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Narrative Review

Dietary protein and protein substitute requirements in adults with phenylketonuria: A review of the clinical guidelines

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SUMMARY

Lifelong dietary treatment is recommended in the management of phenylketonuria (PKU). Accordingly, an increasing adult population require age-specific PKU guidelines on protein requirements to support changing metabolic demands across the lifespan. Given that protein intake for dietary management of PKU is primarily (52–80%) derived from protein substitutes, the prescribing practice of protein substitutes must be underpinned by robust evidence. Whilst dietary guidelines for PKU management is evolving to incorporate adult specific protein recommendations, the scientific evidence underpinning these guidelines is currently limited. Instead, the determination of protein requirements for those with PKU have previously been extrapolated from estimates derived from the general healthy population, based on arguably outdated nitrogen balance methodology. Furthermore, a compensatory factor of 20–40% has been incorporated to account for the reduced uptake and utilisation of the elemental amino acids contained in protein substitutes. However, research informing this compensatory factor has been conducted in younger adults, with the majority of studies in non-PKU individuals. Given extensive evidence that the muscle anabolic response to ingested protein is impaired in older *vs.* young adults, the validity of current dietary protein recommendations for adults and older adults with PKU has been challenged. This narrative review aims to critically evaluate the existing scientific evidence underpinning current guidelines on protein requirements for adults with PKU, highlighting existing gaps in knowledge and directions for future research. We argue that current guidelines on protein requirements need updating to optimise long-term physical and functional outcomes in older adults with PKU.

1. Introduction

Phenylketonuria (PKU; OMIM 261600) is an autosomal recessive inborn error of protein metabolism resulting from a deficiency in the hepatic enzyme, phenylalanine hydroxylase, which converts phenylalanine into tyrosine. The deficiency in phenylalanine hydroxylase leads to an accumulation of phenylalanine in the blood and brain which, if left untreated, results in irreversible intellectual disability, microcephaly, seizures and behavioural problems. In Europe, the majority of PKU cases are diagnosed during neonatal screening and prevalence rates range from 1:3000 to 1:30,000 births [1]. With early diagnosis and treatment initiated in the neonatal period, individuals with PKU no longer risk developing profound and irreversible intellectual disability. However, suboptimal phenylalanine control in childhood and adulthood has been shown to lead to attention deficit, mood disturbance and impaired executive function [2–4]. Furthermore, the long-term effects of high phenylalanine levels on morbidity and ageing are yet to be determined [5].

Despite advances in the pharmacological management of PKU (eg. Sapropterin dihydrochloride [BH4] or Pegvaliase), dietary management remains the mainstay of treatment. The dietary management of PKU was first introduced by Horst Bickel and colleagues [6] in 1953 and since then dietary guidelines in children with PKU have evolved. Dietary guidelines for the management of adults with PKU have also developed, with the emergence of the recommendation for lifelong treatment [7]. Notwithstanding, variations in dietary management guidelines during childhood and adulthood exist both within and between countries [8–12].

Protein requirements for those with PKU are typically met through a combination of protein containing foods in the habitual diet and commercially available protein substitutes. Protein substitutes constitute

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the majority (52–80%) of protein intake in most individuals with PKU [10,13]. Existing guidelines on protein requirements for people with PKU have been extrapolated from estimations of protein requirements for the general healthy population using nitrogen balance methodology, with a compensatory factor accounting for the reduced utilisation of elemental amino acids from ingested protein substitutes [9,10,14]. However, a paucity of studies have directly compared and contrasted estimations of protein requirements between those with PKU and the general population across the lifespan. With growing consensus for lifelong treatment of PKU and dietary management being the mainstay treatment [7–10,15], there is now an increasing adult and ageing population who require age specific guidelines for protein requirements to support protein needs across their lifespan. Accordingly, the main purpose of this narrative review is to critically evaluate the scientific evidence underpinning current clinical guidelines for protein requirements in adults with PKU, with a view to highlighting existing gaps in knowledge for future research.

