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Protein status in phenylketonuria: A scoping review

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ABSTRACT

Background: The physical and functional outcomes of lifelong treatment with a phenylalanine restricted diet for the management of Phenylketonuria (PKU) remain unknown. Given that the mainstay of dietary management consists of modifying the sources of ingested protein, various aspects of body protein status could be compromised.

Objectives: To examine the existing evidence regarding the protein status of people with PKU and identify nutritional and lifestyle variables that influence protein status.

Eligibility criteria: Studies reporting anthropometric, biochemical and/or functional measurements of body protein status in people with PKU were eligible.

Source of evidence: MEDLINE (Ovid), Embase (Ovid), CENTRAL, Web of Science and Scopus, and conference abstracts.

Results: Seventy studies were included in the review. The majority of studies assessing protein status based on anthropometric measurements observed no differences between people with PKU and controls, although deficits in muscle mass were reported within PKU cohorts. Findings for biochemical assessment of protein status were mixed and limited studies assessed protein status using functional measures. Factors such as participant age, sex, metabolic control, protein source, type of protein substitute, and pharmacological treatments were found to modulate protein status of people with PKU.

Conclusions: Findings were inconclusive regarding body protein status in people with PKU. The relationship between diet and protein status outcomes remains unclear and further research is warranted to determine the impact of dietary regimens on physical and functional outcomes, and to understand the best clinical assessments to reliably monitor the protein status in people with PKU.

Keywords: amino acid kinetics; biochemical markers; body composition; muscle function; phenylketonuria; protein status

INTRODUCTION

Phenylketonuria (PKU; OMIM 261600) is an inherited metabolic disorder characterised by an accumulation of phenylalanine in the blood due to a deficiency in phenylalanine hydroxylase that converts phenylalanine to tyrosine. If left untreated, high serum phenylalanine levels result in irreversible neurocognitive disability. Dietary intervention remains the cornerstone management, and consists of limiting dietary phenylalanine intake through adherence to a low protein diet and inclusion of phenylalanine-free or low-phenylalanine protein substitutes to meet protein requirements (1,2).

Protein substitutes predominantly consist of L-amino acids (L-AA) and are an essential component of the dietary management of PKU. Studies that profile the blood amino acid kinetics in healthy individuals following ingestion of elemental amino acids have provided useful insight into increased amino acid oxidation rates and a decrease in whole-body protein retention compared with ingestion of whole protein sources (3–8). Accordingly, and as a compensatory measurement, dietary recommendations advise a correction factor of 20-40% in excess of the protein requirement guidelines for the general population (1,2,9). However, the scientific evidence underpinning protein requirement guidelines for people with PKU is limited and, as such, are based on extrapolations from studies that estimate protein requirements in healthy populations (10). Moreover, these studies utilised nitrogen balance methodology (11) that determines the minimum nitrogen requirement to balance nitrogen losses, as opposed to a requirement that serves to optimise health and functional outcomes in a given population group (12,13).

Whilst current dietary guidelines are effective in maintaining blood phenylalanine concentrations at levels to support neurological development, the impact of a lifelong PKU diet on physical and functional outcomes remains unknown. Concerns have been raised regarding the impact of standard dietary practices on growth, rates of obesity and bone health in people with PKU, with conflicting results reported (14–20). Limited attention has focused on the impact of a PKU diet on functional outcomes such as skeletal muscle mass (SMM) that serves as the major storage site for amino acids. Muscle mass is a key determinant of an individual's physical and functional ability, and plays an underappreciated metabolic role in

reducing risk of cardiometabolic diseases, including obesity, cardiovascular disease, diabetes and hyperlipidaemia (21). Moreover, the age-related decline in SMM begins as early as the fourth decade of life and continues to progress with age (22). However, it remains unknown whether existing protein requirement guidelines for people with PKU adequately offset age-related changes in protein metabolism across the adult lifespan in order to optimise functional and health outcomes (10).

The primary aim of this scoping review is to characterise the protein status of people with PKU across the lifespan, as determined by a combination of anthropometric, biochemical, and functional measurements. The secondary aim is to identify key nutritional and lifestyle variables that influence protein status in people with PKU. Understanding how PKU and associated dietary components facilitate or compromise the protein status of the individual is crucial to inform future research into personalising protein recommendations for individuals of all ages with PKU. In this regard, a scoping review is pertinent to enable the breadth of evidence to be mapped and synthesised given the wide focus of the research questions.

METHODS

This scoping review was undertaken in accordance with the five-stage framework outlined by Arksey and O'Malley (23), and is reported in accordance with the PRISMA Extension for Scoping Reviews checklist (24). A full peer-reviewed protocol is available (25).

Identifying relevant studies

Information sources and search strategy

MEDLINE (Ovid), Embase (Ovid), CENTRAL, Web of Science and Scopus were searched between June and July 2021 to identify relevant literature. No date restriction was applied and all articles up to July 2021 were included. Only English language articles were selected. Abstracts from relevant conferences held between 2010-2020 and reference lists of eligible full-text articles were manually searched for additional literature. Corresponding authors were contacted to request articles not available in full text. The search strategy was developed by

the research team, with guidance from a Librarian (see supplemental material for example of MEDLINE (Ovid) search strategy).

Eligibility criteria

The Joanna Briggs Institute population, concept, context (PCC) strategy was used to develop the inclusion and exclusion criteria (25). Studies were considered for inclusion if they included participants with PKU, and all age categories and study designs were considered *(Population)*. Pregnancy and the presence other co-morbidities that could influence protein intake were excluded. Given that the focus of this scoping review was on body protein status, eligible studies reported anthropometric, biochemical and/or functional measurements of protein status in people with PKU *(Concept)*. Studies from all geographical areas were considered *(Context)*.

Study selection

All records were uploaded to Zotero 5.0 (George Mason University, USA). Following the removal of duplicate records, two independent reviewers (SF, MOK) applied the pre-defined eligibility criteria and independently assessed eligible titles and abstracts. In accordance with the published protocol (25), studies were excluded if they reported body weight, height, growth parameters (height, growth rate and head circumference), body mass index, fat mass (FM), or bone mineral density, with no other measurements of protein status. Full texts of eligible articles were retrieved and independently assessed by the same reviewers (SF, MOK). Studies were included if they reported at least one measurement of protein status (Table 1). Reasons for exclusion of full-text articles are outlined in Figure 1. Following each stage of the selection process, the reviewers compared results and reached a consensus.

[Insert Table 1: Examples of anthropometric, biochemical and functional measurements of protein status]

Data charting process and items extracted

Data from eligible full-text articles were independently tabulated by the reviewers (SF, MOK), using an extended version of the data charting tool outlined in the protocol. The tool was piloted and modified to ensure all relevant information was extracted. Only anthropometric, biochemical and functional data relevant to the measurement of protein status (Table 1) were extracted. Factors modulating protein status or variables (either controlled or stratified by) were extracted if relevant to the assessment of protein status. The majority of studies included multiple outcome measurements but only the findings pertinent to protein status were tabulated. Additionally, primary outcomes and pharmacological interventions (eg. BH4 or sapropterin) were extracted. Due to limited availability, these data were not tabulated, but will be discussed herein. Protein intake data were extracted (see supplemental material).

Data Synthesis

The data is synthesised and reported under three main sections: anthropometric, biochemical, and functional measurements of protein status and variables influencing protein status are discussed under each section. Age categories were defined as: 'children' <10 years of age, 'adolescents' 10-19 years of age, 'adults' >19 years of age, and 'older adults' >60 years of age. Prealbumin, otherwise known as transthyretin, will be referred to as prealbumin throughout the text.

[Insert Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) study flow diagram outlining the process of study selection]

RESULTS

The electronic database search identified 1353 papers and an additional 94 were obtained through manual searching (Figure 1). Following the removal of duplicates, 860 titles and abstracts were assessed resulting in 102 studies for full text review. Thirty-two studies were subsequently excluded, with reasons for exclusion outlined in Figure 1. Full data extraction was performed on 70 studies and sample size ranged from one (7) to 174 participants (26). Of

the 70 studies which reported outcomes relevant to protein status, 24 reported anthropometric, 32 biochemical, and 14 included mixed outcome measurements of body protein status. No studies measured protein status in older adults (>60 years of age). Twenty-six studies included participants that were diagnosed through newborn screening or defined as being early diagnosed for PKU. Six studies included both early and late diagnosed, but provided no further information, and 38 studies did not report time of diagnosis.

The extent to which protein status was reported varied between the 70 studies. Giovannini et al (2014) was the only study to state a primary outcome measurement that was related to protein status. Nineteen studies focused on investigating protein status or specific marker(s) of protein status and, of these, 6 included anthropometric methods (27–32), 11 included biochemical methods (7,33–42), and 2 included multiple methods (43,44). The remaining 50 studies were not primarily focussed on assessing protein status but included relevant markers.

What is the existing evidence of the protein status of people with PKU across the lifespan?

Anthropometric assessments of protein status

Study characteristics

Study characteristics are summarised in Table 2. Thirty-seven studies, including 24 full text papers and 13 conference abstracts, reported anthropometric assessments of protein status. Seven were interventional studies but only three reported the study design; two had a retrospective design (45,46) and one was a 3-year prospective longitudinal study (27). The remaining 30 studies were non-interventional, of which 53% were cross-sectional (28,29,31,43,44,47–57). Nine studies did not state the study design; however, based on the reported methods, all were non-interventional (30,32,58–64).

One study was conducted in children only (47), 15 in children and adolescents (aged 2 months to 19 years) (27,44,48,49,54–56,58,59,62,65–69), 11 in adolescents and adults (age range 15-50 years) (43,45,46,53,57,61,63,64,70–72), and one in adults only (60). Seven studies

included a mixed age cohort (age range 4 to 54.6 years) (28–31,50–52). Two studies did not report participants age (32,73).

How is body protein status measured?

Methods to assess body protein status are presented in Table 3. The majority of studies utilised bioelectrical impedance analysis (BIA) (32,45,46,49–52,57,64,65,70–72), dual-energy X-ray absorptiometry (DEXA) (27,29,30,53,56,62,63,68) or skinfold techniques (31,44,55,58,66,69) for the anthropometric assessment of protein status. Protein status was also measured using deuterium dilution technique (73), total body electrical conductivity (48), peripheral quantitative computed tomography (43), total body potassium (59), and prompt gamma neutron capture analysis for total body nitrogen (TBN) (44). Two studies used more than one method to assess protein status; Dobbelaere et al (2003) used skinfolds and BIA, whilst Wilcox et al (2011) used DEXA and gamma neutron activation analysis. Evans et al (2018) measured fat-free mass (FFM) using both BIA and total body water by deuterium dilution technique (TBWDeut) to determine the validity of BIA as a measurement of body composition in children and adolescents. This study reported no difference in FFM between the two measurements and confirmed that FFM calculated from BIA was correlated with FFM calculated from TBWDeut. Three studies did not report the method used for determining protein status (54,61,67).

Key findings from anthropometric assessments of protein status

Table 4 describes the main findings for anthropometric outcome measurements related to protein status. The majority of studies that compared cohorts of people with PKU to healthy controls reported no significant differences in protein status, regardless of age (32,44,47,48,50–52,56,62,65,71,73). However, in one study, TBN was lower in participants with PKU and, within each age, children with PKU accrued 53g less TBN compared to controls, equating to a six-month lag in TBN in the PKU group (44). Longitudinally, the annual accretion of nitrogen was comparable between groups. Moreover, in a similarly aged PKU population, lower total body potassium z-scores were reported compared to controls (59). A reduction in muscle cross-sectional area (MCA) was also reported in adolescent and adult

participants with PKU, with 30% exhibiting a MCA below the third percentile (43). Dietary adherence in this study was variable, whereby 59% of participants were following a PKU diet plus protein substitutes and a further 7% of participants were following a PKU diet with no supplementation. In contrast, Paci et al (2018) reported a greater FFM and total body water content in children and adolescents with PKU compared to controls, although neither the methods of assessments nor protein intake data were reported.

There was greater variation regarding the assessment of protein status within cohorts of people with PKU. In children and adolescents, total body protein and muscle mass were normal (55), and consistent findings were reported in adults (60). Two studies reported a lower protein status in people with PKU, with 30.8% of child and adolescent participants recording a deficit in fat-free mass index (FFMI) (54), whilst in a mixed-age cohort, a 41.5% overall deficit in muscle mass was identified (31). Of those participants that reported a deficit in muscle mass, the majority (87.5%) were of normal weight-for-age and of adolescent age (78%) (31). Regarding the relationship between body weight and protein status, Rocha et al (2010) found that adolescents and adults with PKU who were classified as overweight and obese reported a lower FFM percentage compared to other participants. No data regarding protein intakes were reported in the above studies; however, Rocha et al (2010) observed no difference in the median intakes of natural protein and protein substitute in participants classified as overweight/obese compared to other participants with PKU. Three studies revealed positive correlations between FFM and bone mineral density (BMD) (56,57) and bone strength (43) in people with PKU, and FFM rather than FM had a greater effect on BMD. In one study, these findings were limited to females only (57).

Factors modulating anthropometric outcomes of protein status

Gender

Several studies focused on factors known to influence protein status. Stratified by sex, Sailer et al (2020) found male children and adolescents to exhibited a lower percentage lean body mass (LBM) compared to healthy controls, whereas Allen et al (1995, 1996) found female children and adolescents to have lower total body FFM and TBN, respectively, when compared

to controls. Sailer et al (2020) reported no differences in total protein intake between people with PKU and controls, and no differences in total protein, natural protein and protein substitute intake were detected between males and females with PKU (see supplemental material). However, when considering percentage of total energy from protein and protein to energy ratio, protein intakes were lower in males with PKU compared with controls. These differences in protein intake were not observed in females. No sex comparisons of dietary protein intake were reported by Allen et al (1995, 1996). In contrast, one study reported no sex differences in body composition, although in the mild hyperphenylalaninemia (HPA) group, increased LBM was identified in pubertal compared to prepubertal participants (56).

Within a PKU cohort, adolescent and adult males reported a higher LBM and appendicular lean mass compared to females (53). However, no sex differences were observed in appendicular lean mass index (ALMI) or ALMI z-scores, although the mean ALMI among younger males was close to a cut-off point previously suggested to identify sarcopenia in older men. The mean total protein substitute consumed was greater in males compared to females but did not reach statistical significance. The intake of protein substitutes, expressed per kilogram of body weight or per kilogram of lean mass, were not statistically different between males and females.

Dietary factors

The impact of natural protein and/or protein substitute intake on protein status has also been examined. In children and adolescents, Huemer et al (2007) reported that natural protein intake correlated with FFM, explaining 47.7% of the variance in FFM. This relationship was also observed in a mixed-age cohort. Jani et al. (2017) correlated high natural protein intake with high FFMI and low FMI:FFMI ratio in adults with PKU, whereas in children, protein substitute and total protein intake were associated with FFMI. In a mixed-aged PKU cohort, the percent of total protein intake, expressed relative to DRI, positively correlated with muscle mass (52). Moreover, a similar relationship between protein intake and protein status in children and adolescents was reported but in the context of percentage of FM rather than percentage of FFM; with a decline in percentage FM there was an assumed proportionate

increase in FFM (65). A negative correlation was reported between total protein, natural protein and protein substitute intake and percentage of FM, that presumably translates to a positive correlation with percentage of FFM (65). In contrast, no differences in body composition parameters were reported in adolescents and adults with PKU that were consuming vegan diets, vegan diets plus protein substitutes, non-restricted diets (72) or PKU-diets (64,72).

Metabolic control

Metabolic control has been reported to impact protein status. Poorer metabolic control (i.e., higher phenylalanine levels) resulted in improved protein status (32,66). The cause for higher phenylalanine levels in participants included in these studies is unclear and could be related to increases in habitual dietary protein intake and / or reduced intake of protein substitutes. In contrast, one study reported no difference in LBM between the pubertal PKU sub-groups with high phenylalanine levels and participants with blood phenylalanine levels within target range (30). However, when analysed using LBM z-scores, a significant difference was reported with lower LBM z-scores in those participants with higher phenylalanine levels. No difference was reported in LBM z-scores between the pre-pubertal and the pubertal subgroup with recommended phenylalanine levels, indicating no impact of pubertal status on LBM z-scores. Choukair et al (2017) reported no relationship between MCA and classification of PKU or phenylalanine levels.

Type of protein substitute

Two studies investigated LBM in children and adolescents with PKU that consumed either casein glycomacropeptide (cGMP) or L-AA (27,68). No significant differences between groups were found at baseline; however, Daly et al (2019) reported higher FFM values in those participants taking cGMP compared to L-AA after 36 months. Whilst not significant, Daly et al (2021) demonstrated improved LBM in people with PKU taking a cGMP-based protein substitute. No differences in body composition parameters were reported in adolescents and adults taking L-AA compared to cGMP (45,46).

