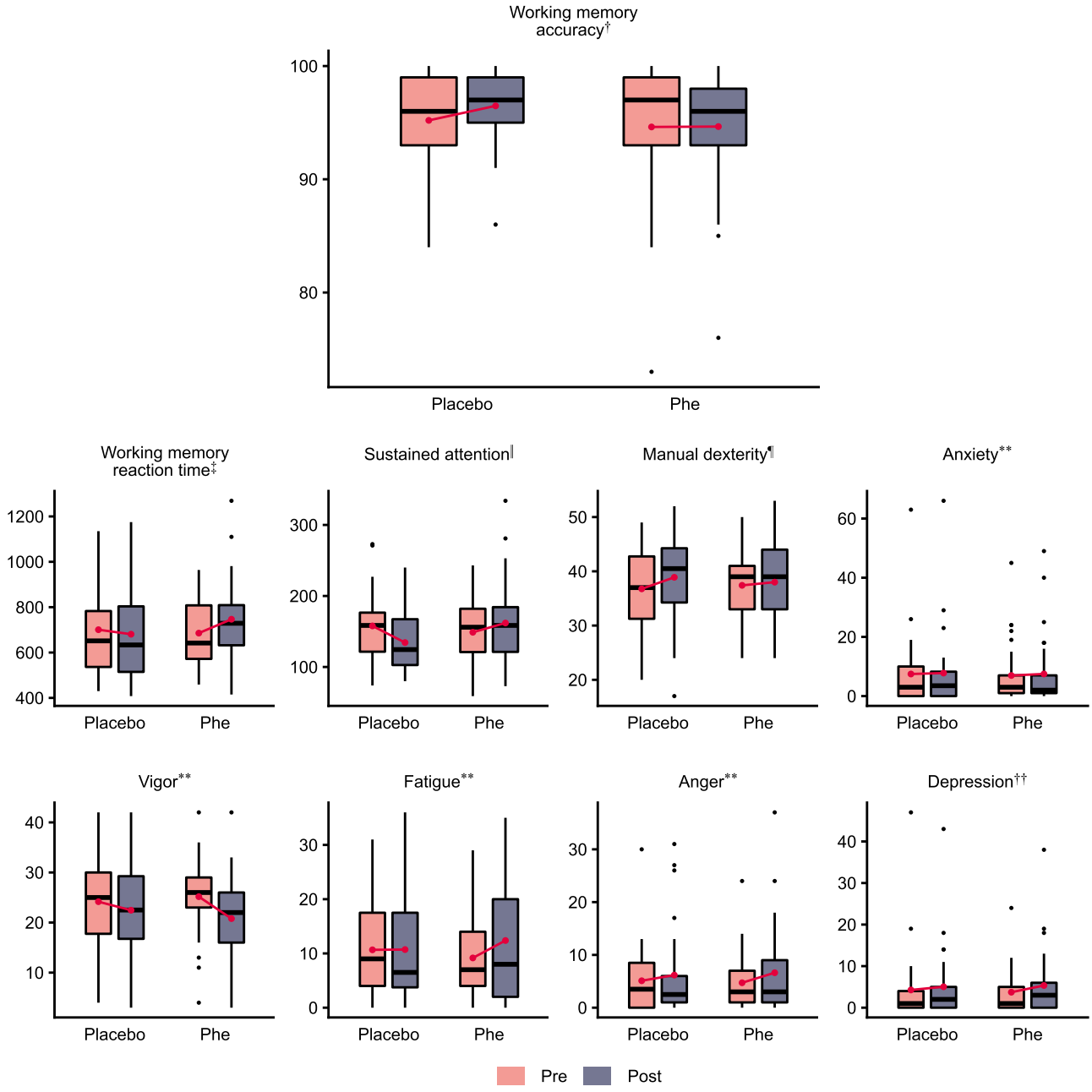

FIGURE 3. Primary and secondary endpoints before (pre) and after (post) the Placebo or Phe period for the ITT analysis presented for all participants (median, mean, IQR, and CI). The red lines reflect the changes during the Placebo or Phe period. †percentage, ‡milliseconds, ‖standard deviation of reaction time, milliseconds, ¶assembly subtest, **Score of Profile of Mood States (POMS; anxiety, vigor, fatigue, and anger), ††Beck Depression Inventory (BDI-II) score. CI, confidence interval; IQR, interquartile range; ITT, intention-to-treat; POMS, Profile of Mood States; PP, per protocol. One extreme observation from the post-placebo period was removed from the figure showing working memory accuracy (50% accuracy). However, this data point was still included in the median, mean, IQR, CI, and the statistical analysis.

interventions, 34% as unlikely to be related to the intervention, and 16% as unrelated to the intervention.