2. Methods

Searches of MEDLINE (Ovid) and EMBASE were carried out in October and November 2019 to identify published literature on protein requirement guidelines for those with PKU across the lifespan (see Box 1). Relevant references from articles retrieved, and conference abstracts and literature known to authors also were reviewed. This review classifies adults as ≥18 years and older adults as ≥60 years. The Recommended Dietary Allowance (RDA) or Recommended Dietary Intake (RDI) for protein are commonly reported in adult protein requirement guidelines (Table 2), herein referred to as RDA. The RDA is defined as meeting the protein needs of 97.5% of healthy individuals and is considered to be the 'safe level' for protein intake by the FAO/WHO/UNU reports.

While the definition of protein requirement may be considered controversial, for the purpose of this review, the protein requirement refers to the metabolic demand and the efficiency of protein utilisation, as detailed by Millward [16]. Specifically, the metabolic demand relates to what the organism needs in terms of amino acids for maintenance plus additional needs for growth, pregnancy and lactation; and efficiency of protein utilisation describes the relationship between dietary protein intake and satisfaction of metabolic demands. Protein requirements for the general population are typically based on nitrogen balance studies [17], and in essence equates to the minimum dietary protein intake necessary to balance nitrogen losses from the body. In contrast, for the purpose of this review, a protein recommendation is defined as the protein intake that serves to optimise metabolic function and improve health outcomes [18,19]. Throughout this review, close consideration was given to the methodology used to establish the protein requirement, including the provision of protein substitutes, and whether these methods are transferable to PKU populations.

3. The evolution of protein requirement guidelines in adults with phenylketonuria

In 1993, the United Kingdom (UK) Medical Research Council (MRC) Working Party on PKU first introduced the concept that a phenylalanine restricted diet should continue into adult life [7]. However, the oldest age group included in these guidelines was children >2 years and the recommendation was to consume a protein substitute at a dose of 2 g/kg body mass/day. Prior to this publication, dietary guidelines for PKU management only referenced data on phenylalanine restriction in children [20,21]. Hence, while the importance of age-specific protein guidelines for those with PKU was recognised, the MRC report was unable to make explicit reference to data on protein requirements tailored to adolescents or adults with PKU. Indeed, it was not until the revised American College of Medical Genetics and Genomics (ACMG) practice guidelines in 2014 [8,15,22] that protein requirement guidelines specific to adults with PKU finally emerged (see Table 1). These guidelines recommended a protein intake equivalent to 120–140% of the RDA [23] for age, and therefore take into consideration the changes in protein requirements as individuals age from 4 years into adulthood. Prior to the ACMG guidelines, a review by MacLeod & Ney [24] on the nutritional management of PKU concluded that insufficient data existed to devise evidence-based protein recommendations for people with PKU over 19 years old.

Box 1. Search terms

phenylketonuria* OR PKU OR hyperphenylalaninemia
AND management OR guidelines OR consensus

Table 1
Summary of clinical phenylketonuria (PKU) guidelines that include protein recommendations for adults with PKU.

Author/date	Population	Protein recommendation	Evidence cited for adult PKU protein requirements
MacLeod & Ney (2010) *[Narrative review]*		15–18 years: 2.0 g/kg/day ≥19 years: NA	Metges CC et al. (2000) Dangin M et al. (2001) van Rijn M et al. (2007)
Camp et al. (2014) *[Conference proceedings]*	United States	>4 years to adults: 120–140% RDA for age	Recommended Dietary Allowances (1989)
Singh et al. (2014) *[Guideline]*	United States	>4 years to adults: 120–140% RDA for age	Recommended Dietary Allowances (1989) Metges CC et al. (2000) Gropper SS & Acosta PB (1991)
Vockley et al. (2014) *[Guideline]*	United States	>4 years to adults: 120–140% RDA for age	Cited guideline by Singh et al. (2014)
Singh et al. (2016) *[Guideline]*	United States	>4 years to adults: 120–140% DRI for age	Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2005) Gropper SS & Acosta PB (1991)
van Wegberg et al. (2017) *[Guideline]*	Europe	FAO/WHO/UNU 2007 plus additional 40% from L-amino acids supplements	Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation (2007) Metges CC et al. (2000) Gropper SS & Acosta PB (1991) Daenzer M et al. (2001) Monch E et al. (1996) Hennermann JB et al. (2013) Schindeler S et al. (2007) Hidalgo IJ & Borchardt RT (1990) Matalon R et al. (2007) Matalon R et al. (2006)
Inwood et al. (2017) *[Guideline]*	Australasia	10–18 years: 1.0–1.5 g/kg/day >18 years: 140% RDA for age	Nutrient reference values for Australia and New Zealand (2006) Cited guideline by Singh et al. (2016) Cited guidelines by van Spronsen et al. (2017)

