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Narrative Review

A food pyramid for adult patients with phenylketonuria and a systematic review on the current evidences regarding the optimal dietary treatment of adult patients with PKU

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SUMMARY

Early dietary treatment is mind-saving in patients with phenylketonuria. A "diet-for-life" is advocated, aimed to prevent effects of chronic exposure to hyperphenylalaninemia.

While adherence to diet is significant during childhood as patients are followed-up at specialized metabolic centers, during adolescence and adulthood percentage of patients discontinuing diet and/or lost at follow-up is still high. The process of passing skills and responsibilities from pediatric team to adult team is defined "transition". The goal of transition clinics is to set up specific multidisciplinary care pathways and guarantee continuity of care and compliance of patients to care.

In 2017, "The complete European guidelines on phenylketonuria" were published. These guidelines, however, do not provide an easy way to illustrate to adult patients how to follow correct dietary approach. The purpose of this review is to evaluate current evidence on optimum dietary treatment of adults with phenylketonuria and to provide food pyramid for this population.

The pyramid built shows that carbohydrates should be consumed every day (3 portions), together with fruits and vegetables (5 portions), extra virgin olive oil, and calcium water (almost 1 L/day); weekly portions can include 150 g potatoes walnuts and hazelnuts (20 g).

At top of pyramid, there are two pennants. The green means that, based on individual metabolic phenotype and daily phenylalanine tolerance, patients need personalized supplementation (specific phenylalanine free amino acid mixtures, vitamins and omega 3 fatty acids); the one red indicates foods that are banned from diet (aspartame and protein foods exceeding individual dietary phenylalanine tolerance).

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1. Introduction

Phenylketonuria (PKU) is a rare autosomal recessive disease due to recessive mutations in the phenylalanine hydroxylase (PAH) gene, which cause a disturbance in amino acid metabolism [1,2]. In PKU, the dietary excess of phenylalanine (i.e. the one not used for protein synthesis) is not converted into Tyr and accumulates in the blood and in some tissues of the body, particularly in the brain [3]. Tyr deficiency, moreover, leads to insufficient production of neurotransmitters, including adrenaline, noradrenaline, dopamine, and DOPA [4]. Healthy individuals have blood Phe levels not exceeding 2 mg/dL [5]. Healthy carriers have slightly higher values than healthy people, again not exceeding 2 mg/dL. In the case of PAH deficiency, hyperphenylalaninemia does occur in newborns, being promptly detected at newborn screening. Based on dietary Phe tolerance, PAH deficiency can be classified in classic PKU, mild PKU, and non-PKU HPA requiring differential dietary treatment for optimal outcome [3]. In particular, classic and mild PKU require restriction of dietary proteins and supplementation with Phe-free amino acid mixtures to maintain blood Phe levels within safe ranges, whereas no dietary restriction is necessary in non-PKU HPA. Dietary treatment, indeed, is a mind-saving therapy for patients with PKU, which effectiveness should be monitored by regular evaluations of blood Phe levels [6,7]. Phe is an essential amino acid present in natural proteins, both of animal origin and in vegetal proteins. In general, 1 g of natural proteins provide approximately 50 mg of Phe. This allows the estimation of foods Phe content by consulting nutritional tables shown on the packages [8]. Dietary treatment of PKU is extremely restricted. Actually, patients with classical PKU tolerate 220–240 mg of Phe per day, corresponding to approximately 4–5 g of natural protein per day [9]. This picture makes evident how difficult it is to re-enter these parameters following a balanced diet, such as that according to the pyramid of the Mediterranean Diet [10]. Moreover, while attention to diet during childhood is warranted by systematic follow-up at specialized metabolic centers, compliance to dietary prescriptions is generally lower in adolescent an adult PKU patients, with increased risk of the newly recognized later-onset complications of the disease [11]. Based on the medical records of specialized centers in the United States, in 2013 it was estimated that 77% of 25–45 years-old PKU patients were lost at follow-up. Recent online surveys, moreover, showed that 32% of patients were no longer actively managed by specialists, a percentage increased to 55% in patients older than 30 years [12].

Cazzorla et al. highlighted the problem PKU management in adults. The survey found that about 40% of patients did not consider PKU as a disease and half of them reported an elevated blood Phe values (>600 umol/l) in the previous 6 months. Poor compliance to dietary treatment of PKU is generally characterized by two lines of failures, namely 1) increased consumption of natural proteins and 2) inadequate supplementation with artificial Phe-free amino acids mixtures, implying the need of more intense and personalized educational measures, as well as structured transitional assistance processes [13]. Among the therapeutic strategies proposed in order to improve metabolic control and patient outcome, the use of long-chain neutral amino acids (LNAA), including tyrosine, tryptophan, threonine, methionine, valine, isoleucine, leucine and histidine, has been suggested as complementary treatment [14,15]. Since all LNAAs share a common transport system with Phe across the blood–brain barrier, high plasma concentrations of these amino acids were hypothesized to competitively limit or block the transport of Phe to the brain [15]. Nutrition of PKU patients, moreover, is hampered by micronutrient deficiencies due to the limited choice and quantity of natural foods: to prevent deficiencies from developing, Phe-free amino acid mixtures (AAMs) generally contain significant quantities of vitamins, minerals and trace elements [16].