Pharmacological management and other factors

Two studies measured protein status outcomes in participants taking BH4 and reported no difference in percent FFM compared to participants on diet only (65), and in branchial muscle area compared to general population reference data (69). Genotype (29,53) nor socioeconomic status (66) were associated with protein status.

Biochemical assessments of protein status

Study characteristics

Forty-four eligible articles, 33 full text and 11 abstracts, included biochemical assessments of protein status (Table 2). Eighteen articles were intervention studies, of which seven reported the study design. Randomised control trials were conducted by Prince et al (1997), Giovannini et al (2014) and Ney et al (2016). Two studies were prospective (74,75), and two were retrospective longitudinal studies (45,46). The remaining 11 studies did not identify the study design; however, based on the reported methods, had an interventional design (7,36,39–41,69,72,76–79). Twenty-six studies were non-interventional and the majority utilised a cross-sectional design (44,56,57,80–82). Thirteen studies failed to state the study design, but were non-interventional based on the methods reported (33,34,38,42,62–64,83–88).

There was greater variation in participant age for studies including biochemical measurements of protein status than anthropometric measurements of protein studies. Six studies were conducted in children (age range 13.7 days to 10 years) (33,38,42,84,85,89), ten studies were in children and adolescents (age range 2.4 months to 18 years) (44,56,62,69,74,76,77,83,86,90), 15 in adolescents and adults (11-49 years) (7,39,40,45,46,57,63,64,70,72,75,79,91–93) and two in adults only (41,82). Seven studies included mixed-age cohorts (age range 7 months to 54 years) (26,36,37,80,81,94,95). Four studies failed to report participant age (34,78,87,88).

How is protein status measured using biochemical assessments?

A range of biochemical measurements were used to assess protein status, and most studies included multiple measurement (Table 3). Serum albumin (36,41,42,56,62,63,69,72,74,78,85,86,95), prealbumin (26,34,37,57,80,81,84,87,89) or both (33,38,40,45,46,76,77,82,83,88,90,91,93,94) were the most common biochemical measurements reported. Total protein (36,40–42,56,63,72,76,78,80,81,85,86,91,93,94) and blood urine nitrogen (BUN) (33,40,45,74,75,78,85,86,93) were also frequently used. Three acute phase studies were included: two studies measured whole-body protein metabolism using valine and leucine stable isotopes (39,41), and one measured nitrogen excretion and leucine kinetics (7).

Key findings from biochemical assessments of protein status

The key findings for biochemical assessments of protein status are summarized in Table 5. In children with PKU, no differences in albumin (33,38,42), total protein (42), retinol binding protein (33), BUN (33) or plasma essential amino acid (EAA) concentrations (except phenylalanine) were reported compared to healthy controls (89). In children and adolescents, studies reported albumin (56,62,83,85), total protein (56,83,85), creatinine (62), BUN (85) and/or plasma amino acid levels (44) were comparable to untreated HPA or controls. However, two studies identified lower concentrations of albumin (77,90), retinol binding protein (77), and higher amino acid ratios (90) in participants on a PKU diet compared to untreated HPA or controls. Furthermore, deficits in prealbumin (38,77,83,90) were found, and participants with low prealbumin levels were more likely to have an EAA deficiency (83). In contrast, Acosta et al (1999) and Prince et al (1997) observed prealbumin concentrations within normal reference ranges despite recording protein intakes below the recommended dietary allowance (RDA) (1980) and the Medical Research Council (1993) protein recommendations, respectively.

In two mixed-age cohorts, increased prealbumin concentrations were observed in PKU participants compared to controls, with no difference in albumin and total protein levels (88,94). In contrast, five studies reported low prealbumin levels in PKU participants (34,37,80,81,87); one study reporting prealbumin ≤20 mg/dL in 60% of participants (34) and

another reported prealbumin levels below the third percentile for 15% of PKU participants, despite average total and natural protein intakes (g/kg/day) of 117% and 48% of RDA, respectively (80). Total protein levels were similar between people with PKU and controls (80). Despite normal levels of total protein, abnormal albumin (high) and BUN (low) were identified in children and adolescents where total protein intake was 62 ± 15 % of RDA (86). Low protein levels in adolescents and adults with low SMM, and normal protein levels in participants with high SMM were also observed (70). In contrast, Modan-Moses et al (2007) reported plasma albumin concentrations to be within range, and Prochazkova et al (2012) reported no significant differences in levels of prealbumin among a large mixed-age cohort of people with PKU and HPA.

Thompson et al (1990) and van Rijn et al (2007) investigated whole body protein metabolism in adolescents and adults with PKU compared to healthy participants. Both studies reported no differences in whole body protein metabolism between groups. In the study by van Rijn et al (2007), participants with PKU were prescribed total protein intakes (natural protein and protein substitute) to meet 120% of RDA. Half of the participants with PKU in the study by Thompson et al (1990) were consuming a normal diet, and plasma amino acid concentrations were at the lower end of the reference range compared to the participants on a PKU diet and controls. Thompson et al (1990) reported no association with plasma phenylalanine concentrations and protein synthesis rates.

Factors modulating biochemical outcomes of protein status

Type of protein substitute

Protein substitutes also modulated various biochemical markers of protein status. Studies reported no difference in BUN (45,74,75,93), albumin (45,46,74) and prealbumin (45,46) concentrations in a mixed age cohort of people with PKU taking cGMP versus L-AA protein substitutes. However, van Calcar et al (2009) reported lower BUN with cGMP compared to L-AA protein substitutes. Ney et al (2016) reported higher albumin concentrations with the ingestion of cGMP than L-AA. When habitual protein substitutes were replaced with a protein substitute utilising prolonged release technology, Giovannini et al (2009) reported an

improvement in prealbumin concentrations within three days, and demonstrated a significant increase in prealbumin (90), plasma protein, albumin, and some amino acids (36) in participants who received the prolonged release protein substitute. These changes were not observed in participants taking only 80% of protein requirements from a prolonged-release protein substitute (36).

Pharmacological management

Studies that implemented BH4 therapy resulting in a corresponding increase in natural protein intake and reduction in protein substitute dose reported no changes in the majority of biochemical markers of protein status (69,76,78,91). However, an increase in prealbumin levels in children on BH4 treatment was reported by Singh et al (2010), despite total protein intakes remaining stable. In contrast, two studies reported lower BUN (78) and prealbumin (81) in participants on BH4 treatment. Adults treated with pegvaliase that recorded protein intakes that met or exceeded the RDA for protein reported no deficiencies in albumin, prealbumin and essential amino acids (82).

Gender, age, metabolic control, dietary and other factors

Sumanszki et al (2019) reported prealbumin concentrations among adolescents and adults with PKU to be higher in males compared to females. Age and metabolic control were reported to correlate with prealbumin levels across all age-categories. Desloovere et al (2014) stated a correlation between prealbumin and age but provided no supporting data. Lower prealbumin levels were reported in participants who were younger (37,81,83,84,94) and those with better metabolic control (80,81,83,84,94). In contrast, Rocha et al (2010) and Desloovere et al (2014) reported no correlations between prealbumin levels and median blood phenylalanine levels. Furthermore, no significant correlations were reported between prealbumin levels and the amount of protein substitute prescribed (34,37) or classification of PKU (37), and between prealbumin or total protein and time of diagnosis (81). Prealbumin levels were observed to correlate with haemoglobin levels (87). Age was reported to positively correlate with serum

total protein levels (81) and albumin concentrations (95), whereas findings for metabolic control were inconsistent (42,81).

No associations between dietary protein intake and biochemical markers of protein status were reported across studies (42,62), although studies were limited. Moreover, whether participants consumed a vegan diet, vegan plus protein substitutes, unrestricted or PKU diets did not alter total protein levels (72). However, plasma amino acid levels were higher in those individuals on a PKU diet and serum urea concentration was low-to-normal in those on restricted protein diets (72), consistent with findings presented by Das et al. (2010). The only study to measure biochemical markers of protein status in response to exercise was conducted by Mazzola et al (2015). At baseline, participants with PKU reported lower levels of BCAA compared to controls, with BCAA levels not modified post exercise in both PKU participants and controls.

Functional assessments of protein status

Study characteristics

Three studies included functional assessments of protein status, with one study specifically related to muscle strength (43) and two studies including assessments of physical performance (71,79) (Table 3). One study was cross-sectional in design (43), and the other two studies did not state the study designs but were interventional according to the methodology. All studies included adolescents and adults with PKU, and made comparisons to healthy controls (71,79) or reference population data (43) (Table 2).

How is protein status measured using functional assessments?

Choukair et al., 2017 used handgrip strength as a functional measurement of protein status, whilst Sumanszki et al (2020) and Mazzola et al (2015) measured VO_2 max as a functional measurement of physical performance (Table 3).

Key findings and factors modulating functional protein status outcomes

Table 6 describes the main findings for functional assessments of protein status. Compared to a healthy population, handgrip strength was reduced in participants with PKU, with over one third of participants recording handgrip force below the third percentile (43). Regarding exercise performance, Sumanszki et al (2020) reported a lower VO_2 max and cumulative workload was lower in the PKU group compared to controls (71). In contrast, Mazzola et al (2015) reported no differences in VO_2 max between PKU and controls.

DISCUSSION

This scoping review is the first to examine the literature that describes the body protein status of people with PKU, with a specific focus on anthropometric, biochemical and functional assessments of protein status. Overall, the findings for anthropometric, biochemical and functional measurements of protein status were inconsistent. Whilst majority of studies reported no discernible differences in anthropometric parameters when comparing participants with PKU to healthy controls, a significant minority of studies reported deficits in muscle mass among PKU cohorts and warrant further investigation. Two studies identified 30-40% of participants with PKU to have deficiencies in muscle mass (31,43). In one study, 87% of participants with reduced levels of muscle mass were of normal body weight, thus emphasising the importance of body composition analysis in addition to body weight (31). This has been discussed in the literature in the context of body fat assessment in people with PKU (96), and our findings also support the recommendation of monitoring FFM in people with PKU across the lifespan.

BIA was the most common method for assessing body composition, likely due its practicality in the clinical setting and cost effectiveness. BIA has been validated for FFM assessment in children and adolescents with PKU (28); however, it remains unvalidated in adults and older adults with PKU. Given the increasing adult PKU population, validation is warranted. Furthermore, data from the general population demonstrates that skeletal muscle mass has an important role in bone health (21) and these findings are now corroborated in people with PKU (43,56,57). Concerns have been raised regarding the impact of the PKU diet on bone health, which will be further exacerbated with ageing. Therefore, monitoring, and optimising muscle mass in people with PKU play an important role in bone health outcomes.

Biochemical measurements of protein status are routinely used in monitoring patients with PKU, but a lack of consistency in the markers utilised has been identified. Most studies measured albumin and/or prealbumin concentrations. Albumin levels were predominantly comparable to healthy controls or reference data, whereas the prealbumin levels were generally lower in people with PKU than healthy controls or reference data. Due to its short half-life, prealbumin is favoured over albumin for monitoring changes in nutritional status

(97), and therefore findings related to prealbumin concentrations may provide greater insight into the protein status of people with PKU than plasma albumin concentrations. However, both markers are influenced by disease states, and therefore their specificity to protein status may be limited (97,98). Measurements such as D3-creatine dilution, 24-hour urinary creatinine and 3-methyhistidine concentrations have been recommended for monitoring protein status (97,99), but no studies have conducted these measurements in people with PKU, and their practicality for clinical use may be limited. Future research is warranted to determine the most effective biochemical markers for monitoring protein status in people with PKU. At the level of whole-body protein metabolism, two studies reported comparable outcomes to healthy controls and mean total protein intake exceeded RDA by 20%, which aligns with current dietary guidelines (1,2,9).

It is clear that the functional measurements of protein status remain scarce, with only one study measuring muscle strength. Two studies measured physical performance as an indirect measurement of protein status. In this regard, both physical performance and muscle strength were compromised in participants with PKU (43,57). Muscle mass does not directly translate to functional ability (100), and therefore outcomes from anthropometric studies in PKU cannot directly be extrapolated to functional ability. Accordingly, future studies are warranted to advance understanding regarding how PKU dietary guidelines translate to functional outcomes. Moreover, physical activity is known to modulate protein status in the general population, with exercise improving nitrogen retention (101) and postprandial rates of muscle protein synthesis (102). With the exception of one study that reported the impact of acute exercise on plasma BCAA concentrations in participants with PKU (79), no studies have directly investigated the impact of physical activity on protein status in people on a PKU diet.

Studies included in this review were conducted between 1980 until 2021, a period that has seen significant advances in the dietary management of PKU. It is now recognised that protein requirements should exceed the RDA by 20-40% to compensate for the reduced uptake and utilisation of protein substitutes. Due to inconsistencies in studies reporting protein intake data, no conclusions could be made on whether differences in findings could be attributed to intakes of natural protein and protein substitutes. However, in studies reporting protein

intake data, dietary patterns did not translate to protein status outcome measurements, whereby protein intakes both above and below RDA had mixed findings. A limited number of studies utilised the current protein recommendation for PKU dietary management of 120-140% of RDA.

Several studies investigated the association between protein intake and protein status outcomes. Two studies reported that higher total protein intakes, as measured by 24 hour recalls and food records, resulted in improved protein status in children, adolescents and adults with PKU (52,65). In contrast, Huemer et al (2007) reported natural protein intake to be an important predictor of protein status in children and adolescents with PKU. Jani et al (2017) observed a similar association in adults with PKU, whereas total protein and protein substitute intake were shown to be of greater importance in children. In the context of a traditional PKU diet, there is limited scope for large increases in natural protein, thus highlighting the importance of preventing over-restriction by reassessing natural protein tolerance (103). Moreover, with pharmacological treatments there are now opportunities to improve natural protein intake. In addition to the amount of protein substitute, the type of protein substitute can modulate the protein status of an individual (27,36,40,45,46,68,74,75,77,90,93); however, findings were inconclusive and further research is warranted. A correlation between metabolic control and protein status was identified, whereby participants with poor metabolic control exhibited improved body composition parameters and higher prealbumin levels, possibility attributed to increases in natural dietary protein intake. Therefore, those individuals who have good metabolic control may be at greater risk of compromised protein status.

In addition to protein intake, participant sex and age were observed to modulate body protein status outcomes in people with PKU, consistent with data from a non-PKU population (104–106). However, the impact of sex on anthropometric outcomes in participants with PKU was inconsistent, whereas age was found to positively correlate with prealbumin and total protein levels, which has previously been described in the general population (107). The majority of studies were conducted in children, adolescents and younger adults, whereas no studies included older adults. This omission is likely due to when newborn screening of PKU was

introduced, limiting numbers within this cohort. However, individuals who have received dietary treatment for PKU are now approaching older age and therefore understanding the impact of dietary management on ageing is a key focus question.

Protocol deviations and study limitations

This scoping review was undertaken in accordance with the published protocol (25), with the only deviation being redefining the age categories to include adolescents. Limited studies had a primary focus on protein status outcomes, and therefore the majority of findings were extracted from studies where protein status measurements formed part of a wider nutritional status assessment or were reported as secondary findings. Almost a third of studies were abstracts and provided limited data. The heterogeneity in studies and outcomes restricted the conclusions that could be drawn on variables modulating protein status. However, studies that specifically investigated associations between natural protein intake, amount and type of protein substitutes, metabolic control and protein status provided some useful insight into areas that require further consideration in optimising the health outcomes of people with PKU. Some studies were impeded by small sample sizes, in that half of the studies had sample sizes of 30 or less participants. Additionally, the same patient populations may have been included in multiple studies and this may have affected the interpretation of results.

CONCLUSION

The maintenance of SMM and function with advanced age is critical in reducing the risk of chronic disease and obesity, as well as optimising bone health. These conditions have been highlighted as concerns in the PKU population. However, limited studies and clinical guidance have specifically focused on monitoring and optimising FFM in people with PKU. Accordingly, it is currently unknown what the functional outcomes are for people with PKU following a phenylalanine restricted diet whereby the majority of protein is derived from elemental amino acids. Further research is warranted to understand both, the impact of PKU diet on functional ability, and the significance of currently used anthropometric, biochemical and functional

markers when assessing protein status in people with PKU. None of the currently used markers adequately assess body protein status; thus, emphasising the urgent need for research to establish robust clinical and biochemical markers of protein status that can be reliably used to monitor the impacts of dietary interventions in people with PKU.

Dietary adherence is known to decline with age, and therefore, conducting research in adults with PKU with a focus on investigating the impact of dietary management of health outcomes can be challenging. This challenge may be a contributing factor to the limited number of studies of protein status conducted in adults with PKU. With the consensus for life long treatment and as a result, a growing adult and ageing PKU population on dietary management, there is an urgent need for research specifically in adults with PKU to ensure dietary management leads to optimal health outcomes across the life course.