Table 2
Protein requirements for the general population (g/kg body mass/day) used to inform the development of PKU dietary management guidelines.

Age	FAO/WHO/UNU (1985) [a]	RDA (1989) [b]	DRI (2005) [c] RDA values	Aus/NZ RDI (2006) [d]	FAO/WHO/UNU (2007) [e]
Male					
18 years	0.86	0.80	0.85	0.99	0.85
Female					
18 years	0.81	0.80	0.85	0.77	0.82
Male					
19–30 years	0.75	0.80	0.80	0.84	0.83
31–50 years	0.75	0.80	0.80	0.84	0.83
51–70 years	0.75	0.80	0.80	0.84	0.83
>70 years	0.75	0.80	0.80	1.07	0.83
Females					
19–30 years	0.75	0.80	0.80	0.75	0.83
31–50 years	0.75	0.80	0.80	0.75	0.83
51–70 years	0.75	0.80	0.80	0.75	0.83
>70 years	0.75	0.80	0.80	0.94	0.83

[a] Food and Agricultural Organization: World Health Organization: United Nations. Energy and protein requirements. Report of a joint FAO/WHO/UMA Expert Consultation. Technical Report Series No. 724. Geneva: World Health Organization, 1985.
[b] National Research Council Recommended Dietary Allowances. 10th ed. Washington, DC: National Academies Press; 1989.
[c] Institute of Medicine. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: The National Academies Press; 2005.
[d] National Health and Medical Research Council. Nutrient reference values for Australia and New Zealand including recommended dietary intakes. Canberra: NHMRC; 2006.
[e] Joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition (2002: Geneva, Switzerland), Food and Agriculture Organization of the United Nations, World Health Organization & United Nations University. Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation. World Health Organization; 2007.

Subsequently, in 2016, Singh et al. [25] published an expanded review and expert consultation on PKU nutrition therapy guidelines that advanced the 2014 ACMG practice guidelines. Importantly, guidelines on protein requirements for those 4+ years contrasted with the 2014 guidelines in that the 120–140% of age appropriate 2005 dietary recommended intakes [26] were used rather than the 1989 RDA [23]. For those with PKU aged 18 years, the 2005 guidelines were higher than the previous 1989 RDA; however, for those >18 years of age, no differences in protein recommendations existed (see Table 2).

The most recent (2017) European PKU guidelines draw on previously published guidelines and survey data of protein requirements used in clinical practice across European countries for adults with PKU [9]. For the majority of countries, no data were collected on protein prescribing practices for adults with PKU. Whilst the ACMG 2014 [8,15,22] and updated 2016 GMDI/SERC [25] guidelines recommend that PKU patients consume a protein intake of 120–140% of age-specific protein RDA [23], this European consensus guideline recommends 140% of FAO/WHO/UNU 2007 age-specific protein requirements [27]. Furthermore, the guideline specifies that the additional 40% of dietary protein is prescribed in the form of L-amino acid supplements, a 20% compensatory factor to account for the digestibility and utilisation of amino acids from the supplement, and a further 20% compensation to optimise phenylalanine control [9].

