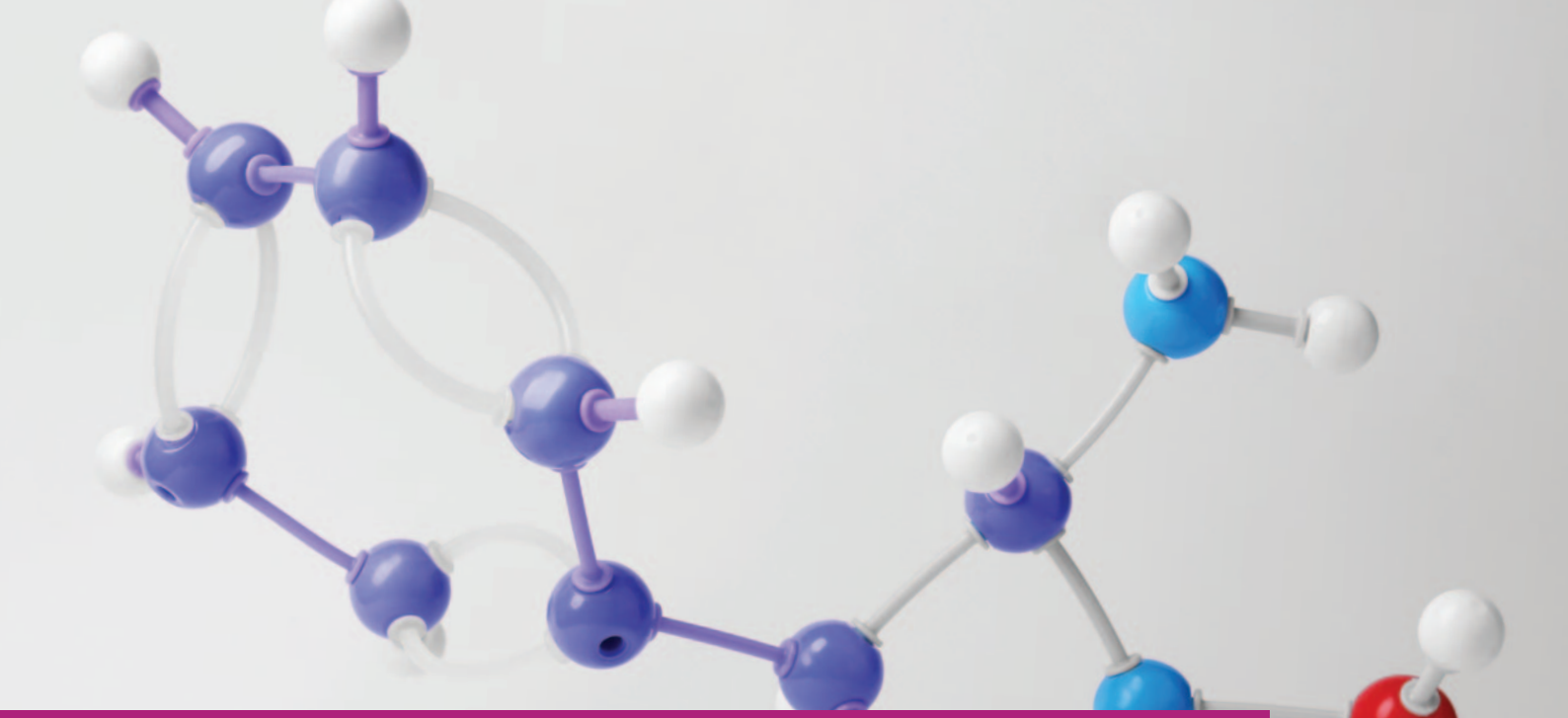

Improving Metabolic Stability in PKU with a Prolonged-Release Protein Substitute



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Phenylketonuria (PKU) is a rare inherited metabolic disorder caused by a deficiency of phenylalanine hydroxylase (PAH) enzyme, resulting in high blood and cerebral phenylalanine (Phe) concentrations, with concomitant tyrosine (Tyr) depletion.[1, 2] Without adequate management, elevated Phe levels exert neurotoxic effects, leading to intellectual disability, impaired executive functioning and neuropsychological disturbances.[3-6] Early diagnosis via newborn screening, and prompt dietary intervention, have dramatically improved outcomes; however, achieving and maintaining optimal metabolic control across the lifespan remains a significant clinical challenge.[7-11]