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Conflict of interest

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Author Contributions

SF, RR, KW, OW and MOK were involved in conceptualisation and development of the protocol. Database and manual searches were conducted by SF, and study selection, full text review and data extraction were completed by SF and MOK. SF led on preparing the original draft with support from MOK who was lead supervisor for this review. RR, KW, OW and MOK provided critical revision of the draft. SF, RR, KW, OW and MOK revised and approved the final manuscript.

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Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) study flow diagram outlining the process of study selection

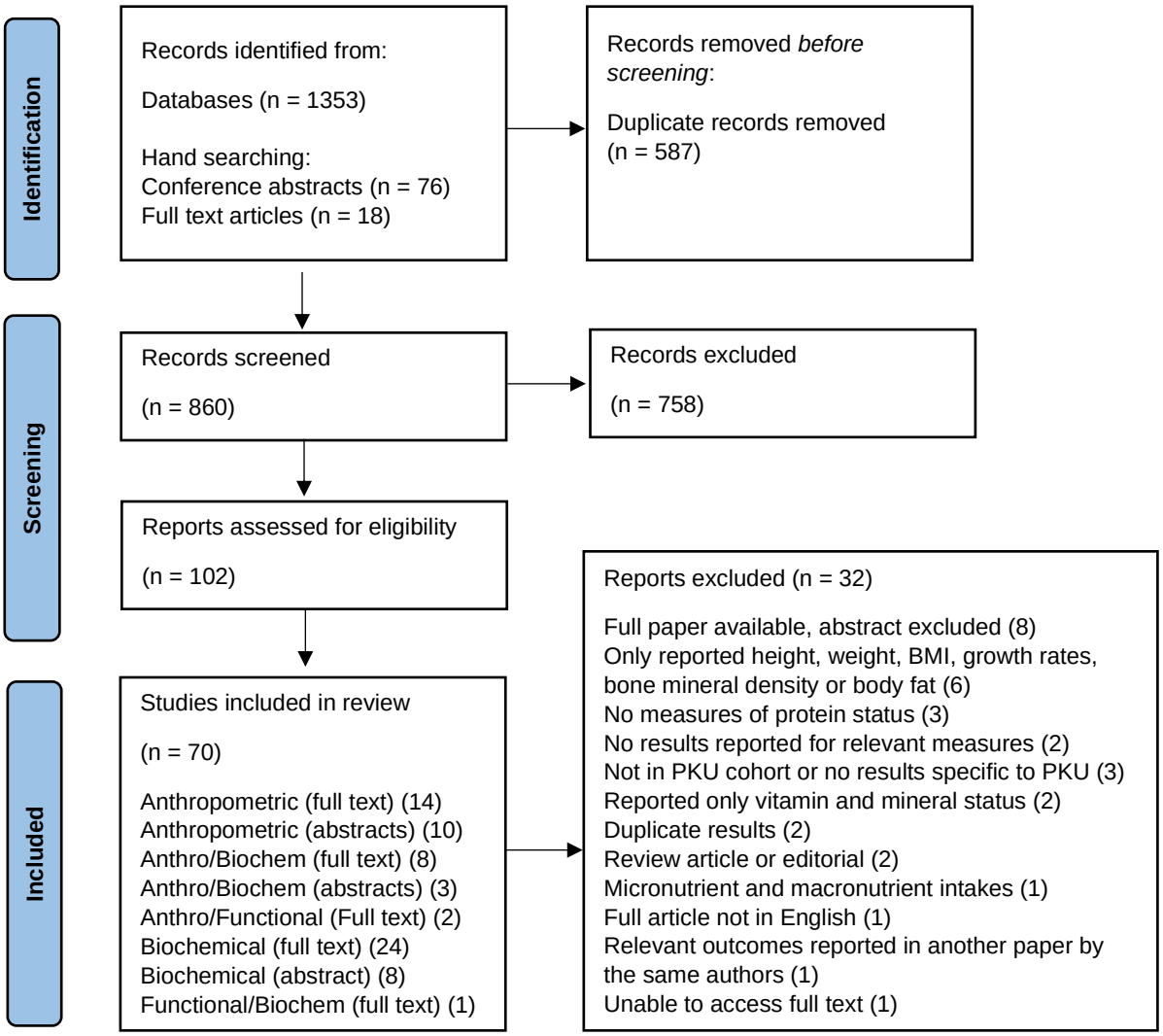

Table 1: Examples of anthropometric, biochemical and functional measurements of protein status

Anthropometric	Body composition (fat free mass, lean body mass and / or skeletal muscle mass) via dual-energy X-ray absorptiometry (DEXA), bioelectrical impedance analysis (BIA), total body electrical conductivity (TOBEC), BodPod whole body air-displacement plethysmography or skinfolds.
Biochemical	3-methylhistidine concentrations, albumin, pre-albumin, transthyretin, retinol-binding protein, urea production, blood urea nitrogen, urinary nitrogen, total body nitrogen, and whole-body protein metabolism. Plasma amino acids concentrations, urea production and creatinine where the author(s) have specifically used these as a measurement of protein status.
Functional	Hand-grip strength, the Short Physical Performance Test (SPPT; including tests of balance, gait speed, and timed sit-to-stand), one repetition max (1RM, or a five-repetition max for older adults), VO_2max tests (or VO_2peak for older adults) and other validated measurements of muscle function (i.e. isokinetic quadriceps strength using dynamometry and vertical jump performance using force platform technology).

Retrieved from the published protocol (Firman et al., 2021)

Table 2: Study characteristics classified by measurement category.

Author(s) (Year) Country	Study design	Participants (N, age, sex)	Intervention (duration of study)
		ANTHROPOMETRIC MEASURES	
Daly et al. (2021) UK	Longitudinal prospective (3y)	50 PKU (28 males, 22 females); 48 completed Age: AA 11.1y (range 5-15y); CGMP50 7.3y (range 5-15y); CGMP100 9.2y (range 5-19y).	2 types of protein supplements, allocation dependent on taste preference and phe levels: (1) AA: protein substitute given as AA only; (2) CGMP50: combination of CGMP-AA and AA; (3) CGMP100: all their protein substitute as CGMP-AA.
Daly et al. **(2019)** UK	Longitudinal prospective (36 mo)	28 PKU (18 on cGMP-AA (9 male) and 10 on L-AA (7 male). Median age at study end: CGMP-A: 11.2y (range 8-19y) and L-AA: 15.9y (range 9-18y).	Not applicable
Evans et al. (2017) Australia	Longitudinal prospective	32 PKU on diet (D-PKU) (10 males, 22 females; Age y ± SD (range) 9.2 ± 4.7y (0.83–18.0)) 5 PKU treated with BH4 ± phe restricted diet (BH4-PKU) (3 males, 2 females; Age y ± SD (range) 8.8 ± 4.6y (0.64–10.9)). 37 All-PKU (13 male, 24 female; Age y ± SD (range) 8.8 ± 4.6 (0.6–18.0))	Not applicable

		21 controls (healthy sex and aged matched siblings): 8 males, 13 females, Age y ± SD 8.8 ± 4.8y	
***Paci et al.* (2018)** Italy	Case-control	25 PKU (aged 5-14y) 25 sex and aged matched controls	Not applicable
Rocha et al. (2013) Portugal	Case control cross-sectional	89 PKU (age 14.4±6.6y; 46% females) 78 controls (age 15.9±7.1y; 58% females). 74.4% of controls were close relatives.	Not applicable
Huemer et al. (2007) Austria	Cross-sectional and longitudinal	34 classical PKU (22 males, 12 females; mean age 8.7 ± 3.9y, range 2 months to 15y) 34 healthy age- and sex-matched controls (mean age 9.2 ± 3.7y, range 1 month to 16y)	Not applicable
Sailer et al. (2020) USA	Cross-sectional	30 PKU (age 5-16y (mean 11.6y ± 3.41); 12 females, 18 males) 30 control age- and sex- matched (mean age 11.75 ± 3.49y; 12 females, 18 males)	Not applicable
Weng et al. (2020) Taiwan	Cross-sectional	22 PKU (ages 8-27y, mean age 15.23 ± 5.23 y; 12 females, 10 males) 22 age- and gender-matched controls (ages 8-39y, mean 19.73 ± 10.6y; 12 females, 10 males)	Not applicable
Evans et al. (2018) Australia	Cross-sectional	16 PKU (7 males, 9 female). Median age 12.5y (range 5–20.6y)	Not applicable

Stroup et al. (2018) USA	Cross-sectional	15 (6 males and 9 females) (12 adults (aged 19-50y) and 3 adolescents (aged 15-17y))	Not applicable
Jani et al. (2017) USA	Cross sectional study	86 PKU (59 children and 27 adults; 60.5% female) Age: Total sample: 16y (4 - 54.6y); Adults: 28.8 y (19.5-54.6), Children: 13.4y (4-18.9y))	Not applicable
***Torriente et al.* (2017)** Cuba	Cross-sectional	12 with PKU (aged 3-18y). Both genders (number not specified)	Not applicable
Mazzola et al. (2016) Brazil	Cross-sectional	27 PKU; 27 controls Aged (6-25y) and gender-matched (14 males and 12 females)	Not applicable
***Kanufre et al.* (2015)** Brazil	Cross-sectional	77 PKU (aged 5-25y)	Not applicable
***Mexia et al.* (2015)** Portugal	Cross-sectional	30 PKU; 57% female; mean age 12.2 ± 3.6y	Not applicable
Dobbelaere et al. (2003) France	Cross-sectional	20 PKU (11 females; 9 males), age 8mo-7y (4.5 ± 1.6y/55 ± 19 mo). Controls: Age (53 ± 19 mo) and sex matched	Not applicable
Nogueira et al. (2021) Brazil	Retrospective cohort study	53 PKU (aged 2-19y; (mean ± SD, 10.4 ± 4.6y); with 33 (62.3%) <12y old. 64% female (34/53)	Not applicable

***Alfheeaid et al.* (2018)** UK	Not reported	13 PKU and 10 health controls (matched for gender, age and BMI)	Intervention not reported, measurements taken over 3 hrs after isocaloric meal.
***Nalin et al.* (2013)** Brazil	Not reported	23 PKU and 17 heathy, aged and gender matched controls	Not applicable
Adamczyk et al. (2011) Poland	Not reported	45 PKU (aged 13.8 ± 5.2y, range 4.9-21.9y), 25 males and 20 females PKU subgroups = 1=pre-pubertal and normal phe levels, n=15 2a=pubertal and normal phe levels (n=18 2b=increased phe levels, n=12. Compared to references for healthy children and adolescents[a]	Not applicable
***Wilcox et al.* (2011)** Australia	Not reported	42 PKU (33 females, 9 males) mean age 32.2 ± 9.5y	Not applicable
***Bonifant et al.* (2010)** Australia	Not reported	20 PKU Data compared with normative data using matched controls	Not applicable
***Rocha et al.* (2010)** Portugal	Not reported	27 PKU (aged 18-38y)	Not applicable
Allen et al. (1995) Australia	Not reported	30 PKU (15 males, 15 females; aged 4.6-17.0y) 76 controls (23 male, 42 females; aged 4.3-18.4y). 7 unaffected siblings and 69 non-familial	Not applicable

BIOCHEMICAL MEASURES			
Ney et al. (2016) USA	2-stage, crossover RCT	30 PKU (18 females and 12 males) included 5 minors (aged 15–17 y) and 25 adults (aged 18–49 y)	11-week protocol: 1 week education; 3 weeks GMP-MF or AA-MF; 3 weeks on usual routine with AA-MF (washout), then cross-over to 1 week education and 3 weeks with either GMP-MF or AA-MF
Giovannini et al. (2014) Italy	RCT	60 PKU (n=30 prolonged release group; n=30 in conventional substitute group); 55 completers (24 males, 31 females; age 9.2y (3.4y)) 60 mild HPA (MHP) (26 males; age 9.3y (3.3y); 60 unaffected (26 males; age 9.2y (3.2y))	Random allocation to prolonged-realise PHE-free protein substitute or current conventional substitute for 30 days. Dose and frequency were tailored to needs of child. Unable to blind participants.
Prince et al. (1997) USA	Phase 1: RCT Phase 2: A historic control design	Phase 1: n=28 aged 4-10y 25 participants continued to phase 2 (completed the 5-y study). Compared to non-PKU data from Armstrong and Stave 1973	Phase 1: Duration 2 years. Interventional: Random allocation to new AA mixture or standard mixture. Phase 2: Compared the safety, efficacy, and acceptance of new treatment products developed with AA mixture tested in phase 1. Products nutritionally incomplete were taken with vitamin/mineral tablets
Ahring et al. (2018) Denmark	Single-blinded, prospective, crossover	8 (7 female, 1 male), age 15–48 y (mean 33.25 ± SD 11.21)	24 h to 1 month Intervention = 4 AA drink mixtures (DM)

	intervention study		DM1: Lacprodan CGMP-20; DM2: FSAA (equivalent AA profile as DM1); DM3: Lacprodan CGMP-20 and synthetic AA; DM4: FSAA (equivalent AA profile as DM3 but without Phe. 4 visits per patient, random allocation. Bloods at T0, T15, T30, T60, T120 and T240 min after meal. Test meal: total protein content was 25% of 1g /kg/day.
Zaki et al. (2016) Egypt	Prospective, self-controlled, clinical trial	10 PKU (6 males, 4 females), aged 4-16y. Median IQR age 6.73 (5.02-11.79y)	Two phases: Phase I was 9 weeks (50% GMP (cheese spread) and 50% AAF) and Phase II was 9 weeks (100% AAF).
Kose et al. (2019) Turkey	Single center, case-control	112 PKU (59 males, 53 females). Age: 136.8 ± 82.1 months (range: 18 to 377 mo) PKU categorised into two groups: Low dietary adherence (n=71, 41 females, 30 males; age 138.9 ± 80.1 mo (18-377 mo)); High dietary adherence (n=41; 12 female, 29 males; age 133.1 ± 84.4 mo(18-207 mo)) 36 healthy controls (18 males, 18 females). Age: 119.7 ± 37.3 mo (73 to 206 mo).	Not applicable

***Prochazkova et al*.** **(2012)** Czech Republic	Prospective	174 patients (113 children, 61 adults)	Not applicable
Viau et al. (2021) USA	Cross-sectional	18 (mean age, SD 38.2 ± 8.8), 11 females,7 males	Not applicable
Andrade et al. (2017) Spain	Cross-sectional	42 PKU (23 males, 19 females); Median age 10y (range 2-36y) 40 age and sex-matched controls	Not applicable
Crujeiras et al. (2015) Spain	Cross-sectional	156 PKU (46.8% male; range age: 7 months–42y old; 27.4% >18y)	Not applicable
van Vliet et al. (2019) The Netherlands	Retrospective	12 with TT1 (mean age 13.5 ± 9.9, 75% male, 25% female) 92 with PKU (mean age 24.5 ± 13.9, 45% male, 55% female)	Not applicable
Rocha et al. (2010) Portugal	Retrospective	69 treated PKU; 30 females (43.5%) and 39 males (56.5%) aged 1–27y (mean = 10y; SD = 6.47y)	Not applicable
Gokmen-Ozel et al. (2009) UK	Audit	34 PKU, 17 female, 17 male, median age of 15y (range 7–54y); 13 participants aged >18y of age.	Not applicable
***Kose et al.* (2016)** Turkey	Not reported	112 PKU 17 controls (age- and sex-matched)	Not applicable

***Desloovere et al*.** **(2014)** Belgium	Not reported	35 PKU	Not applicable
Schulpis et al. (2013) Greece	Not reported	54 PKU divided into groups based on metabolic control Group A: 24 (12 males, 12 females, mean age 6.78 ± 1.5 y) Group B: 30 (15 males, 15 females, mean age 5.0 ± 3.2 y) 50 age-and sex-matched controls (25 males, 25 females, mean age 7.68 ± 2.3 y)	Not applicable
Douglas et al. **(2013)** USA	Not reported	57, unclear how many participants were responders	Sapropterin for 1 month, followed by phe challenge and classified as Definitive (DR) or Provisional (PR) Responders. Non-responders (NR) discontinued drug. Protein status assessed after 1 year
Singh et al. (2010) USA	Not reported	Stage 1: 10 (9 males and 1 female) mean (SD) age 8.7y (2.5y); Stage 2: 6 (6 males).	Stage 1: BH4 response testing. BH4 administered OD 20 mg/kg/d. If plasma phe decreased by at least 30% after 1 wk = responsive. Dietary phe tolerance determined via milk challenge. Stage 2: Protein substitute and protein intake adjusted to keep phenylalanine in target. Follow-up for 24 months as per standard care.