Alongside the European PKU guideline [9], the 2017 Australasian consensus guidelines [28] for those aged 18+ years recently set their protein requirement guideline at the 140% of the US and European PKU dietary management guidelines of age appropriate RDI. The Australasian guidelines reference the Nutrient Reference Values from the Australia and New Zealand 2006 report [29], which includes an increased protein requirement for adults >70 years. The Australasian guidelines for protein requirements in adults with PKU reflects this viewpoint and advocate an increased protein requirement for the elderly PKU population [28]. To this end, the Australasian consensus is the only guideline with increased protein requirements for older patients.

With the initiative to continue dietary management for PKU into adult life, tailoring protein guidelines for older adults with PKU is crucial to the lifelong health and well-being of this patient group. Moreover, given that modifying the source of protein consumed is integral to phenylalanine control, establishing evidence-based protein recommendations to meet their metabolic demand is essential. In terms of progress, protein requirements for adults were initially devoid, but more recently, guidelines have developed to incorporate protein requirements specific to adults with PKU, as outlined in Table 1. However, these recommendations are extrapolated from the general healthy population with an additional compensatory factor of 20–40% and the scientific evidence underpinning these guidelines warrants further review.

4. Determining protein requirements in adults with phenylketonuria

4.1. Extrapolation from general population protein requirement estimations

The majority of studies used to inform national and international guidelines on protein requirements for adults and older adults in the general population utilise data generated using nitrogen balance methodology [17,23,26,27,30]. A more contemporary and valid approach to estimating protein requirements is the Indicator Amino Acid Oxidation (IAAO) method [31]. Using this method, Courtney-Martin and colleagues [32] reported that protein requirements for healthy adults across the lifespan were greater than previously published [17]. Accordingly, the use of nitrogen balance studies in older adults has been challenged given that older adults have been shown to adapt to a lower protein intake by breaking down lean tissue mass to meet nitrogen equilibrium [33]. Therefore, it may be argued that the protein RDA, based on nitrogen balance data, is inadequate to meet the changing metabolic demands of adults for maintaining fat-free mass and functional capacity [33–36]. Furthermore, this definition does not account for different health conditions and specialist dietary modifications, such as those experienced by people with PKU. As detailed above, adult specific PKU protein guidelines have evolved since the publication of MacLeod & Ney [24] to incorporate age appropriate guidelines from a range of reports (see Table 1). However, these reports have been established for the general healthy adult population [17,23,26,27,29,30], as summarised in Table 2, and do not account for sub-populations with health conditions such as PKU.

Current guidelines for protein requirements in PKU utilise guidelines set for the general population that are age-specific until 19 years,

after which protein requirements remain consistent across the adult lifespan (Table 2). Given that age-related changes in metabolic and physiological needs of the musculoskeletal system exist, concerns have been raised regarding how appropriate a "one size fits all" approach is to devising protein requirements for adults with PKU across their lifespan. Regarding protein requirements for muscle health, there is extensive evidence that the muscle anabolic response to ingested dietary protein is impaired in older adults compared with their younger counterparts [37,38]. This phenomenon has been termed muscle anabolic resistance. Accordingly, increasing dietary protein intake has been advocated as an important lifestyle strategy to overcome age-related anabolic resistance and by extension ameliorate the loss of skeletal muscle mass associated with advancing age [33–37]. It follows that consideration has been given to increasing protein requirements of older adults, as evidenced by the Nutrient Reference Values for Australia and New Zealand 2006 report [29] that recommends a 25% increase in protein intake for adults >70 years of age. Support for this initiative stems from studies demonstrating that protein intakes equivalent to the RDA (0.8 g/kg body weight/day) are inadequate to meet metabolic demands for older adults [39,40]. In this regard, Campbell et al. [39] reported a significant reduction in mid-thigh muscle area and an associated decrease in urinary nitrogen excretion in 10 healthy participants (aged 55–77 years) who adhered to a protein intake of 0.8 g/kg body weight/day over 14 weeks. The Nutrient Reference Values derived from the Australia and New Zealand 2006 report informs the Australasia PKU protein guideline [28]. With the exception of this PKU protein guideline, other protein recommendations for adults with PKU remain consistent across the lifespan and therefore are not likely adequate to meet the metabolic and physiological needs associated with ageing [41].