Background

Although several pharmacological treatments for PKU are now licensed, including sapropterin dihydrochloride (sapropterin), pegvaliase and sepiapterin,[12-14] their use is not universal. Access and reimbursement differ across countries,[15] and these therapies are not suitable for all individuals with PKU. Eligibility varies by age (for example, pegvaliase is approved from 16 years in Europe and Canada, and from 18 years in the United States), as well as by phenotype and residual PAH activity. Only a minority of individuals with classical PKU respond to sapropterin, further limiting applicability.[15, 16] Consequently, dietary management comprising strict Phe restriction supplemented with low Phe protein substitutes and special low protein foods, remains the cornerstone of treatment for the majority of patients.[17]

Conventional protein substitutes are largely based on free amino acids, which are rapidly absorbed in the gastrointestinal tract, leading to variable plasma amino acid concentrations, particularly when intake is unevenly distributed throughout the day.[18, 19] While effective in preventing protein and micronutrient deficiency, their non-physiological absorption profile may contribute to metabolic instability, including diurnal variation in blood Phe levels (i.e. higher concentrations early in the morning, likely reflecting fasting-induced protein catabolism exceeding protein anabolism overnight).[19-22]

Emerging evidence suggests that variability and instability in blood Phe levels may independently influence intellectual, neurocognitive and psychological outcomes in early and continuously treated individuals with PKU.[23, 24] This effect appears particularly pronounced in patients with classical PKU.[19, 20, 25, 26] These observations have driven growing interest in evidence-based, innovative dietary solutions that aim not only to reduce overall Phe exposure but also to improve metabolic stability by more closely mimicking physiological protein digestion, without increasing burden for patients and caregivers.[27-30]

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Blood phenylalanine control: Are blood Phe levels 'out of target'? Evidence from international long-term studies

Long-term and effective metabolic control remains a major challenge in the management of PKU. Although good blood Phe control is generally achievable in childhood, adherence to dietary treatment frequently declines with age, usually beginning in early adolescence and often persisting into adulthood.[8, 10, 31-33]

Studies from the UK, Europe, Australia and the USA consistently show a marked deterioration in metabolic control after adolescence.[7-10, 34-36] The most recent and largest multicentre study, involving over 1,300 patients from nine European and one UK PKU centres, reported that more than 70% of blood Phe measurements were within the recommended target range up to the end of adolescence.[11] This proportion declined substantially in young adulthood (19-30 years) and continued to decrease with advancing age, falling to approximately 40%. Patients with classical PKU, the most severe and prevalent phenotype, face the greatest difficulty in maintaining metabolic control, with only just over half of blood Phe measurements within the recommended range.[11]

Overall, a substantial proportion of adolescents and adults with PKU experience blood Phe concentrations above the recommended therapeutic range, which has been associated with subtle yet clinically meaningful neurocognitive, psychological, behavioural and emotional impairments.[6, 24, 37-53]

Reduced Phe variability: An emerging strategy to improve cognitive performance

The primary goal of PKU treatment is to prevent neurocognitive and psychological complications by maintaining blood Phe concentrations within recommended target ranges. However, emerging evidence suggests that stability of blood Phe levels may be as important as absolute Phe concentrations.[23, 24] Research in children and adolescents with PKU indicates that greater Phe variability and higher Phe/Tyr ratios may be associated with lower IQ scores,[23, 26, 54, 55] poorer executive functioning,[56-58] and increased symptoms of anxiety and depression.[59, 60] Phe variability also appears to be a stronger predictor of cognitive and executive outcomes, particularly during later developmental stages.[23] Evidence further indicates that fluctuations in Phe levels in childhood or adolescence can predict adult neuropsychological and language performance.[24, 61, 62] In adults with PKU, greater Phe variability has also been linked to deficits in specific neurocognitive domains and possibly to more severe brain structural changes.[63] In response to this growing body of evidence, recent European PKU guidelines emphasise the importance of minimising Phe fluctuations in addition to controlling mean Phe concentrations.[15]