Giovannini et al. **(2009)** Italy	Not reported	28 treated HPA (aged 6–18 y) 56 untreated mild HPA 56 controls matched for age and gender.	Slow-release protein substitute given to 28 treated HPA and dietary intake and biochemical parameters reassessed after 3 days.
Rocha et al. **(2009)** Portugal	Not reported	60 PKU	Not applicable
van Calcar et al. (2009) USA	Not reported	11 subjects (age range: 11–31y; 7 males and 4 females)	Two dietary treatments of 4 d each: AA diet (days 1–4) and GMP diet plus multivitamin as GMP nutritionally incomplete (days 5–8). Dietary menu (24-h) repeated on all days of diet treatment. Meals were timed to usual routine and PA was permitted but limited. Bloodspots taken d1-2. All blood samples drawn daily 3 h after the start of breakfast or 2.5 h after eating breakfast (days 3–8).
van Rijn et al. (2007) The Netherlands	Not reported	6 PKU (27 ± 7 y; 3 females and 3 males) 6 controls (32 ± 4 y, 4 females and 2 males)	Isocaloric meal for 1 day providing 0.8g protein/kg/day, plus additional 20% from PKU3 supplement Baseline bloods and breath samples taken. T0-T60min whole-body $NaH_{13}CO_3$ production was measured using a primed constant infusion of $NaH_{13}CO_3$. Regular breath samples taken.

			T60-T420 min: $NaH_{13}CO_3$ infusion replaced with L-[1-^{13}C]-valine bolus followed by a continuous infusion Regular blood and breath samples taken. At 1200 h, the meal period was started by consumption of the first meal and continued for 4 h by consumption of a meal every 60 min. After the start of the meal period, blood and breath samples were taken every 30 min for 3 h and during the last hour samples were taken every 15 min.
Giovannini et al. **(2006)** Italy	Not reported	13 PKU (7 females, 6 males; mean age 14y, range 5-26y)	Participants randomised to new AA mixture for 6 mo: Group 1: 100% daily N needs Group 2: 80% daily N needs Blood markers measured at baseline and 6mo.
Arnold et al. (2002) USA	Not reported	38 PKU (mean age was 8.9y)	Not applicable
Arnold et al. (2001) USA	Not reported	41 PKU (24 males, 17 females); age 1-16y; Age- and gender-matched patients (non-familial). Prealbumin controls were not available.	Not applicable
Mönch et al. (1996) Germany	Not reported	Study 3: 10 (aged 12-23y) Study 4: 1 adult with PKU (female)	Study 3: Day 1: AA mixture in two divided portions; Day 2: AA mixture in three divided portions Study 4: ^{13}C-L-leucine (3 mg/kg) was given as a single

			bolus together with the AA mixture taken at breakfast. Day 1: AA mixture taken in one single dose at breakfast. 5 days later: repeated with dividing the AA mixture into three portions per day.
Acosta et al. (1999) USA	Not reported	35 with PKU (15 females and 20 males). Subjects entered the study at 13.7 (±1.9 SEM) days of age. Data was compared to normal reference data	Not applicable
***Graffin et al.* (1995)** USA	Not reported	6 PKU (3 males,3 females, aged 3-16y)	Not applicable
Thompson et al. (1990) Australia	Not reported	10 classical PKU (8 male, 2 females; mean age 19.3 y, range 14-24) 2 HPA (1 male, 1 female; ages 22 and 45 y) 6 age-matched normal controls (all male, mean age 20.8 y, range 19-23)	Radioisotope infusion: Isotope: priming bolus doses of L-[1-^{13}C]leucine and sodium [1-^{13}C]bicarbonate followed by continuous infusion of L-[1-^{13}C]leucine was then given over the next 4-6 h (6 h PKU and HPA; 4 h controls). Blood and expired air samples were collected at 15- to 20-min intervals in the final 2 h of each infusion and at 1/2-h intervals from 2-4 h in PKU participants.
Nord et al. (1988) USA	Not reported	50 children with PKU 13 children with HPA	Not applicable

Shenton et al. (1983) UK	Not reported	20 treated PKU (age 2-9y) 58 controls (age 1-15y). Control group = children with mild neurological disease, or children for elective operations or other admissions.	Not applicable
Pena et al. (2018) Portugal and UK[b]	Systematic review & meta-analysis of observational & interventional studies	72 participants included in the meta-analysis	Not applicable
ANTHROPOMETRIC + BIOCHEMICAL MEASUREMENTS			
***Boros et al.* (2015)** Hungary	Case-control	27 PKU (aged 16-44y). Compared to age and sex matched reference values	Not applicable
Allen et al. (1996) Australia	Cross sectional with longitudinal cohort	37 PKU (aged 7.3 ± 2.0y, range 3.9-11y; 21 male, 16 female) 27 control children (aged 8.1 ± 1.9y, range 4-11.5y; 15 male, 12 female)	Not applicable
***Sumanszki et al.* (2019)** Hungary	Cross-sectional	80 PKU (41 premenopausal women, 39 males (aged 18-49y))	Not applicable

Doulgeraki et al. (2014) Greece	Cross-sectional	48 PKU (25 males,23 females; mean age 10.9 ± 3.43y) 32 mild mHPA (18 male, 14 females; mean age 10.85 ± 3.6y) 57 age and sex-matched controls	Not applicable
Pena et al. (2021) Portugal	Retrospective longitudinal[c]	11 PKU (8 females, 3 males). Mean age at CGMP-AA onset 28y (range 15-43y).	cGMP-AA (mean of 29 months) cGMP-AA either fully or partially replaced L-AA; cGMP-AA: 100%, n = 4, 50% to <100%, n=4, <50%, n = 3.
Pinto et al. (2017) Portugal	Retrospective, longitudinal	11 PKU (8 females, 3 males) with PKU had a mean age of 27 ± 10y (2 patients <18y)	cGMP-AA (either completely or partially) replaced AA, depending on patient preference when refusing to take AA (mean of 13 ± 7 mo).
Das et al. (2013) Germany	Not reported	51 PKU (age range 16–44y, mean ± SD 26.6±6.6y; 32 females, 26.5± 6.2y; 19 males, 26.8 ± 7.7y)	All patients not taking AAM at the beginning of the study subsequently agreed to supplement their original diet with an AAM.
***Das et al.* (2010)** Germany	Not reported	51 PKU (age 17–44y, 31 females, 20 males)	Not applicable
Modan-Moses et al. (2007) Israel	Not reported	31 PKU (18 females, 13 males; mean age 25 ± 5.3y (range 19–41))	Not applicable

Lambruschini et al. (2005) Spain	Not reported	14 PKU for BH4 treatment; 11 responders (aged 0.2-12.2y, 7 females, 4 males). Compared to age- and sex-specific percentiles for healthy population.	BH4 treatment for 1 year. Initial dose 5mg/kg/day. Phe restricted diet was progressively liberalised by adding 200mg Phe/day/week for 2 mo, while formula was gradually reduced until complete removal was achieved.
Hillman et al. (1996) USA	Not reported	11 PKU (mean age 10.9 ± 4.2y; 5 male, 6 female), 64 controls (mean age 11.4 ± 4.2y; 32 male, 32 female) (11 were matched to the PKU children for sex and age)	Not applicable
ANTHROPOMETRIC + FUNCTIONAL MEASUREMENTS			
Choukair et al. (2017) Germany	Cross-sectional study	56 PKU (16 male, 40 female), aged 26.0 ± 8.9y (range, 11.8–41.5y). 700 reference population data also used	Not applicable
Sumanszki et al. (2020) Hungary	Not reported	12 PKU males (median age 26 (18-41y) 10 healthy controls (median age 26 (24-27y))	Day 1: Stress stimuli: cold pressor test (CPT) and isometric handgrip test (HGT). Day 2: peak treadmill test to exhaustion.
FUNCTIONAL + BIOCHEMICAL MEASUREMENTS			
Mazzola et al. (2015) Brazil	Not reported	9 PKU (7 males and 2 females; age 21 ± 4y) 17 controls (12 males, 5 females; aged 22 ± 4y)	2 days intervention. Day 0: BMR test in fast state. VO2 peak test. Minimum 1 week interval. Day 1: Blood sampling (Moment 1 (M1), breakfast and

	30-min rest, aerobic exercise session (30 min at a prescribed VO2) and blood sampling (M2).

Note: Authors in italics are abstract only papers. Not applicable: Not applicable, none interventional; PKU: phenylketonuria; HPA: hyperphenylalaninemia; Phe: phenylalanine; MUAC: Mid-upper arm circumference; UAMA: upper arm muscle area; ALMI: appendicular lean mass index; ALM: appendicular lean mass; PA: phase angle; BCM index: body cell mass index; NDR: nitrogen deposition rate; LBMDR: lean body mass deposition rate; MCA: muscle cross-sectional area; MM: muscle mass; LTM: lean tissue mass; TBW: total body water; FFMI: fat-free mass index; FMI: fat mass index; BUN: blood urine nitrogen; MF: medical food; PPS: Pretrial Protein Substitute; LPS: Liquid Protein Substitute.

[a] Płudowski P et al (2005) Reference values for the indicators of skeletal and muscular status of healthy polish children. J Clin Densitom 8:164–177

[b] Studies in the meta-analysis by Pena et al (2018) included participants from Denmark and USA.

[c] Study includes data from Pinto et al. (2017) but an extended follow-up period of 2.9 years if patients remained on CGMP-AA.

Table 3: Protein status outcome measures

Reference	Anthropometric									Biochemical												Functional	
	DEXA	BIA	Skinfolds	TOBEC	Deuterium Isotopic Dilution	Total body potassium	Total body nitrogen	pQCT	Method not reported	Albumin	Prealbumin	Total protein	Retinol-binding protein	Urea production	Blood urea nitrogen	Whole-body protein metabolism	Amino acids	Creatinine	Nitrogen excretion	13C-leucine in CO2	Method not reported	Grip strength	VO_{2max}
Mazzola et al, 2016		•																					
Adamczyk et al, 2011	•																						
Weng et al, 2020		•																					
Rocha et al, 2013		•																					
Dobbelaere et al, 2003		•	•																				
Huemer et al, 2007				•																			
Daly et al, 2021	•																						
Nogueira et al, 2021			•																				
Sailer et al, 2020		•																					
Jani et al, 2017	•																						
Stroup et al, 2018	•																						
Evans et al, 2017		•																					
Evans et al, 2018		•			•																		

Allen et al, 1995			•																					
Bonifant et al, 2010						•																		
Daly et al, 2019	•																							
Rocha et al, 2010									•															
Nalin et al, 2013		•																						
Wilcox et al, 2011	•						•																	
Alfheeaid et al, 2018					•																			
Torriente et al, 2017			•																					
Mexia et al, 2015									•															
Boros et al, 2015		•																				•		
Paci et al, 2018									•															
Kanufre et al, 2015			•																					
Allen et al, 1996			•				•										•							
Doulgeraki et al, 2014	•									•		•						•[a]						
Pena et al, 2021		•								•	•				•									
Hillman et al, 1996	•									•								•						
Lambruschini et al, 2005			•							•														
Pinto et al, 2017		•								•	•													
Modan-Moses et al, 2007	•[a]									•		•												
Das et al, 2010		•[b]												•										

Sumanszki et al, 2019		•									•												
Das et al, 2013		•[a]								•[a]		•		•			•	•[a]					
Choukair et al, 2017								•														•	
Sumanszki et al, 2020		•																					•
Singh et al, 2010										•	•	•											
van Vliet et al, 2019										•	•	•											
van Calcar et al, 2009										•	•	•			•		•						
Andrade et al, 2017											•	•											
Arnold et al, 2001										•	•	•					•						
Gokmen-Ozel et al, 2009										•													
Viau et al, 2021										•	•						•						
Shenton et al, 1983										•	•												
Arnold et al, 2002											•												
van Rijn et al, 2007										•		•				•	•						
Thompson et al, 1990																•	•						
Acosta et al, 1999										•	•		•		•		•						
Giovannini et al, 2014										•	•		•				•						
Schulpis et al, 2013										•		•											
Rocha et al, 2010											•												
Prince et al, 1997											•						•						

Mönch et al, 1996																			•	•			
Crujeiras et al, 2015											•	•											
Kose et al, 2019										•	•	•											
Nord et al, 1988										•		•			•								
Kose et al, 2016										•	•												
Prochazkova et al, 2012											•												
Giovannini et al, 2006										•		•					•						
Desloovere et al, 2014											•						•						
Giovannini et al, 2009										•	•		•				•						
Graffin et al, 1995										•		•			•								
Rocha et al, 2009											•												
Douglas et al, 2013										•		•			•			•					
Ahring et al, 2018															•		•						
Pena et al, 2018															•								
Zaki et al, 2016										•					•		•						
Ney et al, 2016										•	•	•			•		•						
Mazzola et al, 2015																	•						•
Total of measures included in studies	9	15	7	1	2	1	2	1	3	27	23	17	3	2	10	2	18	4	1	1	1	1	2

Authors in italics are abstract only papers; DEXA: Dual-energy X-ray absorptiometry; BIA: Bioelectrical impedance analysis; TOBEC: total body electrical conductivity; IVNAA: Gamma neutron activation analysis; pQCT: Peripheral quantitative computed tomography

[a] Included in methods, but results not reported

[b] Reports body composition measured by BIA, but unclear if FM or FFM measured

Table 3: Anthropometric measurements of protein status and key findings

Author (Year)	Method used	Modulating factors	Key findings: Protein status outcomes	Variable	Group 1	Group 2	Group 3
Daly et al. (2021)	DEXA	Type of protein substitute	No significant differences in LBM between the Tx groups, although a trend for improved LBM was observed in the CGMP100 group. All body composition parameters increased over 3 years.		**AA (n=19):**	**GMP50 (n=13):**	**GMP100 (n=16):**
				Lean mass at baseline (kg)	26.7 (16.9-34.2)	16.3 (14.2-17.7)	20.1 (16.5-21.9)
				Lean mass at end of study (kg)	32.6 (5.9-40.5)	23.9 (22.7-26.5)	31.3 (25.6-35.9)
				Lean mass change (kg)	+5.9 (9.0-6.3)	+7.6 (8.4-8.8)	+11.2 (9.1-13.9)
***Daly et al.* (2019)**	DEXA	Type of protein substitute	FFM not significantly different between groups at baseline. At 36 mo, FFM was significantly greater in the CGMP-AA than for LAA group (p = 0.01)		**CGMP-AA:**	**L-AA:**	
				FFM (kg) at 36 months	9.05	6.47	
Evans et al. (2017)	BIA	BH4, dietary parameters	%FFM was only measured at baseline. No significant difference in %FFM between groups (p=0.148 All-PKU vs Controls) Neither %EBMR nor P:E ratio contributed significantly to %FFM		**Diet-PKU:**	**BH4-PKU:**	**All PKU:**
				%FFM at baseline (range)	84.1 ± 7.4 (64.1-92.1)	83.5 ± 3.1 (80.8-87.5)	84 ± 7 (64.0-92.1) **Controls:** 80.9 ± 4.2 (71.8-86.2)
***Paci et al.* (2018)**	Method not stated	None assessed	Total body water and FFM (kg) were significantly higher in PKU vs. controls.	No data shown			

Boros et al. **(2015)**	BIA	None assessed	Decreased SMM were found in 2 patients (7%). In 7 patients (26%), SMM was higher than matching normal reference values.			
Rocha et al. **(2013)**	BIA	None assessed	No significant differences were found between patients and controls. Data as median [25th and 75th percentiles]		**PKU:**	**Controls:**
				%FFM	78.0 [71.1–85.7]	77.0 [71.2–83.7]
				%Muscular mass	48.3 [43.0–53.3]	46.7 [43.5–53.2]
				PA	5.9 [5.2–6.5]	6.0 [5.5–6.6]
Huemer et al. **(2007)**	TOBEC	Dietary parameters	Cross sectional: No significant difference in FFM between PKU and age- sex-matched controls. Longitudinal: %FFM remained unchanged over time Natural protein intake (g/kg per day) explained 47.7% of the variance of FFM ($R2 = 0.477$; $p < 0.0001$). Total protein intake (g/kg per day) and natural or total protein intake (g/day) without reference to body weight explained 8.8%, 7% and 26.1%, respectively of the variance of FFM.		**PKU:**	**Controls:**
				%FFM at baseline	84.6 ± 16.9	85.2 ± 9.7
				%FFM at 6 mo	83.6 ± 15.8	
				%FFM at 12 mo	85.5 ± 13.8	
Allen et al. **(1996)**	Skinfolds PGNA for total body nitrogen	Sex	Cross Sectional Data No diff in LBM between PKU and controls. Sig diff in TBN between PKU and controls ($p<0.005$) TBN lower in females (data not shown)		**PKU:**	**Controls:**
				LBM (kg) (mean ± SD)	19.7 ± 4.5	22.0 ± 4.8
				TBN (g) (mean ± SD)	575 ± 200	710 ± 215