Protein recommendations for patients with PKU also must consider the sources of protein consumed alongside the total daily intake. Data from nitrogen balance studies consisting of animal, vegetable or mixed proteins were used to inform the most recent FAO/WHO/UNU 2007 [27] RDA for protein in the general healthy population. Whilst vegetable and fruit protein constitute a portion of protein intake for those with PKU, animal proteins would be devoid for the majority. Protein substitutes consisting of elemental amino acids or casein glycomacropeptide (CGMP), a peptide derived from the whey fraction during cheese production supplemented with additional amino acids, provide the major protein source for most individuals on dietary treatment [10,13]. Due to the digestibility and bioavailability profile of vegetable- and fruit-based protein, and the utilisation of protein substitutes, the protein RDA for the general healthy population could not be generalised to the PKU population. Accordingly, recent PKU guidelines advocate exceeding protein requirements for the general population by 20–40% to account for reduced utilisation of amino acids and to optimise circulating phenylalanine levels. However, utilising IAAO methodology, Turki et al. [42] reported protein requirements in four children (9–18 years of age) with mild hyperphenylalanemia to be 1.85 g/kg body weight/day which could be 39–55% higher than the upper end of the current recommendation for those with PKU, depending on age. A study is currently underway to determine protein requirements from L-amino acid vs. CGMP protein substitutes in adults with PKU utilising the IAAO method [43]. However, to date, no published studies have utilised the IAAO approach to determine protein requirements in adults with PKU .

4.2. Amino acid uptake and utilisation of protein substitutes

Several studies referenced in the PKU dietary guidelines provide evidence for the difference in utilisation of elemental amino acids versus whole protein, as detailed in Table 1. Specifically, Gropper et al. [44] compared plasma amino acid profiles and urea nitrogen content in 10 healthy male volunteers (age range 18.5–33.9 years) who consumed a test meal of either cottage cheese (whole protein), L-amino acids matched in composition to cottage cheese, or a mixture of cottage cheese and L-amino acids. The transient rise in plasma amino acid (total and essential amino acids) concentrations was more rapid and of greater magnitude in the L-amino acids groups *vs.* the whole protein group. Moreover, amino acid concentrations returned to baseline levels more rapidly with ingestion of L-amino acids. In contrast, no difference in plasma urea nitrogen content was observed between test meals. These data provide insight into the impact of ingesting L-amino acids on plasma amino acid kinetics and warrants a follow up study in PKU patients, particularly when combined with other measurements of whole-body protein metabolism.

Several experimental studies also provide insight into the metabolic impact of a more rapid and greater rise in plasma amino acid availability on protein synthesis rates [45–48]. For instance, Metges et al. [45] compared the uptake and utilisation of labelled leucine with the ingestion of an isolated whole protein (casein derived from goats milk) source *vs.* a free amino acid mixture that simulated the amino acid composition of casein in 14 healthy young adult volunteers (mean age range of 20.6–26.8 years). Although study diets were isocaloric, isonitrogenous and matched for leucine content, the ingestion of casein (whole protein) was associated with lower leucine oxidation rates, and increased nonoxidative leucine disposal and net protein synthesis rates, compared with free amino acids. Taken together, these data [44,45] suggest that the increase in plasma amino acid concentrations with free amino acids is of greater magnitude and more rapid than intact protein sources. Thus, amino acid oxidation rates are higher and protein retention lower when consuming free amino acids *vs*. a whole protein source. Whilst these studies are cited in current guidelines, the rationale that protein requirements of those with PKU exceeds the general population by 20–40% remains unclear. In this regard, these studies fail to provide insight into whether findings would differ if additional amino acids were provided to those consuming free amino acids to compensate for the differences in protein metabolism when compared to whole protein. Furthermore, these studies were conducted in healthy adults and the test diets did not reflect what is typically consumed in a PKU diet, thus limiting the translation of findings to individuals with PKU.