Variability in blood Phe concentrations may influence cognitive outcomes through several interrelated mechanisms. In PKU, brain Phe levels depend both on blood Phe concentrations (typically representing 16-44% of blood levels) and on transport across the blood-brain barrier via the L-type amino acid transporter 1 (LAT1), which is influenced by the presence of other large neutral amino acids (LNAAs).[20] Notably, Phe peaks in the brain occur later in blood, are less steep, but persist for longer in patients with PKU.[64, 65] Consequently, short-term fluctuations in blood Phe may result in sustained cerebral Phe exposure, which may be particularly relevant for neurocognitive outcomes. In addition, elevated or fluctuating blood Phe concentrations competitively inhibit the transport of other LNAAs (e.g. Tyr and tryptophan) across the blood-brain barrier via LAT1. As these amino acids are precursors for dopamine, serotonin and norepinephrine, reduced cerebral availability may compromise monoaminergic neurotransmitter synthesis, thereby contributing to cognitive and mood disturbances.[66-71]

A study by Romani *et al.*[24] examined which metabolic variables (mean Phe *vs.* Phe variability) were most predictive of cognitive outcomes. The authors reported that increased Phe variability was particularly detrimental during childhood and that both childhood Phe variability and adult mean Phe concentrations together predicted approximately 40% of the variance in cognitive scores. Romani *et al.* further proposed that Phe peaks may be especially harmful to the developing brain, potentially through adverse effects on white matter structural integrity. This hypothesis is supported by findings from Hood *et al.*,[54] who demonstrated that prolonged exposure to elevated and variable Phe concentrations was associated with poorer white matter integrity in children with PKU. In contrast, cumulative Phe exposure, reflected by mean Phe concentrations, may be more detrimental in adulthood, partly due to its impact on neurotransmitter availability.[24]

Collectively, these findings suggest that avoiding blood Phe peaks (defined as Phe SD ≤180 µmol/L throughout life) may be as important as maintaining low mean Phe levels (≤360 µmol/L in childhood; ≤600 µmol/L from 12 years onwards).

Achieving 'good' and 'stable' blood Phe levels over a 24-hour period remains particularly challenging in individuals with classical PKU, who are highly sensitive to dietary changes and protein catabolism. These patients often experience marked intra- and inter-day fluctuations in blood Phe levels, despite adherence to prescribed dietary protocols.[11, 19, 25, 26] Given the detrimental effects of cumulative day-to-day Phe fluctuations on cognitive performance over months and years, there is a clear need for practical, evidence-based strategies that minimise Phe fluctuations without increasing treatment burden for patients or caregivers.

Prolonged fasting periods, particularly overnight, promote endogenous protein catabolism, leading to increased circulating Phe levels, an effect that is more pronounced in individuals with classical PKU.[21, 25, 72] Uneven intake or suboptimal adherence to protein substitutes during the day can further exacerbate Phe fluctuations. This is largely related to the rapid and non-physiological absorption of conventional free amino acid mixtures compared with intact proteins.[73] Such absorption kinetics result in early plasma amino acid peaks followed by rapid declines, contributing to metabolic instability and increased nitrogen losses when amino acid supply exceeds the body's capacity for protein synthesis.[74, 75]

Prolonged-release protein substitutes have emerged as a promising dietary strategy to address diurnal blood Phe variability in individuals with classical PKU. These formulations use Phe-free amino acids embedded in a fibre-based matrix, allowing gradual gastrointestinal release that more closely mimics the absorption kinetics of intact natural proteins.[28-30] In contrast to conventional free amino acid substitutes, prolonged-release protein substitutes provide a slower, more sustained amino acid supply. This may support improved nitrogen retention, greater Tyr availability and reduced amino acid losses.[27, 28, 76]

Current evidence on prolonged-release protein substitutes in PKU has been limited and has largely focused on protein status, nitrogen balance, product acceptability and gastrointestinal tolerance. However, their potential role in improving metabolic control, particularly in reducing morning Phe rises following overnight fasting, the longest catabolic period of the day, has not been specifically investigated.