	UAMA were derived from the MUAC and skinfolds		PKU: had 35g less TBN than controls for same LBM. At each age children with PKU had 53g less TBN than controls, representing 6mo lag in PKU vs. controls Control: TBN significantly correlated LBM, weight, height and age (r = 0.97, 0.95, 0.88, respectively, p<0.001). UAMA similar between PKU and controls (data not shown). UAMA significantly correlated with TBN in both PKU and controls (r_s = 0.84, r_s = 0.89, P < 0.001, respectively). <u>Longitudinal Data</u> (n=29 PKU, n=17 controls) Similar increase in TBN and LBM in both groups between the two time periods Annual accretion of nitrogen, NDR and LBMR: Similar between groups. NDR was significantly correlated with the LBMDR in the control but not in PKU.	Annual accretion of nitrogen (g/y) NDR (g/y) Cross-sectional Longitudinal LBMDR (kg/y) Cross-sectional Longitudinal	86 ± 45 93 86.1 ± 45.1 2.1 1.8 ± 0.6	77 ± 58 98 77 ± 57.9 2.3 2.2 ± 0.9
Sailer et al (2020)	BIA	Sex, dietary parameters	No difference in LBM% in females (p = 0.34), but males with PKU had significantly lower LBM% (p = 0.02) than male controls.	Lean body mass % males	**PKU:** 85.38 ± 4.9	**Controls:** 89.09 ± 5.94

				Lean body mass % females	77.87 ± 9	76.50 ± 9.87
Weng et al. (2020)	BIA	Dietary parameters	Muscle mass %: No significant difference between PKU and controls (p = 0.37), and between PKU patients on phe-free formulas and those taking no PS (p=0.95), although natural protein intake was significantly different. Significant positive correlation between total protein intake % of DRIs and muscle mass (r = 0.491, p= 0.020) in PKU. % natural protein had no correlation to muscle mass (r = − 0.007, p = 0.974).	Muscle mass % Muscle mass %	**PKU:** 73.39 ± 8.79 **PKU + PS:** 73.15 ± 8.5	**Controls:** 75.51 ± 7.42 **PKU + no PS:** 73.68 ± 9.56
***Sumanszki et al.* (2019)**	BIA	Sex	Females: both lumbar spine and femoral BMD correlated positively with LBM (r = 0.53; p< 0.001, r = −0.31; p = 0.042, respectively). Lean mass had a greater effect on BMD than fat mass. Observations not reported in males			
Evans et al. (2018)	BIA TBW by deuterium dilution.	None assessed	No difference between FFM measured by Deut and BIA (p = 0.111) Correlation analysis showed that FFM by BIA correlated significantly with FFM by TBWDeut (r = 0.984, p < 0.0001)	FFM (kg): Mean (SD)	**FFM by Deut:** 31.81 (± 12.77)	**FFM by BIA:** 32.93 (± 13.93)

Stroup et al. (2018)	DEXA	Sex, genotype	Males had significantly more lean mass (p = 0.0008) and ALM (p=0.0002) compared to females. No significant difference in the ALMI (and ALMI z-scores) between males and females. Mean ALMI for our young male participants was close to the cut point of ≤7.26 kg/m2, a suggested cut-off to identify sarcopenia in older men. All the above are not significant for genotype.	Total Lean mass kg (mean ± SE) ALM kg (mean ± SE) ALMI kg/m2d (mean ± SE)	**Male:** 55.3 ± 2.8 24.8 ± 1.5 7.99 ± 0.31	**Female:** 41.9 ± 1.4 18.0 ± 0.8 6.96 ± 0.35
Choukair et al. (2017)	pQCT to measure MCA	Sex, classification of PKU, metabolic control	Mean MCA was decreased compared with the ref. population (z-score −0.98 ± 1.19; p < 0.0001); observed both in females and males. 30% had MCA <3rd percentile. No relationship between MCA (z-scores) and PKU type or mean phe concentrations. Bone strength were significantly correlated to MCA. In PKU, the regression line slope between SSI and MCA was significantly (p < 0.0001) less steep than in the reference population.			
Jani et al. (2017)	DEXA	Dietary parameters, genotype	In adults (n=17), high intact protein intake was associated with high FFMI (r_s = 0.75, p = 0.008) and low FMI:FFMI (r_s = −0.59, p = 0.04). In children, protein substitute (r_s = 0.38, p = 0.04) and total protein intake (r_s = 0.39, p = 0.04) were directly	Median(min,max) **Adults** Lean mass (kg) **Children** Lean mass (kg)	**Males (n = 9):** 52.2 (45.5, 61.8) **Males (n = 25):** 25.7 (13.3, 64.4)	**Females (n =17):** 38.9 (30.8, 64.3) **Females (n=32):** 32.7 (15.5, 50.7)

			associated with FFMI. Genotype was not associated with body composition.	FFMI	**Total (n=83):** 14.8 (11.3, 24.2)	**Adults (n=26):** 17.4 (13.4, 24.2)	**Children (n=57):** 14.2 (11.3, 23.7)
***Torriente et al.* (2017)**	Skinfolds	None assessed	Muscle area: normal 100%				
Mazzola et al. (2016)	BIA	None assessed	No differences in FFM, ECM/BCM ratio, and PA between PKU patients and controls. 3 patients with PKU and 3 controls were below the cut-off values for PA	FFM %	**PKU:** 80 ± 7	**Controls:** 78 ± 9	
***Kanufre et al.* (2015)**	Skinfolds	None assessed	32 (41.5%) had muscle mass deficit of which 28 (87.5%) were normal weight-for-age and 25 (78%) were adolescents				
***Mexia et al.* (2015)**	Method not stated	None assessed	Deficit of FFMI was found in 30.8% of patients				
Doulgeraki et al. (2014)	DEXA	Sex, pubertal status, classification of PKU	No difference in LBM in PKU and controls. No effect for correction for height. MM of mHPA were comparable to controls. No significant difference was detected in body composition parameters between patient with PKU and mHPA. No effect of gender on body composition Pubertal status associated with increased in LBM in adolescents (pubertal) in mHPA ($p<0.01$) but not in PKU.	LBM z-scores (mean ± SD)	**mHPA pre-pubertal:** -0.65 ± 1.5 **PKU pre-pubertal:** -0.23 ± 1.1	**mHPA pubertal:** 0.7 ± 1.1 **PKU pubertal:** -0.1 ± 1.3	

			PKU: a significant positive correlation between BMD and MM.			
Dobbelaere et al. (2003)	Skinfold thickness BIA	None assessed	No difference between PKU and controls.	FFM (kg) via skinfold FFM (kg) via BIA	**PKU:** 12.4±3.2 14.1±1.4	**Controls:** 12.8±2.1 12.9±2.3
Nogueira et al. (2021)	Skinfolds Arm muscle area	Metabolic control, socio-economic status	Association between metabolic control and AMA (linear trend chi square; p=0.042). AMA classified as above average or adequate was associated with worse % of metabolic control. No association between AMA with SES.	Metabolic control with ≥70% adequate Phe levels	**Low AMA:** 70%	**High AMA:** 18.5%
Pena et al. (2021)	BIA	Type of protein substitute	No difference in LM% and PA for all patients taking L-AA compared with CGMP-AA	LM (%) n=9 PA (°) n=9	**CGMP-AA:** 71.1 ± 13.4 6.8 ± 0.6	**L-AA:** 74.5 ± 16.1 6.8 ± 0.7
Pinto et al. (2017)	BIA	Type of protein substitute	All parameters remained unchanged	LM (%) (n =9) PA (°) (n =9)	**CGMP-AA:** 71.4±15.0 6.7±0.7	**L-AA:** 74.5±16.1 6.8 ±0.7
Sumanszki et al. (2020)	BIA	None assessed	No difference in body composition parameters in PKU and controls (p = 0.497)	FFM (%)	**PKU:** 47.5 (42.1–49.3)	**Controls:** 47.3 (45.3–48.5)

***Alfheeaid et al.* (2018)**	Deuterium dilution technique	None assessed	No difference in FFM between PKU vs. controls.	Data not shown.			
Das et al. (2013)	BIA	Dietary parameters	LBM/FFM: No results reported. PA: Normal in all dietary groups, no significant difference between groups. BCM increased in vegan + L-AA and reduced in vegan patients compared to PKU-diet patients (non-significant).	No data shown			
***Nalin et al.* (2013)**	BIA	Metabolic controls	% FFM and PA: No difference in PKU vs. controls. A positive correlation between PA and phenylalanine levels in PKU (r=0.457, p=0.032)	% FFM PA (°):	**PKU:** 80.9±7.7 6.35	**Controls:** 80.7±7.3 6.89	
Adamczyk et al. (2011)	DEXA	Pubertal status, metabolic control	No significant differences between LBM in 2a and 2b (both pubertal). Increased LBM for body height SD scores in adolescents with normal Phe levels. LBM Z-scores: Sign. differences between 2a and 2b. No difference between subgroups 2a and 1.	LBM (g) LBM SD scores LBM Z-scores	**Group 1:** prepubertal/ normal phe -0.02 ± 1.31 -0.21 ± 1.26	**Group 2a:** pubertal/ normal phe Data not shown +1.94 ± 3.21 +0.51 ± 1.59	**Group 2b:** pubertal/high phe Data not shown -0.20 ± 1.52 -0.69 ± 0.71
***Bonifant et al.* (2010)**	TBK	None assessed	TBK z score was significantly lower ($p < 0.05$) in patients with PKU				

***Das et al.* (2010)**	BIA	Dietary parameters	Body composition within target levels	No data shown		
***Rocha et al.* (2010)**	Method not stated	BMI	Patients classified as overweight and obesity had lower FFM% ($p<0.001$)	FFM%	**Overweight/ obese:** 63.7	**Normal weight:** 78.3
***Wilcox et al.* (2011)**	IVANA for total body protein DEXA	None assessed	SMM was measured as height-adjusted ALTM; TBP measured as age and sex adjusted nitrogen index (NI) SMM and TBP were normal PKU patients	ALTM (kg/m^2): Nitrogen Index:	**Females:** 5.77 ± 2.35 0.98 ± 0.12	**Males:** 8.24 ± 0.45 1.13 ± 0.13
Modan-Moses et al. (2007)	DEXA	None assessed	No data reported for lean body mass or FFM.	No data shown		
Lambruschini et al. (2005)	Skinfolds: Brachial muscular area	BH4	All values within age- and sex-specific percentiles for a healthy population after 1 yr treatment. No difference in brachial muscular area (BMA).	BMA (mm^2) (range)	**Before BH4:** 984-4505	**After BH4:** 1167-4819
Hillman et al. (1996)	DEXA	None assessed	No difference between PKU and controls ($p = 0.53$).	Lean body weight (kg)	**PKU:** 27.98 ± 12.43	**Controls:** 30.71 ± 17.47
Allen et al. (1995)	Skinfolds	Sex	No difference in FFM between males with PKU and control participants. Females with PKU were younger than controls ($P=0.006$) and had a lower FFM ($p=0.007$).	FFM (kg) males FFM (kg) females	**PKU:** 27.2 ± 7.6 23.1 ± 8.1	**Controls:** 27.1 ± 7 30.9 ± 8.2

Note: Authors in italics are abstract only papers

PKU: phenylketonuria; HPA: hyperphenylalaninemia; Phe: phenylalanine; DEXA: Dual-energy X-ray absorptiometry; BIA: Bioelectrical impedance analysis; PGNA: Prompt gamma neutron capture analysis; pQCT: Peripheral quantitative computed tomography; IVANA: Gamma neutron activation analysis; LBM: lean body mass; LM%: lean mass percentage; FFM: fat-free mass; TBW: total body water; FFMI: fat-free mass index; FMI: fat mass index; PA: phase angle; BCM: body cell mass; ECM: extracellular mass; MUAC: Mid-upper arm circumference; UAMA: upper arm muscle area; ALMI: appendicular lean mass index; ALM: appendicular lean mass; NDR: nitrogen deposition rate; LBMDR: lean body mass deposition rate; MCA: muscle cross-sectional area; MM: muscle mass; SMM: skeletal muscle mass; SSI: Strength-Strain Index; TBP: Total body protein; TBK: Total body potassium; BUN: blood urine nitrogen; PAA: plasma amino acids; L-AA: L-amino acids; cGMP-AA: casein glycomacropeptide amino acid; PS: protein substitute

Table 5: Biochemical measurements of protein status and key findings

Author (Year)	Method used	Modulating factors	Key findings: Protein status outcomes	Variable	Group 1	Group 2	Group 3	Group 4
Ney et al. (2016)	PAA, BUN, albumin, prealbumin and total protein	Type of protein substitute	No sig. differences between BUN, total protein and prealbumin for L-AA compared with cGMP-AA. Albumin was sig. higher with cGMP-AA than L-AA (p = 0.027). Mean values were within the normal range for both diets. PAA: Except for Phe, all mean conc of AAs were within the normal range for both groups. Thr (mean ±SE) showed sig. increase with cGMP-AA from 103 ± 4 mmol/L to 149 ± 10 mmol/L (p <0.001). L-AA diet: sig. change from baseline to 3 weeks for Arg (increased), Leu (increased), Phe (reduced) cGMP-AA diet: sig. change from baseline to 3 weeks for Gly (reduced), Lys (reduced), Met (increased), Thr (increased), Val (reduced)	Blood urea nitrogen, mg/dL	**L-AA:** 11.2 ± 0.6	**cGMP-AA:** 10.6 ± 0.6		
				Total protein, g/dL	7.35 ± 0.08	7.42 ± 0.07		
				Albumin, g/dL	4.24 ± 0.04	4.35 ± 0.04		
				Prealbumin, mg/dL	27 ± 1.0	27 ± 1.1		

Giovannini et al. (2014)	Albumin, prealbumin, RBP, PAA	Type of protein substitute	Baseline: PKU had lower albumin, prealbumin, and higher AA ratio than MHP (maximum $p = 0.01$) and unaffected children (maximum $p < 0.001$). Albumin was within the reference range for all children. Children with MHP and unaffected children: prealbumin within reference range, n=2 PKU had prealbumin levels below ref range. 29 (52.7%) PKU had prealbumin <20 mg/dL. The AA ratio ranged from 1.7-3.5, 1.5- 3.0, 1.5-2.5 in PKU and MHP and unaffected children, respectively. End of study: PKU has albumin and prealbumin within range, except n=1 receiving the conventional substitute (prealbumin 18.3 mg/dL). No overall sig. difference between PKU groups was found for protein status. Within-group analysis: sign. increase of prealbumin in children who received the test substitute ($p = 0.017$). The change in albumin was close to statistical sig. in the test group ($p = 0.068$).	Mean ± SD Albumin g/dL Baseline Albumin g/dL End of study Transthyretin (mg/dL) Baseline Transthyretin (mg/dL) End of study AA ratio Baseline AA ratio End of study	**Prolonged-release substitute (n=27)** 4.5 ± 0.2 4.6 ± 0.2 19.1 ± 6.4 20.7 ± 6.8 2.6 ± 0.9 2.7 ± 0.8	**Conventional substitute (n=28)** 4.5 ± 0.2 4.5 ± 0.2 19.0 ± 6.3 19.2 ± 6.0 2.6 ± 0.8 2.6 ± 0.8