To our knowledge, the review by MacLeod & Ney [24] is the only report to cite a study by van Rijn et al. [49] that investigated rates of protein utilisation in young adults with PKU (mean age of 27 ± 7 years) *vs*. healthy young adults (mean age of 32 ± 4 years). In this study, healthy volunteers consumed the protein RDA (0.8g protein/kg/day) from standard protein sources (67% milk protein and 33% vegetable protein) whereas PKU participants consumed protein prescribed to meet RDA from dietary protein allowance (0.1–0.2 g protein/kg per day) and amino acid supplements, plus an additional compensatory 20% from amino acid supplements. In tracing the fate of labelled L-[1–^{13}C]-valine to quantify amino acid uptake and utilisation, this study revealed no differences in whole-body protein turnover, amino acid oxidation and net protein balance between groups after consuming their respective diets. This study is the first to demonstrate that the recommendation for adults with PKU to consume the protein RDA plus an additional 20% from amino acid supplements resulted in a comparable response of whole-body protein metabolism to healthy participants consuming the protein RDA (0.8 g/kg/day). Moreover, these data highlight that for adults with PKU following a PKU diet, protein requirements should include a minimum increment of 20% from protein substitutes to be comparable to the protein requirements of their healthy counterparts.

The most recent European PKU consensus guidelines [9] recommend that ingested L-amino acids should be distributed as three or more equal portions throughout the day, equating to ~20g of protein equivalent per serving for adults. This guideline cites a study by Monch et al. [50] that investigated the metabolic effects of different doses of L-amino acids using healthy volunteers and patients with PKU. In healthy participants (aged 26–46 years), multiple boluses, compared to a single bolus, resulted in a markedly reduced appearance of amino

acids into the circulation, as well as lower urea and insulin concentrations. Consistent with this observation, urinary nitrogen studies in PKU participants (9 with classical PKU and 1 with hyperphenylalaninemia) reported higher nitrogen excretion rates when L-amino acids were administered in two doses (6.3–12.4g/24 h) than three doses (4.7–10.8g/24 h). Whilst this study provided insight into the biochemical and metabolic response after consumption of L-amino acids in those with PKU, the sample sizes were small and no statistical analysis was reported which limits the inference of findings.

Protein digestibility is recognised as an important factor when considering amino acid requirements [51]. Protein requirement guidelines for people with PKU refer to studies that measured the metabolic fate of 'fast' (i.e., L-amino acids and whey protein) vs. 'slow' (i.e. casein and whey ingested in repeat doses) digestible protein sources [47,52]. Using a rodent model, Daenzer et al. [52] investigated the metabolic fate of L-amino acids compared to casein when a 9-day adaption period with standardised meals was followed on day 10 with the administration of either a single meal of ^{13}C-labeled casein or an amino acid mixture containing labelled leucine and lysine. Consistent with previous findings [45], higher urinary nitrogen excretion rates were observed with the ingestion of L-amino acids vs. casein protein, indicating reduced net protein synthesis. Furthermore, the rate of appearance of free ^{13}C-leucine into the intestinal mucosa amino acid pool was greater with the L-amino acids, indicating a faster transit through the stomach compared to casein protein. While this study provides useful mechanistic insight into differences in amino acids kinetics between amino acid sources, follow up clinical studies are warranted in those with PKU.