A study by Cochrane et al. showed that PKU patients appear to face obstacles both in the provision of food for special medical purposes and in primary health care. This may indicate a lack of understanding of PKU management in primary care or poor communication between specialists and primary health care professionals. Alternatively, this may simply reflect the lack of scientific evidence demonstrating the usefulness and efficacy of low protein staple foods (LPSF) in the management of people with PKU [17].

Considering the correlation between phenylketonuria and overweight-obesity, numerous publications have devoted to this topic, but obtaining conflicting and not always clear results [18–22].

As the management of PKU is very complex, “The complete European guidelines on phenylketonuria: Diagnosis and treatment” were published with the aim of optimizing treatment of PKU [11]. In summary, it is suggested that nutritional follow-up requires monitoring anthropometric data, BMI, clinical signs of nutritional deficiencies, nutritional intake and biological biomarkers, to investigate an excess or subclinical deficiency of micronutrients. While study designs and patient numbers are not optimal, many claims are compelling, important, and relevant. In addition, knowledge gaps are identified that require further research in order to target better care for the future. The key recommendations that should be prioritized for the implementation of the available guidelines mainly concern the initiation of treatment, the target levels of Phe for treatment and follow-up. However, these guidelines do not provide an easy way to teach adult patients how to follow the correct dietary approach.

The purpose of this article is to narrative review the current evidences regarding the optimal dietary treatment of adult patients with PKU and to provide a food pyramid for PKU management. The food pyramid proposal will serve to guide energy and dietary intake in order to prevent and treat nutritionally related complications commonly occurring in PKU patients.

2. Methods

This narrative review was conducted by the following steps [23]:

(1) Configuration of the working group: three operators skilled in clinical nutrition (one acting as a methodological operator and two participating as clinical operators); (2) formulation of the revision question on the basis of considerations made in the abstract: “the state of the art on ideal dietary therapy in adult PKU patients”; (3) identification of relevant studies: a research strategy was planned on PubMed (Public MEDLINE run by the National Center of Biotechnology Information (NCBI) of the National Library of Medicine of Bethesda (Bethesda, MD, USA)) as follows: (a) definition of the keywords (PKU, foods, nutrients, diet) grouped in inverted commas (” …“) and used separately or in combination; (b) use of the Boolean (a data type with only two possible values: true or false) AND operator, which allows the establishments of logical relations among concepts; (c) research modalities: advanced search; (d) limits: time limits: papers published in the last 30 years; humans; languages: English; (e) manual search performed by senior researchers experienced in clinical nutrition through the revision of reviews and individual articles on dietary therapy for PKU patients published in journals qualified in the Index Medicus; (4) analysis and presentation of the outcomes: the data extrapolated from the “revised studies” were collocated in tables; in

particular, for each study, Authors and year of publication and study characteristics were reported; (5) the analysis was carried out in the form of a narrative review of the reports. At the beginning of each section, the keywords considered and the kind of studies chosen have been reported. We evaluated, as suitable for the narrative review, the studies of any design that considered the relevance of diet, foods, nutrients and food for special medical purposes for adult PKU patients.

This review identified 49 eligible studies; a dedicated flowchart (Fig. 1) represents proper nutrition for PKU, specifying the quality and amount of food, in order to provide an ideal dietary management and to construct a food pyramid for PKU patients.

2.1. Foods for special medical purposes (protein substitutes)

This research was carried out based on the keywords: "phenylketonuria" OR "PKU", AND "protein substitutes" OR "aminoacidic mixtures" OR "amino acids". 15 articles were sourced: 1 cross-sectional study, 3 randomized cross-over studies, 1 randomized controlled clinical trial, 1 observational study, 1 double-blind clinical trial, 6 clinical studies and 2 narrative reviews (Table 1).