Prince et al. (1997)	Prealbumin, EAA	None assessed	Phase 1: Baseline: All EAA (except phe) uniformly low compared with controls. Mean EAA remained low compared to control. No sig diff in serum EAA between control and experimental groups at entry or end. Phase 2: Despite reductions in protein substitute intakes, mean serum protein levels were not sig. different and remained in the healthy (non-PKU) age-matched reference range throughout the study.	Mean (±SD) Prealbumin, mg/dL	**Baseline:** 17.3 ±2	**End of study:** 21.0±4	**Controls (range):** 17– 42	
Ahring et al. (2018)	BUN, PAA	Type of protein substitute	AUC (adjusted for baseline) for total AA: No differences between DM1 and DM2 (p = 0.852), or between DM3 and DM4 (p= 0.06). Significant differences for AUC for some individual AA: DM1 and DM2: Lys (p = 0.0287), Asn (p = 0.0210), and Asp (p =0.0047) and DM3 and DM4: citrulline (p = 0.0162). BUN: no sig change from baseline to 240 min after meal and DM.	Peak AA concentrations	**DM1:** after 30 min for 90% of AAs.	**DM2:** after 15 min for 71% of AAs	**DM3:** after 30 min for 67% of AAs.	**DM4:** after 15 min for 71% of AA
Zaki et al. (2016)	Amino acids,	Type of protein substitute	Phase I vs Phase II: Individual amino acids were generally lower in phase I, with sig. low level of aspartic acid and citrulline. Levels of all amino acids in phases I and II were	Mean (SD) (min - max) BUN:	**Baseline**	**Phase I**	**Phase II**	

	albumin, BUN		not significantly different. BUN and albumin: no sig. difference between phases of the study.	Albumin:	20.6 (7.66), (6-30) 4.6 (0.35), (4.2-5.2)	17.63 (5.18), (8-23) 4.5 (0.52), (4.0-5.3)	19.67 (9.75), (10-37) 4.52 (0.31), (4.0- 4.8)
Kose et al. (2019)	Prealbumin, albumin and total protein	Dietary adherence, metabolic control and age	Prealbumin was sig. higher in PKU compared to controls (p =0.013). Frequency prealbumin above the ref range was higher in PKU than the controls (p=0.02). Albumin and total protein were not sig. different between PKU and controls. Prealbumin was sig. lower in those with high adherence to diet compared to those with low adherence (p = 0.011). Positive correlation between plasma phe level and prealbumin (r = 0.256, p = 0.003) and between age and prealbumin in PKU (r = 0.556, p < 0.0001) and control groups (r = 0.682, p < 0.0001).	Serum prealbumin, mg/dL (Ref range: 21-41) Prealbumin, (<21 mg/dL), n% Serum prealbumin, mg/dL (Ref range: 21-41)	**PKU (n=112):** 24.1 ± 4.6 (10.5-35.5) 21 (21.1) **High dietary adherence:** 22.5 ± 4.4	**Con (n=36):** 21.9 ± 3.9 (15.9-29.8) 14 (38.8) **Low dietary adherence:** 24.9 ± 4.6	

***Boros et al.* (2015)**	Method not reported	None assessed	Decreased protein levels in 2 found to have low SMM. Normal protein levels in 7 with SMM above normal ref values.	No data provided		
***Prochazkova et al*. (2012)**	Prealbumin	None assessed	No sig. difference in the levels of serum prealbumin among the respective groups.	No data provided		
Allen et al. (1996)	PAA		No difference in PAA between PKU and controls, except for phe.	No data provided		
Viau et al. (2021)	EAA, prealbumin and albumin	Blood phe levels	No deficiencies were identified in EAA, prealbumin, or albumin. No sig differences in protein intake or prealbumin in patients with blood Phe <30 μmol/L vs. ≥30 μmol/L.	Prealbumin, mg/dL	**Phe < 30 μmol/L (n=11):** 27.0 ± 1.7	**Phe ≥ 30 μmol/L (n=7):** 30.6 ± 1.1
***Sumanszki et al.* (2019)**	Prealbumin	Sex	Pre-albumin levels were higher in males compared to females with PKU (p = 0.008)	Prealbumin, mg/dL (mean ± SD)	**Females:** 28 ± 4.1	**Males:** 30.5 ± 3.8
Andrade et al. (2017)	Prealbumin and total protein	Metabolic control	Prealbumin: 15% were below the normal range. Patients with values >97th or <3rd percentile: 1/39>P97; 6/39<P3 Plasma phe sig. correlated with prealbumin (r=0.479, P=.002)	Prealbumin, mg/dL (ref range 20–40)	**PKU:** 22.6 [11.2–53.1]	

				Total protein, g/dL (ref range: 6–8)	7.2 [6.0–7.8]	
Crujeiras et al. (2015)	Total protein and prealbumin	Age, dietary adherence, time of diagnosis, classification of PKU and BH4	Total protein was in the normal range in almost all patients. Prealbumin was <21mg/dL in 34.6% of patients, (74% from PKU) with 94.4% of them <18y, 96.3% having an adequate adherence and 12.96% were on BH4 treatment. Prealbumin was sig. lower in mHPA vs. PKU (p = 0.024) Significant positive correlation between age and prealbumin (p < 0.001) and total protein (p = 0.002). Total protein (p = 0.0072) and prealbumin (p <0.001) were found sig. lower in the patients with high adherence vs. low adherence to diet. More significantly altered in low adherence and >18 years compared to other participants. Time of diagnosis: no differences found	Prealbumin, mg/dL (ref range: 21–41)	**mHPA:** 21.2	**PKU:** 24.5
				Prealbumin, mg/dL	**Aged ≤18 years:** 21.9	**Aged >18 years:** 28.5
				Total protein, g/dL (ref range: 6.3-8.5)	7.05	7.35
				Prealbumin, mg/dL	**High dietary adherence:** 22.6	**Low dietary adherence:** 29

				Total protein, g/dL	7.1	7.3
				Prealbumin, mg/dL	**Low adherence + > 18 years:** 28.91	**Other participants:** 22.81
				Total protein, g/dL	7.41	7.09
Doulgeraki et al. (2014)	Total protein, albumin	None assessed	All biochemical measures were within normal limits.	No data provided		
Pena et al. (2021)	Albumin, prealbumin, and BUN	Type of protein substitute	No difference in the biochemical parameters.	BUN (mg/dL): Prealbumin (mg/dL)	**L-AA:** 1.68 ± 0.63 240 (224–278);	**cGMP-AA:** 1.90 ± 0.55 272 (200–293)
Pinto et al. (2017)			All parameters remained unchanged.	Albumin (g/dl)	**L-AA:** 4.69± 0.33	**cGMP-AA:** 4.69±0.21

	Albumin and prealbumin	Type of protein substitute		Prealbumin (mg/dl)	249± 28	245±53
van Vliet et al. (2019)	Albumin, total protein, and prealbumin	BH4, dietary parameters	No significant differences between groups with relevant biochemical measures. No correlations were found between natural protein intake and relevant biochemical markers	Min-max (median)	**PKU-nBH4:**	**PKU-BH4:**
				Albumin, g/L	43-53 (48)	44-53 (49)
				Patients with deficiency:	0	0
				Patients with excess:	4(24%)	3 (23%)
				Total Protein, g/L	58-84 (73)	66-81 (74)
				Patients with deficiency:	1 (2%)	0
				Patients with excess:	1 (2%)	3 (10%)
				Pre albumin, g/L	0.15–0.51 (0.30)	0.17–0.45 (0.30)

				Patients with deficiency:	6 (11%);	1 (4%)	
				Patients with excess:	4 (8%)	3 (11%)	
Rocha et al. (2010)	Prealbumin	Age, classification of PKU, amount of protein substitute, metabolic control	Prealbumin z-score was –0.5248 (1.09), significantly below z-score = 0 (p<0.001). 9 patients (13%) had a prealbumin z-score < 5th percentile (–1.64), and one > 95th percentile (+1.64). All patients with z-score of prealbumin <5th percentile were <15 years. No association between classification of PKU and prealbumin z-score <5th percentile (p = 0.237). No sig correlation between prealbumin z-score and the amount of protein substitute prescribed (R2 = 0.01; p = 0.38). Prealbumin z-score and blood phe were not sig. correlated (R 2 = 0.003; p = 0.65). Prealbumin < 20 mg/dl: 38 patients (55%) Association between prealbumin <20 mg/dl and age <15 years (p < 0.0001). Prealbumin sig. higher in the group of older patients vs. group of patients < 15 years (p< 0.001).	Classification of PKU with prealbumin z-scores < 5th percentile Prealbumin, mg/dL	**Classical PKU** 5% **Aged <15 years:** 18.38 ± 4.12	**mild PKU** 16% **15 years and over** 25.53 ± 4.38	**HPA** 21%

Gokmen-Ozel et al. (2009)	Albumin	Type of protein substitute, age	>18 years taking LPS: sig. increase in albumin ($p < 0.05$), although within the reference ranges 7–18 years taking LPS: median albumin sig. improved ($p < 0.01$), median values were within the reference range.	Median (range) Albumin (g/L): Baseline: On LPS: Median diff between PPS and LPS	**Aged ≤18 years:** 43 (35–48) (n=21) 45 (42–49) (n=20) 2.5 (-3.0 to 9.5)	**Aged >18 years:** 43.5 (38.5–48.0) (n=9) 47 (41–49) (n=11) 2.8 (-2 to 7)
***Kose et al.* (2016)**	Albumin, prealbumin	None assessed	Albumin was not sig. different between PKU vs. controls. Prealbumin was significantly higher in PKU vs. controls.	No data provided		
Mazzola et al. (2015)	BCAA	Exercise	In rest and fasted state, patients showed lower levels of BCAA in comparison to controls ($p = 0.001$). Levels of BCAA were not modified with exercise in PKU and controls.	BCAA (μmol/L)	**PKU:** 332 ± 50	**Controls:** 456 ± 86
***Desloovere et al.* (2014)**	Prealbumin PAA	Age, protein substitute,	Prealbumin levels were <20 mg/dL in 60 % of participants Prealbumin correlated with age. No sig, correlation was	No data provided		

		metabolic control	seen between prealbumin, protein substitute, PAA profile or phenylalanine level.					
Schulpis et al. (2013)	Total protein, albumin	Metabolic control	No sig difference in total protein or albumin between each group and controls. Natural protein intake differed sig. between groups (see supplemental material).	Total Protein (g/L)	**Group A: poor metabolic control** 7.2 ± 0.8	**Group B: good metabolic control** 7.5 ± 1.0	**Controls:** 7.4 ± 1.0	
				Albumin (g/dL)	4.7 ± 0.3	4.8 ± 0.3	4.7 ± 0.3	
Das et al. (2013)	Total protein, PAA, albumin, urea	Dietary parameters	PAA: Valine was higher in PKU-diet than in subjects consuming normal food. All patients had reduced or low normal urea.	Total protein (g/L) (ref range 65–80)	**Normal food:** 74	**Vegan + no PS:** 76	**Vegan + PS:** 76	**PKU diet:** 73
				Urea (mM) (ref range 3.3–6.7)	3.8	3.6	3.8	3.3
				Albumin	No data provided			

***Douglas et al*. (2013)**	Creatinine, BUN, albumin, total protein	BH4, dietary parameters	Classified as Definitive (DR) or Provisional (PR) Responders to BH4. Non-responders (NR) discontinued drug. Protein markers did not differ between groups or with increased intact protein intake in Responders. Baseline creatinine was sig. lower in PKU vs controls, but within normal ranges. BUN declined in DR and albumin, globulin, and total serum protein declined in NR over time ($p < 0.05$).	Baseline urine creatinine(mg/dL)	**PKU:** 74±40	**Controls:** 141±61		
***Das et al.* (2010)**	Urea	Not reported	Urea concentrations were lower than normal in all patients.	No data provided				
Singh et al. (2010)	Prealbumin, total protein and albumin	BH4	Albumin and total protein: within reference range for all patients and remained stable Prealbumin: lower end of ref range at baseline and increased during the first 12 mo of follow-up ($p<0.001$), then remained stable.	**Baseline:** Prealbumin (mg/dl): (ref range: 19.0–38.0) 19.8 (3.3) Albumin (g/dl):	**6 months:** 21.0 (2.8) 4.6 (0.2)	**12 months:** 21.7 (3.1) 4.7 (0.1)	**18 months:** 23.0 (7.1) 4.9 (0.2)	**24 months:** 23.3 (3.4) 4.6 (0.4)

				(ref range: 3.5–5.0) 4.7 (0.1)				
				Total protein (g/dl): (ref range: 6.3–7.9) 6.9 (0.3)	6.8 (0.2)	6.9 (0.3)	6.9 (0.2)	6.9 (0.3)
***Giovannini et al.* (2009)**	Albumin, prealbumin, RBP, amino acid ratio	Type of protein substitute	Treated HPA vs. mild untreated HPA and controls: lower albumin, p = 0.012, prealbumin, p = 0.005, RBP, p = 0.001, and amino acid ratio, p < 0.0001, respectively. Three days after introducing the new protein substitute prealbumin improved (p = 0.02).	No data provided				
***Rocha et al.* (2009)**	Prealbumin	Age	8 (13%) revealed prealbumin z scores <5th percentile Significant linear correlation between prealbumin and haemoglobin conc (adjusted for age) (R_2=0.446; p=0.017) Prealbumin z-score average was significantly lower in the group with low haemoglobin (p=0.051).	No data provided				

van Calcar et al. (2009)	PAA, prealbumin, albumin, total protein, BUN	Type of protein substitute	BUN was sig. lower with GMP diet on both day 7 and day 8 than with the AA diet on day 4. No sig. differences among albumin, prealbumin, or total protein on the last day of the AA diet (day 4) compared with the GMP diet (day 8). Total PAA was sign. greater, and BUN was sig. lower, with the GMP diet compared with the AA diet when measured 2.5 h after eating breakfast. GMP vs AA diet led to 2.25- to 2.47-fold increase in postprandial conc of isoleucine and threonine within 24 h of ingesting the GMP diet (consistent with the high concentrations of these AAs in GMP). No further sig increases in isoleucine and threonine after days 5 and 7, respectively.	Blood urea nitrogen (mmol/L) Total protein (g/L) Albumin (g/L) Prealbumin (g/L)	**AA diet:** 4.2 ± 0.3 68 ± 1.4 44 ± 0.9 317 ± 7.5	**GMP diet:** 3.4 ± 0.2 67 ± 1.4 44 ± 0.8 310 ± 7.3
Modan-Moses et al. (2007)	Total protein and albumin	Dietary adherence	Total protein and albumin were normal in all patients and did not differ between diet-adherent and non-adherent.	No data provided		
van Rijn et al. (2007)	Amino acids,	None assessed	Both groups were comparable in baseline albumin and total protein.		**PKU:** 43 ± 2	**Controls:** 45 ± 2

albumin, total protein, whole-body protein metabolism	Sig differences in PAA concentrations between the two groups in the pre-prandial period for phe and cystine. Sig higher valine, isoleucine, leucine, phe, and lysine in PKU vs. controls at the end of the meal period. Whole-body protein metabolism: The Ra of valine did not differ between groups before and after meals. Sig higher oxidation rate during the prandial vs pre-prandial period in PKU group ($p < 0.01$). Prandial period, sig. difference in the Ra values of dietary valine into the peripheral circulation in PKU vs controls ($p = 0.02$). Meal decreased whole-body protein breakdown in both groups to a similar extent. Net protein balance: no sig. difference between groups during pre-prandial or prandial phase.	Albumin (g/L)		
		Total protein (g/L)	70 ± 3	71 ± 3
		Valine (Ox)	24 ± 6	28 ± 4
		Pre-prandial		
		Prandial	35 ± 2	33 ± 7
		R_a (dietary valine into peripheral circulation), µmol valine/kg/h	51 ± 8	42 ± 4
		Net protein balance, µmol valine/kg/h	-17 ±6	-21 ±4
		Pre-prandial		
		Prandial	23 ±8	16±9

***Giovannini et al.* (2006)**	PAA, total protein and albumin	Type of protein substitute	Baseline: AA profiles and blood levels of protein were comparable between groups. Plasma proteins and albumin levels sig. increased from T0 to T1 in Group 1. Changes in plasma protein and albumin in Group 2 were not significant. From T0 to T1 the amino acid profiles showed an increased in methionine, lysine and arginine concentrations (all p=0.02). At T1 there was a sig. difference in tyrosine in Group 1 vs Group 2.	Mean ± SD Plasma proteins, mg/dL T0 T1 Albumin, mg/dL T0 T1 Tyrosine (µmol/L) T1	**Group 1 (100% N daily needs)** 6.9 ± 0.3 7.3 ± 0.2 4.3 ± 0.1 4.5 ± 0.2 80 ± 18	**Group 2 (80% N daily needs)** No data provided No data provided No data provided No data provided 47 ± 17
Lambruschini et al. (2005)	Albumin	BH4	No sig. difference in albumin before and after BH4 treatment.	Albumin, g/L	**Before BH4:** 46 ± 5	**After BH4:** 45 ± 3