Overall, patients reported significantly more AE during the Phe period (2.48 ± 2.68) than during the placebo period (1.45 ± 1.43; incidence rate ratio 1.53, $P = 0.037$, 95% CI: 1.03, 2.28). This result is driven by the higher rate of AE in the first intervention period, as shown in Supplemental Table 2 [35]. Independently of the sequence, 62% of AE were reported during the first intervention period compared with 26.4% during the second (2.3% in the run-in, 7.8% in the washout, and 1.6% within 4 wk after the second intervention period).

One serious AE was related to a participant being admitted to a psychiatric ward. After careful consideration and in agreement with the treating psychiatrist, a relation to the intervention was classified as unlikely; hence unblinding was not necessary. Unblinding of data after the completion of the study revealed that the serious AE occurred during the placebo period.

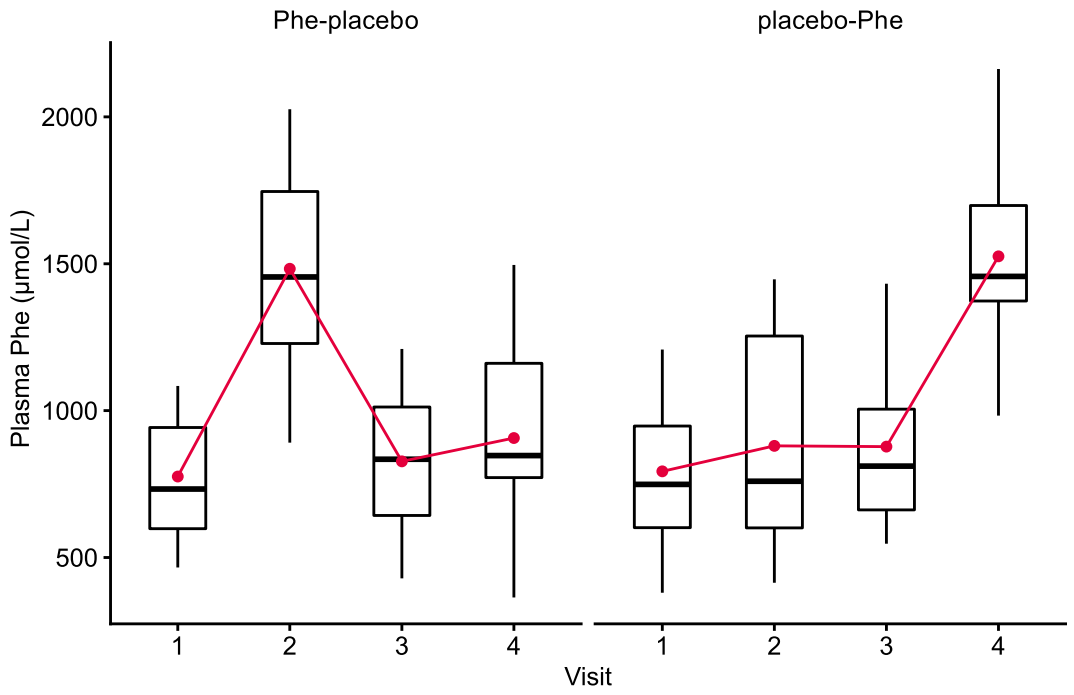

FIGURE 4. Phe levels at the 4 study appointments (median, mean, IQR, minimum, and maximum). In the Phe–placebo group (left side), Phe was given between visits 1 and 2. In the placebo–Phe group (right side), Phe was given between visits 3 and 4. IQR, interquartile range

Discussion

To the best of our knowledge, this is the first sufficiently powered randomized controlled study on the effect of an increased Phe intake - simulating normal dietary Phe consumption - on cognition, mood, and depression in early-treated adult patients with PKU. A 4-week high Phe level was noninferior to continuing the Phe restriction in terms of the primary endpoint, i.e., working memory accuracy, indicating that working memory accuracy did not deteriorate after Phe. Furthermore, working memory reaction time, manual dexterity, mood (anxiety, vigor, fatigue, and anger), and depression did not statistically worsen after 4 weeks of high Phe levels. However, there was a significant difference between the intervention periods in sustained attention performance.

The current study showed that a 4-week increase in Phe level did not cause a deterioration of working memory accuracy, manual dexterity, mood, or depression. This contrasts with previous cross-sectional studies, which associated higher Phe levels with worse performance in the abovementioned domains [3,4,9,10,12,13,16,36]. Methodologic differences across studies (i.e., age range, choice of cognitive task, degree of Phe hydroxylase enzyme rest-activity, and national guidelines) might partly explain the divergent results. However, cross-sectional association studies are unable to distinguish between past effects of elevated Phe levels during childhood and a potentially ongoing negative impact of Phe on cognitive performance in adulthood. Furthermore, findings concerning the association between Phe and cognition could reflect the expectations of patients and caregivers regarding higher Phe levels. Expectations are known to influence the effect of an intervention [37]. Standardly, early-treated patients with PKU adhere to a strict Phe restriction diet throughout childhood and adolescence and are advised to continue the diet into adulthood to prevent neurologic disturbances.