Improved morning metabolic control with a prolonged-release protein substitute in children and adolescents with PKU: A randomised crossover study

To address this important clinical question, a randomised, controlled, crossover study was conducted at Birmingham Children's Hospital in 2023.[77] Children on a low-Phe diet supplemented with conventional protein substitutes were randomly assigned to two treatment sequences: in one sequence, participants received the prolonged-release amino acid protein substitute (PR-AA) as a single evening dose for seven days, followed by a two-week washout, then transferred to their usual free amino acid protein substitute (AA) for all daily doses for another seven days. In the other sequence, the order was reversed. All other daily protein substitute doses remained unchanged throughout the study. Metabolic and clinical outcomes assessed included: blood Phe, Tyr and branched-chain amino acid levels measured from dried blood spots taken at 05:00, 06:00 and 07:00 a.m. prior to breakfast, on two consecutive days before and after each treatment period; Phe/Tyr ratio; urinary nitrogen excretion; tolerability and patient acceptability.

Of 16 eligible patients, 13 children with classical PKU (8 females; mean age 11.8 years, range: 7-16 years) completed the study. The results showed that a single evening dose of the PR-AA formulation resulted in an approximately 18% reduction in early morning blood Phe levels, achieving a mean concentration of 294 µmol/L. In contrast, use of a conventional protein substitute (AA) was associated with higher morning Phe levels (mean 442 µmol/L), exceeding the recommended target range (120-360 µmol/L). Although short term, these findings highlight the limitations of rapidly absorbed protein substitutes for overnight metabolic control and suggest that PR-AA may improve metabolic stability during the fasting period, a time that is usually associated with Phe accumulation in classical PKU. Subgroup analyses suggested greater benefits in children under 12 years and in those with higher baseline Phe (>360 µmol/L).

In addition to lowering morning blood Phe levels, PR-AA consistently increased blood Tyr across all age groups. This contrasted with the variable or minimal Tyr responses observed with the conventional protein substitute. The combined reduction in Phe and increase in Tyr led to a clinically

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meaningful improvement in the Phe/Tyr ratio, a potential biomarker of metabolic control. Other metabolic parameters remained unchanged. The product was well tolerated and adherence was high.

Overall, once-daily evening administration of a PR-AA protein substitute produced favorable changes in blood Phe, Tyr, and the Phe/Tyr ratio, suggesting potential benefits for long-term outcomes. Although current European guidelines recommend dividing protein substitutes into three or more daily doses to limit Phe fluctuations,[15] adherence to such regimens often declines with age,[10, 78, 79] further exacerbated by sensory aversions to conventional substitutes.[80] Prolonged-release protein substitutes may therefore represent a valuable adjunct to PKU management, supporting metabolic control without increasing dosing frequency, while their neutral taste and odour may improve long-term adherence.

Conclusion

Strategies that reduce Phe variability and improve the Phe/Tyr ratio are increasingly recognised as critical for preserving long-term neurocognitive function. This short-term randomised, controlled crossover study conducted at a single metabolic centre showed that a once-daily evening dose of a prolonged-release protein substitute improves early morning blood Phe stability in children with classical PKU. Compared with conventional free amino acid-based substitutes, the prolonged-release formulation was associated with lower morning Phe levels and reduced variability during overnight fasting, alongside increased Tyr levels and an improved Phe/Tyr ratio. These findings suggest a potential role for prolonged-release protein substitutes in supporting nocturnal Phe control as a useful alternative for patients with classical PKU who struggle to maintain stable Phe. Longer-term studies are needed to confirm its sustained effects, adherence and impact on neuro-cognitive outcomes.

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