Arnold et al. (2002)	Prealbumin	Height, age, metabolic control	Mean prealbumin conc: 20.5 mg/dL Prealbumin conc was positively correlated with both height and age: children with higher prealbumin were taller (r = 0.38, P < .02) and older (r = 0.65, P < .001). Prealbumin was also positively correlated to plasma phe levels (r = 0.38, P < .03). Both lower phe levels and younger age were found in the low prealbumin group. Multiple regression analysis: After controlling for age, BMI, and mean phenylalanine level, children with prealbumin <20 mg/dL had a mean height decrease of 44.9 percentiles.			
Arnold et al. (2001)	Prealbumin, albumin, total protein and PAA	Age, metabolic control	Albumin and total protein levels: normal in all 41 patients, and did not differ sig. from the normal ranges. Prealbumin was sig. lower in younger children (p = 0.03). Sig. positive correlation between prealbumin and phe level (r = 0.38, p = 0.02). Prealbumin deficiency (<15 mg/dL) n= 2, both poorly compliant with protein substitutes. Marginal prealbumin (<20 mg/dL) n=12. All age ranges	Prealbumin, mg/dL Deficiency in at least one EAA:	**Aged <6 years:** 19.5 **PKU (n):** 9	**Aged >6 years:** 22.1 **Controls (n):** 1

			and were among the most compliant patients. The PKU children with low prealbumin were more likely to have an EAA deficiency (p = 0.05).			
Mönch et al. (1996)	24h urinary nitrogen excretion, 13C-enrichment of expired CO2	Dose of protein substitute	Study 3: Reduced nitrogen excretion with PS taken in 3 vs. 2 doses. Study 4: Higher and later maximum 13C-enrichment of expired CO2 and high oxidation with PS taken as one large dose vs only one-third of PS taken. Increased nitrogen excretion when taking one large portion compared to only one-third of the total daily amount of PS.	Study 3: 24-h urinary nitrogen excretion (g/24 h)	**PS in two portions:** 6.3-12.4	**PS in three portions:** 4.7-10.8
				Study 4: Maximum 13C-enrichment of expired CO2	**One large dose:** 12 ‰ after 3 h	**1/3 of dose taken:** 7‰ after 2 h
				13C-leucine (Ox) after 5 h	19.5%	9.5%
				24-h urinary nitrogen	6.9	4.3

				excretion (g/24 h)				
Acosta et al. (1999)	PAA, albumin, BUN, RBP, prealbumin	None assessed	All mean plasma indices of protein status were in normal reference ranges. Amino Acids: Mean conc of all amino acids except Cys, Gly and Phe were in the reference ranges. Amino acids below the lower limit of the ref: Arg (12%), Cys (71%), Ile (12%), Lys (14%) and Thr (11%). Dietary intakes of Arg (month 3, r = 0.36, p = 0.05), Met (month 4, r = 0.43, p = 0.05), Phe (months 1, 2 and 5, r = 0.65, p = 0.01; r =0.42, p = 0.05; r = 0.33, p = 0.07), Trp (month 1, r = 0.51, p = 0.05), Tyr (month 4, r = 0.37, p= 0.05) and Val (month 3, r = 0.51, p = 0.01) were positively correlated with the respective PAA conc.	Mean (SD) [n=] Albumin, g/dL (ref range: 3.0-4.6) **Baseline** 3.7 (0.1) [22]	**1mo** 3.6 (0.1) [24]	**3mo** 4.1 (0.1) [23]	**6mo** 4.1 (0.1) [26]	**During study** 3.9 (0.1)
				RBP, mg/dL **Baseline:** NE	NE	3.38 (0.2) [22]	3.74 (0.2) [21]	3.56 (0.2)
				Prealbumin, mg/dL (ref range: 6.7-21)	NE	17.6 (0.8) [22]	17.9 (0.9) [22]	17.8 (0.8)

				Baseline: NE Urea nitrogen, mg/dL (ref range: 5-17) **Baseline:** 12.9 (1.2) [23]	12.3 (0.8) [23]	12.0 (0.6) [24]	11.9 (0.5) [24]	12.1 (0.6)
Hillman et al. (1996)	Albumin, creatinine	Dietary parameters	Serum albumin and creatinine were similar in children with PKU and controls. No sig. correlations between total protein intake, protein intake/kg, or serum albumin after age correcting.	Albumin, g/dL Serum creatinine	**PKU:** 4.9 ± 0.5 (n=11) 0.97± 0.19 (n=11)	**Controls:** 5.1 ±.58 (n=35) 0.82±.0.10 (n=18)		
Graffin et al. **(1995)**	Total protein, albumin, BUN	None assessed	Children had normal levels of total protein; but abnormal values for albumin (high) and BUN (low)	No data shown				

Thompson et al. (1990)	PAA, whole-body protein metabolism	Plasma phe levels, classification of PKU	Rates of protein synthesis in PKU were similar to or above control values, as were rates of protein catabolism. Net protein loss during fasting tended to be lower in PKU than in controls (not statistically different). Protein turnover values were similar in HPA to PKU and controls. Protein synthesis did not change sig. with plasma phenylalanine concentration. Amino Acids: Mean conc of many AAs were in the lower normal range in PKU on normal diets without PS. Those with protein-restricted diets and PS, the mean conc of all AAs other than phe were similar to controls.			
Nord et al. (1988)	Total protein, albumin and BUN	Dietary adherence, diagnosis	Total protein, albumin and BUN were within normal limits and no sig diff between groups.	No data provided		
Shenton et al. (1983)	Prealbumin and albumin	None assessed	No sig difference in albumin between groups. Significant difference in prealbumin between groups ($p<0.01$)	Mean (SD) (range) Albumin (g/l)	**PKU:** 44.4 (3.3) (40-53)	**Control:** 44.6 (3.1) (37-50)

				Prealbumin (mg/L)	168 (33) (105-219)	216 (47) (128-332)
Pena et al. (2018)	BUN	Type of protein substitute	Meta-analysis for BUN reported no sig. differences between GMP-AAs and AAs (MD = -0.22 mg/dL (-1.49, 1.04); I2 = 0%; p = 0.73)			

Note: Authors in italics are abstract only papers

PKU: phenylketonuria; HPA: hyperphenylalaninemia; Phe: phenylalanine; SMM: skeletal muscle mass; BUN: blood urine nitrogen; PAA: plasma amino acids; AA: amino acids; L-AA: L-amino acids; RBP: retinol-binding protein; cGMP-AA: casein glycomacropeptide amino acid; PS: protein substitute; PPS: Pretrial Protein Substitute; LPS: Liquid Protein Substitute; AUC: area under the curve; NE: Not evaluated

Table 6: Functional measurements of protein status and key findings

Author (Year)	Method used	Modulating factors	Key findings: Protein status outcomes	Variable	Group 1	Group 2
Choukair et al. (2017)	Maximal isometric grip force (hand dynamometry)	Sex, MCA, classification of PKU, metabolic control	Mean grip force z-score was significantly decreased compared with the reference population (−0.64 ± 1.26; p = 0.0004); observed both in female and male patients. 38% a grip force <3rd percentile. A significant linear correlation between MCA and grip force was found for PKU patients (r = 0.827, p < 0.0001) and reference population (r = 0.66, p < 0.001). No relationship between grip force (z-scores) and PKU type or mean phe.			
Sumanszki et al. (2020)	Maximum physical stress test: evaluated VO_2max	None assessed	Duration of aerobic or anaerobic exercise: No significant difference between groups (PKU vs controls, p = 0.883 and p = 0.247, respectively). VO_2max: Significantly lower in the PKU group vs. controls (p = 0.004); relative VO_2max (adjusted for body weight) was similar between groups. Cumulative workload (watts): Significantly higher in the control compared with the PKU group (p=0.002). Handgrip test: Used as a stress stimuli intervention and no outcome data provided	VO_2max (ml/min) Relative VO_2max (ml/kg/min)	**PKU:** 3080 (2813–3768) 45 (36.3–52.8)	**Controls:** 3970 (3758–4108) 48.5 (46.5–59)
Mazzola et al. (2015)		Metabolic control	Patients showed similar aerobic capacity and workload peak in the VO2peak test in comparison to controls. PKU patients and controls showed similar values of	VO2peak (mL/kg/min)	**PKU:** 28 ± 8	**Controls:** 31 ± 6

VO2peak Workload peak	prescribed and actual VO2 during exercise. Poorly controlled patients showed the lowest percentage of actual VO2 during exercise in relation to the prescribed value (not sig)	Workload peak (W)	203 ± 31	216 ± 49
		Prescribed VO2 (mL/kg/min)	21 ±6	22 ±4
		Actual VO2 (mL/kg/min)	18 ± 5	22 ± 4

Supplemental material: Protein intake data

Author (Year)	Protein requirements	Total protein intake	Natural protein intake	Protein substitute intake	Additional comments
			ANTHROPOMETRIC MEASURES		
Daly et al. (2021)	Not reported	Not reported	Median amount of prescribed natural protein: 5.5 g protein/day (range 3–30 g) or 275 mg/day of phenylalanine (range 150–1500 mg)	Median daily dose of protein substitute: 60 g/day (range 40–80 g)	cGMP-AA and L-AA groups. All were adherent with protein substitute
Alfheeaid et al. (2018)	Not reported	Not reported	Not reported	Not reported	Unclear what patients' normal dietary patterns are.
Dobbelaere et al. (2003)	RDAs (Comite de Nutrition 2001)	Protein intake varied from 1.2 to 2.1 (mean 1.67±0.23) g/kg per day, representing 109-191% (146%±25%) of RDA	Phe intake ranged from 204-768mg/day (based on 4DDD)	Not reported	
Huemer et al. (2007)	RDA + 20-40% (DACH 2000)	Mean total protein intake: 1.2 ± 0.3 g/kg per day (median 1.1, range 0.8–2.4) in PKU patients. Mean total protein	Mean natural protein intake: 0.3 g/kg/day (SD 0.2, median 0.24, range 0.1–1.1)	Mean Phe-free L-AA mixture intake: 0.9 g/kg/day (SD 0.2, median 0.84, range 0.6–1.4)	Mean total protein intake in PKU patients was 124% (range 77–193%) of the RDA

		intake: 33.7 ± 10.3 g/day (median 32.5, range 11.3–32.5).			
Sailer et al. (2020)	Not reported	g/kg: Female: PKU 1.3 ± 0.56 vs Con 1.9 ± 0.96 Male: PKU 1.44 ± 0.48 vs Con 2 ± 0.77 No diff in the total grams of protein/ kg in PKU compared to controls.	g/kg: PKU Female: 0.36 ± 0.31 Male: 0.41 ± 0.32 (no sig. difference)	g/kg: PKU Female: 0.95 ± 0.65 Male: 1.03 ± 0.37 (no sig. difference)	% total energy of protein among PKU male subjects was lower than male control subjects (10.4 ± 2.1% vs. 14.5 ± 3.7; p=0.003), but % energy from protein was similar between PKU female participants and controls (10.1 ± 2.6% vs. 11.0 ± 5.2%; p = 0.63). Protein to energy ratio was significantly lower among PKU male subjects compared to controls (2.51 ± 0.58 vs. 3.62 ± 0.92; p = .0003). This difference was not seen in females.
Allen et al. (1995)	Not reported	Not reported	Not reported	Not reported	
Evans et al. (2017)	FAO/WHO/UNU recommended safe levels	Total protein: g/kg/d (median ± SD (range)) D-PKU: 2.05 ± 0.60	Natural protein: g/kg/d (median ± SD (range)) D-PKU: 0.50 ± 0.18 (0.18–0.80);	AAF g/kg/d: median ± SD (range) D-PKU: 1.54 ± 0.50 (0.80–	Reported that the median total-protein intake exceeded the

		(1.00–3.50); BH4-PKU: 1.90 ± 0.16 (1.70–2.10); All-PKU: 2.00 ± 0.56 (1.00–3.50)	BH4-PKU: 1.10 ± 0.60 (0.55–2.00); All-PKU: 0.50 ± 0.37 (0.17–2.00)	2.70); BH4-PKU: 1.00 ± 0.61 (0.00–1.30); All-PKU: 1.43 ± 0.59 (0.00–2.70)	FAO/WHO/UNU recommended safe levels (data not shown).
Nogueira et al. (2021)	Not reported	Not reported	Not reported	not reported	
Evans et al. (2018)	Not reported	Not reported	Not reported	Not reported	Reported participants used phe-free AA formula
Adamczyk et al. (2011)	Not reported	Not reported	Not reported	Not reported	L-AA PKU supplements
Mazzola et al. (2016)	Not reported	Not reported	Not reported	Not reported	No access to protein-enriched low-phe food
Rocha et al. (2013)	Not reported	Mean (SD): 1.92 (0.57) g/kg/day for patients aged <19 years (n=63) and 1.43 (0.35) g/kg/day for patients aged ≥19 years (n=26)	Mean (SD): 0.76 (0.46) g/kg/day for patients aged <19 years (n=63); 0.57 (0.39) g/kg/day for patients aged ≥19 years (n=26)	Mean (SD): 1.39 (0.44) g/kg/day for patients aged <19 years (n=63); 1.03 (0.45) g/kg/day for patients aged ≥19 years (n=26)	Total protein (g/kg/d) HPA and mild PKU 1.92 ± 0.61 Classical PKU 1.88 ± 0.34 Natural Protein (g/kg/d): HPA and mild PKU 0.82 ± 0.49 Classical PKU 0.49 ± 0.14 g/kg/d

					AA Mix (g/kg/d): HPA and mild PKU 1.33 ± 0.45 Classical PKU 1.67 ± 0.3
Weng et al. (2020)	DRI	1.265 ± 0.592 g/kg/d (%DRIs 105.448 ± 33.41)	0.874 ± 0.602 g/kg/d	12 (55%) PKU pts phe-free formula; 10 non-phe-free formula (getting 100% of DRI from natural protein)	71% (70.90±31.24) of protein consumed by PKU patients was from medical food and 29% from natural food
Stroup et al. (2018)	Not reported	Not reported	Not reported	g PE from AA-MF: Male: 67 ± 6; Female: 52 ± 4 (p(sex)=0.057; p(gt)=0.09) g PE from AA-MF/kg: Male 0.89 ± 0.09 Female: 0.77 ± 0.08 (p(sex)=0.46; p(gt)=0.21) g PE from AA-MF/kg lean mass: Males: 1.20 ± 0.08 females: 1.24 ± 0.10 (p(sex)= 0.54; p(gt)= 0.14)	Discussed the negative impact on BMD with increase protein substitute

Jani et al. (2017)	120-140% RDA	Total Protein (g/day; g/kg/day): Total (n=83): 59.3 (10.1, 119.7); 1.09 (0.19, 2.20) Adults (n=25): 69.9 (42.1, 119.6); 0.92 (0.86, 0.76) Children (n=58): 53.1 (10.1, 119.7); 1.12 (0.63, 0.97) Median total protein intake (g/d) was highest among males (57.5, range: 46.8, 76.2) compared to PKU patients	Intact protein (g/day); g/kg/day: Total: 13.4 (3.6, 79.4); 0.25 (0.07, 1.46) Adults: 25.1 (8.2, 79.4); 0.33 (0.17, 0.50); children: 11.3 (3.6, 74.7); 0.24 (0.23, 0.61). **Adults:** higher median intakes of intact protein (25.1 vs. 9.9 g/d, U = 73.5, $p < 0.001$) compared to prescribed intakes. **Children:** actual median intact protein intake was higher than prescribed (6.0 vs. 11.3 g/d, U=2549.5, $p < 0.001$).	MF protein (g/day; g/kg/day): Total (n=73): 45.0 (5.0, 90.0); 0.83 (0.31, 0.57)) g/d; Adults (n=19): 53.8 (25.0, 90.0); 0.71(0.51, 0.57) g/d; Children (n=54): 43.2 (5.0, 79.6); 0.91 (0.31, 0.65) g/d Adults: consuming lower actual median intakes of MF (53.8 vs. 60.0 g/d, U = 133.5, $p = 0.03$) compared to prescribed intakes	Majority of participants consumed higher intact proteins (n= 76, 92.7% vs n = 6, 7.3%) than as prescribed. A similar proportion consumed medical food protein as per prescription (n = 35, 51.5%) or lower than as prescribed (n=33, 48.5%).
Paci et al. (2018)	Not reported	Not reported	Not reported	Not reported	Dietary data focus on glycaemic index and glycaemic load
Mexia et al. (2015)	Not reported	Not reported	Not reported	Not reported	