As further insight into the impact of protein source on amino acid uptake and utilisation, Dangin et al. [47] conducted a study in 22 healthy male volunteers (aged 25 ± 1 years) where 'slowly' digested proteins resulted in improved postprandial protein retention rate compared to 'fast' digested protein. These data suggested that protein digestibility affects amino acid utilisation, independent of amino acid composition. To test this theory, human participants were infused with L-[1–^{13}C] leucine and then consumed one of the following 30g protein meals: casein, free amino acids simulating the amino acid composition of casein, whey proteins, or repeated meals of whey proteins. Consistent with previous study findings [45–48], a more profound and transient rise in plasma leucine concentrations and higher oxidation rate was observed in the free amino acid group. When comparing the intake of free amino acids ('fast protein') with a whey protein test drink consumed in small doses to mimic a slower digestion rate ('slow protein'), free amino acids moderately reduced postprandial rates of protein breakdown whereas the whey protein group exhibited a more profound and sustained inhibition of protein breakdown. These data raise an interesting practical consideration for those with PKU on dietary treatment regarding whether the consumption of protein substitutes ('fast' protein source) could mimic a slower digested protein if consumed in smaller repeated doses. While current guidelines recommend the spacing of protein substitutes evenly throughout the day, the implications of recommending more frequent smaller protein doses need to be balanced against the inherent practical difficulties. Furthermore, in terms of long-term health, meeting the protein threshold by provision of adequate per-meal protein dosing presents a promising strategy for reducing age-associated muscle loss [53], and warrants consideration when recommending protein substitute dosing and frequency for adult and ageing individuals with PKU .

The majority of studies demonstrate that amino acid uptake and utilisation is reduced with free amino acids compared to equal intakes of whole proteins. With the exception of van Rijn et al. [49], studies administered matched protein quantities and amino acid composition, and therefore fail to elucidate whether provision of an increased free amino acid dose improves net protein synthesis rates. It is unclear why the study by van Rijn et al. [49] has not been cited by all recent PKU dietary management guidelines as evidence for the recommendation to increase the protein RDA by 20%. A limitation of all studies investigating amino acid uptake and utilisation relates to the tightly controlled nature of laboratory conditions with trial meals provided over a maximum of 24 hours, thus not reflecting the diets and lifestyles of free-living adults with PKU. Furthermore, all studies are limited by a small sample size and were conducted in young healthy adults. It also has been suggested that older adults may exhibit a greater amino acid utilisation with 'fast' absorbed proteins compared to younger adults [46], and therefore the findings may not be directly applicable to older adults. Whilst further research and long-term studies are warranted to draw any definitive conclusions, these data raise an interesting consideration for older adults with PKU whose protein intake could predominately consist of L-amino acids. Moving forward, future studies should be designed to determine the impact of modifying amino acid kinetics with the ingestion of protein substitutes on rates of whole-body and muscle protein synthesis in people with PKU . This information will be important in establishing the metabolic and physiological needs of those with PKU and will help inform protein recommendations for optimal metabolic function in this population across the lifespan.

There is a common misconception that high protein intakes (i.e. 2-3 times the RDA) are harmful to kidney function in otherwise healthy individuals [54,55]. However, a recent review of co-morbidity in people with PKU highlighted that kidney function may be impacted by the high protein intake from amino acid supplements [5]. Highlighted in this review and the European PKU consensus guidelines [9], a cross-sectional study of 67 PKU patients (aged 15–43 years) demonstrated a reduction in glomerular filtration rate (GFR) in those individuals with dietary protein and L-amino acid intakes in excess of the RDA over an extended period (i.e. years) [56]. However, the reduction in GFR remained within the target physiological range. Although proteinuria was detected in 31% of PKU patients, no control group was included in the study and protein intakes were measured at a single-time point, thus limiting the strength of findings from this study. With guidelines on protein requirements for adults with PKU on dietary treatment exceeding the protein RDA, longitudinal studies are warranted to determine if any negative health outcomes, including kidney function, are associated with higher protein intakes in adults with PKU.