The neurotoxic effect of Phe differs substantially between children and adults [38]. A child's brain is exceptional in its developmental capacity but also in its vulnerability to noxious agents [38]. If left untreated during childhood, PKU leads to severe disabilities [39]. Previous studies in early-treated adolescents with PKU suggested that after childhood development, the brain may be sufficiently mature to withstand the neurotoxic influence of high Phe levels [40]. Consequently, in adults, higher Phe levels have only been associated with mild cognitive alterations [3,41] or none at all [42]. In previous investigations, a causal relationship between currently elevated Phe levels and cognitive functions, mood, and depression appeared to be difficult to establish. Unblinded reintroduction of Phe restriction was associated with cognitive improvements [36]. However, a substantial proportion of early-treated adult patients with PKU live their everyday lives with inconsistent Phe restriction or even discontinued diet [7]. Nevertheless, current guidelines recommend maintaining strict protein restriction in adulthood [5,6]. It is worth noting that the long-term safety of lifelong dietary restrictions has not been established in patients with PKU, even though they might have adverse biopsychosocial effects [43,44].

The results of this study support the notion that some cognitive domains might be more susceptible to high Phe than others. Sustained attention performance decreased significantly more during the Phe than the placebo period. However, this result is based on an improvement in performance during the placebo period, whereas the performance slightly worsened during the Phe period (see Figure 3). Whether the extent of the performance decrease during the Phe period is clinically relevant and impacts patients' everyday lives is not yet known. Furthermore, the decrease in attention performance will need to be weighted against the burden of dietary restrictions. Comparisons of cognitive outcome measures across studies are difficult as a multitude of tasks was previously used to assess the same cognitive domain. Standardization of the neuropsychological assessments to regularly monitor the impact of Phe on patients' cognition and mood could be helpful for research and clinical purposes. For example, this study has not shared any cognitive task with the large study by Aitkenhead et al. [3]. Likewise, a recent meta-analysis had to deal with the variance in

outcome measures; however, it revealed that across 220 different cognitive measures ($n = 757$ patients with early-treated PKU), effect sizes were larger for speed than accuracy tasks [4]. Hence, a speed measure as the primary endpoint could be considered for future studies.

Significantly more AEs were reported during the Phe than during the placebo administration. The difference relates to the increased number of AEs during Phe administration in the first intervention period (Supplemental Table 2). In the second intervention period, Phe administration came along with fewer AEs than placebo administration in the first or second intervention period. Moreover, 50% of the AEs were categorized as not or unlikely related to Phe administration or had resolved before the end of the intervention period. Hence, the clinical relevance of the higher rate of AEs during the Phe administration seems questionable.

The strength of this study is its methodology, statistical approach, and the fact that no patient was lost to follow-up. Some limitations should be considered, which reduce the generalizability of our study results and ask for caution when interpreting this study. First, Phe levels were elevated for 4 weeks. In adults, clinically relevant cognitive impairment could occur only after prolonged exposure. Second, Phe was increased without suspension of dietary restrictions or amino acid supplementation to allow participants' blinding. Third, the median Phe level was slightly above 700 μmol/L at baseline and increased into the range of untreated patients during the Phe period. In adults, adverse effects of high Phe may reach a plateau at lower Phe levels than 700 μmol/L. In addition, the patient group was homogenous with respect to the diagnosis (classical PKU), but the extent of dietary restriction varied between patients. Some patients did not perform a strict dietary restriction as 2 baseline Phe levels were slightly above 1200 μmol/L (1205 and 1208 μmol/L, respectively). And fourth, the results only apply to the age range studied and the primary and secondary outcomes under investigation. How the brain of older early-treated adults with PKU reacts to higher Phe levels should be investigated at a time when enough patients are available for this purpose.

In conclusion, in early-treated adult patients with PKU, a 4-week increase in Phe intake was noninferior to continuing Phe restriction with respect to working memory accuracy, and secondary outcomes did not differ except for sustained attention performance. Future randomized, double-blinded intervention studies should address the impact of a long-term increase in Phe intake across different periods of adulthood. It might also be crucial to identify patients with particular vulnerability to high Phe, which would allow treatment recommendations to be individually adapted.

Author contributions

The authors' responsibilities were as follows – RT: designed research, conducted research, wrote paper, had primary responsibility for final content; RM: designed research, conducted research, wrote paper; SM-A: conducted research, wrote paper; AGH: designed research, performed statistical analysis; MH: designed research; RE: designed research, conducted research, wrote paper, had primary responsibility for final content; and all authors: read and approved the final manuscript.

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Data availability

With publication of the article, the anonymized individual participant data, a data dictionary defining each field in the set, and additional related documents (that is, study protocol, statistical analysis plan, informed consent form) will be made available from the corresponding author by e-mail (regula.everts@insel.ch) and upon reasonable request and after signing a data sharing and data access agreement.

Conflict of interest

The authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.11.007.

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