Torriente et al. (2017)	Not reported	Not reported	Not reported	Not reported	
Daly et al. (2019)	Not reported	Not reported	Not reported	Not reported	
Kanufre et al. (2015)	Not reported	Not reported	Not reported	Not reported	
Bonifant et al. (2010)	Not reported	Not reported	Not reported	Not reported	
Rocha et al. (2010)	Not reported	Not reported	Not reported	Not reported	Medians of natural protein and protein substitute intake were not significantly different in patients with overweight/obesity compared to the others.
Wilcox et al. (2011)	Not reported	Not reported	Not reported	Not reported	
Nalin et al. (2013)	Not reported	Not reported	Not reported	Not reported	
BIOCHEMICAL MEASURES					
Prince et al. (1997)	(Phase 2) RDA minus exchanges and prescribing	Not reported	Not reported	AA Intakes (g protein/kg) Prescribed by clinician Entry (yr. 1): 1.15 ±0.5 End (yr. 5): 0.67 ± 0.2	Mean ages associated with these intakes were 6.9 years at entry and 11.1 years at end, which results in the mean 'Received Intakes' of AA =

	difference as protein substitute			P<0.001 Reported by participant Entry (yr. 1): 1.31 ± 0.8 End (yr. 5): 0.68 ± 0.2 P<0.001 Received by participant Entry (yr. 1): 0.91 ± 0.4 End (yr. 5): 0.4 ± 0.3	75% RDA protein and 40% RDA protein, respectively. Observed no significant reductions in protein status, with 'Received Intakes' 50–100% below prescribed intakes recommended by Medical Research Council (1993) for the mean age of subjects.
Singh et al. (2010)	Not reported	Total protein intake remained at approximately 1.0±0.08 g/kg per day (43.7±4.2 g/day) throughout the 24 months of the study.	At 3mo, phenylalanine increased from a baseline average of 11.9± 4.1 mg/kg to 39.9 ± 11.5 mg/kg (p=0.001), and phenylalanine intake from food increased from 15.9 ± 5.3 mg/kg to 34.2 ± 13.8 mg/kg (p=0.007).	By 3mo of BH4 therapy, n=3 were consuming a reduced MF prescription (50%, 20%, and 38%, respectively). n=3 no longer required MF. n=1 who initially discontinued MF consumption experienced a growth spurt, MF was reintroduced	Stage 2: MF was reintroduced as needed to stabilize plasma phe <360 μmol/L and to keep serum transthyretin within normal limits
Giovannini et al. (2014)	Italian RDA	Children who received the test or conventional substitute: Baseline protein intake was 1.9 (0.8) vs 2.0 (0.9)	Baseline mean (SD) PHE intake was 403 (213) vs 392 (227) mg/day in children who received the test or conventional substitute, respectively. End of the trial the	41 (68.3%) children had 3 doses/day (test substitute 21/30; conventional substitute 20/30) and 19 (31.7%) had 4 doses/day	Unaffected children: protein intakes were 150% higher than the Italian RDA

		g/kg/day; end of trial was 1.8 (1.0) vs 2.0 (0.9) g/kg/day	corresponding values were 392 (207) vs 400 (208) mg/day.	(test substitute 9/30; conventional substitute 10/30).	PKU: mean protein intake was around 120% of RDA
Zaki et al. (2016)	All patients received the same total protein intake to match recommended protein requirement for their age and weight*	Not reported	Not reported	Phase I: 50 % GMP and 50% AAF and Phase II: 100% AAF (amounts not provided)	Required to be compliant to treatment for at least two months prior to start of study.
Mönch et al. (1996)	Not reported	Not reported	Not reported	Not reported	Took their usual prescription divided in 2 and 3 doses
Thompson et al. (1990)	Not reported	Mean Protein (g/kg/d) PKU: 1.06 ± 0.34 Range: Free diet: 0.76-1.64 g/kg/day Diet+AA: 0.5-1.36 g/kg/day HPA: 1.4 & 1.1 (n=2)	Unclear of current dietary protein intake	Not reported	n = 5 were on free diet; n = 5 were on PKU diet + AA No alteration in dietary intake of any subject in the 3 mo. prior to the study

		Control: 1.25 g/kg/d (range 0.9-1.5)			
van Calcar et al. (2009)	DRI	Not reported	Phe allowance ranged from 5.8 mg/kg (subject 10) to 26.7 mg/kg (subject 2)	Not reported	Two dietary treatments of 4 d each: the AA diet (days 1–4) and the GMP diet (days 5–8).
Ney et al. (2016)	Not reported	Protein g/d: AA-MF: 80 ± 3 GMP-MF: 79 ± 4 Protein g/kg/d: AA-MF: 1.15 ± 0.05 GMP-MF: 1.14 ± 0.06	Classical: mean± SE 0.34 ± 0.04 g protein from natural food/kg/day; 15 ± 2 mg Phe/kg/d/; Variant: 0.50 ± 0.07 g protein from natural food/kg/day, 22 ± 3 mg Phe/kg/day	Mean ± SE prescribed dose was 0.85 ± 0.03 g PEs from AA-MF/kg/d. AA-MFs or GMP-MFs provided 66–68% of total protein intake or 0.74–0.76 g protein/kg/d	Reduce intake of natural foods that contain Phe to offset the Phe intake in GMP-MFs and maintain constant Phe intake. Medical food logs: intake was higher for GMP-MFs during both stages of the study and significantly higher during visits 3 and 4 than it was with AA-MFs (3.74 servings GMP-MFs/d compared with 2.43 servings AA-MFs/d; P = 0.001)
Ahring et al. (2018)	Not reported	In each test meal, the total content of protein was equivalent to 25% of 1 g/kg/d	Not reported	Given test protein substitute to provide 25% of requirements	Intervention included four different drink mixtures: DM1: 100% cGMP DM2: 100% L-AA (equivalent AA profile to DM1) DM3: cGMP + L-AA (to ensure nutritionally complete)

					DM4: L-AA (equivalent AA profile as DM3, but without phe)
van Rijn et al. (2007)	RDA + 20%	Protein intake (g /kg/d) (mean, SD) PKU: 1.1 ± 0.1 Controls: 1.2 ± 0.1	Tolerances of dietary Phe: based on daily intake of natural protein at 5 y of age were: 21± 9 and 11 ±4 mg Phe/kg/d at the time of the test.	Not reported	
Giovannini et al. (2009)	Italian RDA	Reports nutrient intake consistent with the Italian RDA	Not reported	Not reported	
Giovannini et al. (2006)	Not reported	Not reported	Not reported	Not reported	Compliance with the new protein substitute mixture was 100%
Douglas et al. (2013)	Not reported	Definitive responders (DR): decrease in total protein intake (Baseline: 1.4±0.7, 1 year:1.0±0.7; $p<0.001$) due to 75% decline in medical food (MF) intake. Total protein intake declined significantly in NR and PR without change in Phe tolerance	DR increased intact protein (g/kg (Baseline:0.58±0.4, 1 year:0.75±0.3)	Intake not reported, in DR group there was a 75% decline in medical food intake	

		or prescribed MF, indicating nonadherence			
Acosta et al. (1999)	RDA (1980)	Protein (g/day) 17.3 ± 0.6 Protein (g/kg/day) 2.7 ± 0.1	Not reported	Medical Food (g/day) 79 ± 4	17% of Phenex-fed infants had protein intakes below 100% of 1980 RDA. Mean intake of EAA/kg were greater than recommended.
Shenton et al. (1983)	Not reported	Not reported	Not reported	Not reported	
Schulpis et al. (2013)	Not reported	Total protein (g) Group A: 72 ± 20 Group B: 70 ± 18 Controls: 73 ± 17 P= NS	Natural protein (g) Group A: 40 ± 20 Group B: 9 ± 1.2 Controls: 73 ± 17 A vs C P<0.001 B vs C p<0.001 A vs B p<0.001	Phe free formula dose depended on age, weight and residual activity of Phe hydroxylase as related to their molecular analysis	Group A - 'loose diet' and Group B strictly adhered to diet
Arnold et al. (2001)	Not reported	Children ages 1-4 were prescribed approx. 30 g protein/day. Children aged 4-7: 35 grams protein; Children aged 7-11: 40 grams protein; Females ≥12 years: 50	Not reported	Not reported	

		grams protein; Males ≥12 years: 55 grams protein			
Arnold et al. (2002)	Not reported	Not reported	Not reported	Medical foods prescribed accordingly: ages 2-4 years, 30 g/d; ages 4-7 years, 35 g/d; ages 7-11 years, 40 g/d; ages ≥12 years, 50 g/d for female and 55 g/d for male.	
Nord et al. (1988)	Not reported	Not reported	Not reported	Not reported	
Rocha et al. (2010)	MRC on PKU, adopted by the Portuguese guidelines	Not reported	Not reported	Not reported	
Kose et al. (2019)	Not reported	Not reported	Not reported	Not reported	L-AA used as protein substitutes
Andrade et al. (2017)	RDA (0.8–1.3 g/kg/day)	Total proteins, g/kg/day PKU 1.0 [0.2–2.0] 117% RDA	Natural proteins, g/kg/day PKU 0.5 [0.2–2.0] 48% RDA	Not reported	
van Vliet et al. (2019)	Not reported	Significantly higher natural protein intake for PKU-BH4 patients vs. PKU-nBH4 ($p < 0.001$))	Not reported	Not reported	

Crujeiras et al. (2015)	RDA (recommendation was 1.3-1.5x RDA)	Not reported	Not reported	Not reported	
Viau et al. (2021)	DRIs 0.8 g protein/kg/d	Mean protein intake: 73.2 ± 17.6 g/d (range: 46.9–125.4 g/d) and 1.0 ± 0.3 g/kg/d (range: 0.5–1.8 g/kg/d). Majority (16/18) of participants' intake met or exceeded the DRI, two male participants consumed less at 0.5 and 0.6 g/kg/d.	Intact Protein, g/day Phe < 30 µmol/L (n=11): 72.2 ± 11.4 Phe ≥ 30 µmol/L (n=7): 72.5 ± 25.8 P=0.97 Intact Protein, g/kg Phe < 30 µmol/L (n=11): 1.0 ± 0.2 Phe ≥ 30 µmol/L (n=7): 1.0 ± 0.4 P=0.84	None	FFQ: participants ate a median of 92.8% (IQR 70.0 – 111.1%) of the recommended daily servings of protein foods (e.g., meat, poultry, seafood, eggs, soy, nuts, seeds and legumes) and 55.4% (IQR 32.1–85.7%) of dairy foods. On average, animal proteins comprised 62 ± 10% of total protein intake.
Pena et al. (2018)	Not reported	Not reported	Not reported	Not reported	
Gokmen-Ozel et al. (2009)	Not reported	Not reported	Median phenylalanine exchanges were 6 x 50mg daily (range 3–15). n=6 adults were not following measured phenylalanine exchanges but avoided high protein foods	Median protein substitute dose was 60 g PE daily (range 45–75 g day) both before and during the study.	

Graffin et al. (1995)	Not reported	Mean protein intake: 62 ± 15 % of their recommended levels	Not reported	Not reported	
Prochazkova et al. (2012)	Not reported	Not reported	Not reported	Not reported	
Rocha et al. (2009)	Not reported	Not reported	Not reported	Not reported	
Desloovere et al. (2014)	Not reported	Not reported	Not reported	Not reported	
Kose et al. (2016)	Not reported	Not reported	Not reported	Not reported	
ANTHROPOMETRIC + BIOCHEMICAL MEASURES					
Lambruschini et al. (2005)	Not reported	Not reported	Phe-restricted diet initially. Phe tolerance increased significantly from 356 ± 172 mg/day (mean ± SD; range: 201–600) to 1546 ± 192 mg/day (range: 1240–1801) (Wilcoxon test; p = 0.004)	Stopped in 11/14 patients with BH4 treatment	All participants initially were treated with phe-restricted diet and protein substitutes, prior to BH4 treatment. Phe-restricted diet unknown duration
Pena et al. (2021)	Not reported	Not reported	Natural protein intake (g/kg/day) Baseline: 0.41 (0.26–0.62) When on cGMP-AA 0.34 (0.21–0.69)	Amount of protein equivalent from protein substitute remained unchanged [(0.86 ± 0.24 g/kg/day	At the last ANSE, CGMP-AA contributed a mean of 66 ± 31% (range 23 to 100) to the total protein substitute intake.

				vs 0.74 ± 0.23 g/kg/day; p = 0.126) and (50.8 ± 16.3 g/ day vs 44.6 ± 12.8 g/day; p = 0.118)].	
Das et al. (2013)	DACH-recommendations (German-Austrian-Swiss dietary association) (DGE 2012)	PKU: protein intakes were below DACH-recommendations Protein (g/kg/d) Baseline Protein reduced + AAM: 1.1 Vegan + AAM: 0.9 Vegan – AAM: 0.5 Normal Food: 0.6 Follow-Up Protein reduced + AAM: 1.0 Vegan + AAM: 1.0 Vegan – AAM: 0.7 Normal Food: 1.0	Not reported	Not reported All patients not taking AAM at the beginning of the study agreed to supplement their original diet with an AAM subsequently.	Participants were grouped into the following: 1. Normal food (“normal food”) - 36% 2. Vegan without amino acid mixture (“vegan”) -14% 3. Vegan with amino acid mixture (“vegan + AAM”) - 8% 4. Protein reduced with amino acid mixture which is the recommended form of nutrition (“PKU-diet”) - 42%

Pinto et al. (2017)	Not reported	Not reported	Natural protein intake (g/kg/day) AA diet: 0.47 ± 0.27; GMP diet: 0.59 ± 0.49 (p = 0.241)	Protein substitute (g/kg/day) AA diet: 0.85 (0.73–1.08); GMP diet: 0.75 (0.61–0.99) (p = 0.182)	Mean GMP contribution to the total protein substitute intake was 57% (27 to 100%) providing an additional 34 ± 12 mg of PHE per day.
Allen et al. (1996)	FAO/WHO/UNU, 1985	Protein intake was similar for the PKU and controls (median): PKU: 2.11 g/kg equivalent to 209% of recommended Controls: 1.9 g/kg equivalent to 193% of recommended FAO/WHO/UNU 1985	Median phe intake was significantly lower in the PKU group (23 mg/kg) than in the controls (92 mg/kg)	Not reported	
Doulgeraki et al. (2014)	Not reported	Not reported	Not reported	Not reported	PKU - L-AA supplements
Hillman et al. (1996)	RDA	Mean intake: 46.1 ± 12.1 g/d (1.5 ± 0.6 g/kg)	Not reported	Not reported	Protein intakes were above the RDA
Modan-Moses et al. (2007)	RDA	Protein intake (g/day): All: 72.7 ± 34.9; Diet-adherent: 86.8 ± 30;	PHE intake (mg/day). All: 1394 ± 982; Diet-adherent: 1097 ± 1063; non-adherent: 1859 ± 624 (NS)	Not reported	All diet-adherent patients achieved protein intake above RDA, only 3

		non-adherent: 45.3 ± 22.2 (p = 0.011) Protein intake was significantly higher in the diet-adherent patients			non-adherent patients met the RDA for protein intake
Boros et al. (2015)	Not reported	Not reported	Not reported	Not reported	
Das et al. (2010)	Not reported	Not reported	Not reported	Not reported	41% of PKU-patients followed recommended protein restriction supplemented with AM. 14% said they follow a less restricted 'vegan' diet supplemented with AM. 45% claimed to have normal eating habits without AM.
Sumanszki et al. (2019)	Not reported	Not reported	Not reported	Not reported	
ANTHROPOMETRIC + FUNCTIONAL MEASURES					

Choukair et al. (2017)	Not reported	Not reported	Not reported	Not reported	33 (4 adolescents and 29 adults) were on a PKU diet + protein substitute 16 (2 adolescents and 14 adults) did not follow a diet or protein substitute 3 adults had protein substitute only; 1 adolescent and 3 adults followed a PKU diet exclusively.
Sumanszki et al. (2020)	Not reported	Not reported	Not reported	Not reported	
FUNCTIONAL + BIOCHEMICAL MEASURES					
Mazzola et al. (2015)	Not reported	Not reported	Not reported	Not reported	

Note: Authors in italics are abstract only papers

* V. R. Young and S. Borgonha, “Nitrogen and amino acid requirements: the Massachusetts Institute of Technology amino acid requirement pattern,” The Journal of Nutrition, vol. 130, no.7, pp. 1841S–1849S, 2000

Supplemental material: Search strategy

Database: MEDLINE (Ovid)

(exp Phenylketonurias/ OR Phenylketonurias.mp. OR PKU.mp. OR Hyperphenylalanin?emia.mp. OR exp Phenylalanine Hydroxylase/ OR "Phenylalanine Hydroxylase".mp. OR "Phenylalanine Hydroxylase deficiency".mp.) **AND** (exp Nutritional Status/ OR "nutritional status".mp. OR "protein status".mp. OR exp Muscle Proteins/ OR "muscle proteins".mp. OR "protein metabolism".mp. OR "muscle protein metabolism".mp. OR exp Body Composition/ OR "body composition".mp. OR exp Muscle Strength/ OR "muscle strength".mp. OR "muscle function".mp. OR exp Prealbumin/ OR prealbumin.mp. OR Transthyretin.mp. OR exp Albumins/ OR albumin.mp. OR "3-methylhistidine concentrations".mp. OR exp Retinol-Binding Proteins/ OR "retinol-binding protein".mp. OR "urea production".mp. OR exp Nitrogen/ OR nitrogen.mp. OR exp Creatinine/ OR creatinine.mp. OR “VO2max” OR exp Physical Exertion/ OR "physical exertion".mp. OR exp Exercise Test/ OR "exercise test".mp.) **[Limit to English language]**