4.3. Plasma phenylalanine control with higher doses of protein substitutes

The measurement of phenylalanine control discussed below refers to circulating phenylalanine concentrations. The role of protein substitutes in reducing plasma phenylalanine concentrations is well established, as evidenced by recent European consensus guidelines [9]. These guidelines were the first to recommend an incremental factor for protein substitutes in an attempt optimise phenylalanine control. Although studies in children with PKU report improvements in phenylalanine control with higher intake of protein substitutes [57], a limited number of studies demonstrate the benefits of increasing intakes of protein substitutes on phenylalanine control in adults with PKU. In this regard, Duran et al. [58] demonstrated reduced plasma phenylalanine levels in five maternal PKU patients who received hospitalisation to support adherence to their prescribed protein substitute dose. However, the impact of a 20% increase in provision of protein substitute on phenylalanine concentrations in adults with PKU remains unclear from these studies and warrants further investigation. Future studies investigating requirements should also consider other factors influencing protein substitute intake; country specific phenylalanine targets and variable adherence in adulthood.

Sufficient dietary protein intake is a pre-requisite for protein anabolism and may be linked with improvements in phenylalanine control observed with higher doses of L-amino acids. However, it has also been suggested that the intake of large neutral amino acid leads to reduced plasma phenylalanine concentrations, as mediated by an alteration in the transportation of phenylalanine across the intestinal mucosa [59-61].

5. Practical implications

National and international guidelines on protein requirements for the general healthy population form the basis of protein requirements in adults with PKU. However, there is ongoing debate around "one size fits all" for protein requirements in all adult and ageing populations given the age associated changes in metabolic and physiological demands, particularly with regards to the musculoskeletal system. The protein RDA is based on the protein intake required to meet nitrogen equilibrium rather than optimal health outcomes and does not consider long-term physical and functional outcomes. For the general population, higher protein intakes have been suggested to overcome age-related muscle anabolic resistance [37]. However, when devising recommendations across the lifespan, multiple factors need to be considered, including physical activity level, type of protein intake (fast versus slow protein), distribution of protein over the day and energy intake, all of which need consideration for people with PKU.

The most recent PKU guidelines recommend that protein intakes exceed the RDA for age by 20–40%. This incremental factor serves to compensate for the reduced uptake and utilisation of amino acids from protein substitutes, which constitute the majority of protein intake in individuals on a PKU diet. As detailed above, the evidence underpinning this notion is primarily based on inference from relatively small-scale experimental studies conducted in healthy young adults that compare the metabolic fate of free amino acids *vs*. whole protein sources. Accordingly, it is unclear from these studies whether this incremental factor is adequate to meet the potential increased needs of older adults with PKU. Future PKU dietary management guidelines need to address this issue and incorporate specific recommendations for ageing patients with PKU.

6. Future research directions

With inherited metabolic disorder services now providing guidance on dietary treatment for adults with PKU across their lifespan, future research is urgently needed to establish evidence-based protein requirements and recommendations to overcome age-related anabolic resistance and prevent skeletal muscle mass loss associated with advancing age for those with PKU. Guidelines on protein requirements should consider the metabolic demands of adults with PKU across their lifespan and the efficiency of protein substitutes to meet their metabolic needs. Factors influencing protein metabolism such as the dose of protein substitute ingested, daytime distribution of protein substitutes, digestibility of protein sources and energy intakes, and the impact these factors have at different ages, warrants further investigation in a PKU population. Historically, protein requirements for the general population are determined by the outdated nitrogen balance technique and have been extrapolated to protein requirements for people with PKU. However, recent studies utilising the more advanced method of IAAO suggest that protein requirements should be increased in both the general and PKU population. Furthermore, establishing biochemical and functional markers that represent protein status in adults with PKU will improve the monitoring and management of PKU, allowing for a more individualised approach to determining protein requirements in this patient group.

Author contributions

SF provided conceptualisation, data/evidence collection, visualisation and preparing the original draft. OW provided critical revision of the draft and final manuscript, and expertise on protein metabolism. RR provided clinical supervision, conceptualisation, medical oversight, critical review of the draft and final manuscript. MOK provided academic supervision, conceptualisation, design of methodology, guidance of evidence collection, and critical revision of the draft and final manuscript. All authors approved the final manuscript. ∗MOK and RR contributed equally to this article and share final authorship.

Guarantor

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Conflict of interest

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