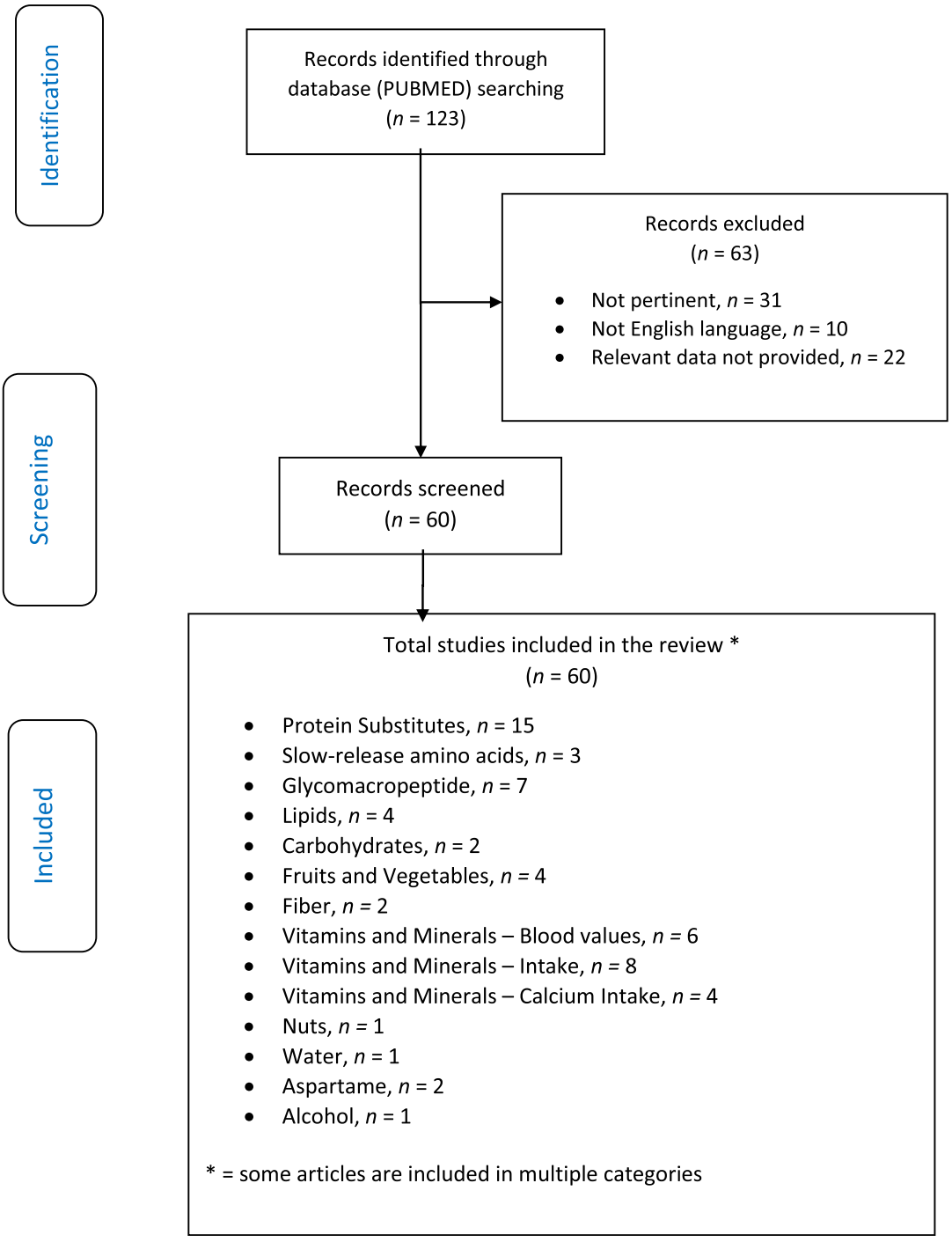

Fig. 1. Flowchart of the study.

Table 1
Foods for special medical purposes (protein substitutes).

Author, year	Type of study	Study period	Supplementation (type of aminoacid mixture …)	Subjects	End point	Results	Strength of evidence
Rohde C. et al., 2014	Open multicenter cross-sectional study	3 days	Phe-free amino-acid mixture (from a detailed 3-day diet record)	67 (6–45 years)	Protein supply and consumption of essential amino acids and several micronutrients (compared with the current recommendations and data for the healthy population)	Severe micronutrient deficiencies in all patients without AAM supplement. PKU patients under a relaxed diet are at risk of an insufficient nutrient supply, if they have: no substitution with AAM; a protein supply less than 0.5 g per kg body weight from AAM; a total protein supply less than 120% of the recommendations.	Moderate
MacDonald A. et al., 2003	Randomized crossover study	Two phases of 12 weeks each	Aminogran Food Supplement tablet (1.3 g) containing: 1 g amino acids, 0.3 g carbohydrate, 66 mg tyrosine/g amino acids.	21 (15 F–6 M; 8–25 years; median age 15 years)	Effectiveness and acceptability of tablets and comparison with conventional protein substi- tute drinks	Better compliance with the new tablets than with patients' usual protein sub- stitutes; lower plasma phenyalanine and increased tyrosine with the amino acid tablets.	Moderate
Rohr F.J. et al., 2001	Observational study	24 weeks	Phlexy-10 (SHS North America, Gaithersburg, MD 20884, USA): drink mix, fruit-flavoured bars and capsules (one sachet, one bar or 20 capsules providing 10 g of amino acids).	11 (8 F–3 M; 10–32 years)	Acceptability and tolerance of a newly developed flexible medical product for PKU	The powder drink was the favourite module used. Mean weekly blood phenylalanine decrease of 40% from mean baseline levels. Normal blood concentrations of vitamins and minerals, except for a low zinc concentration in two subjects and a low vitamin B12 concentration in another.	Moderate
Prince A.P. et al., 1997	Randomized control clinical trial and historic control design	5 years	A new amino acid-containing nutritionally complete drink mix (isoleucine 40 mg/g protein, leucine 89 mg/g, lysine 91 mg/g, methionine plus cysteine 40 mg/g, phenylalanine plus tyrosine 93 mg/g, threonine 47 mg/g, tryptophan 15 mg/g, valine 67 mg/g; total 482 mg)	25 (4–10 years at the enrolment)	Long-term safety, efficacy, and acceptance of a new amino acid formulation and a variety of treatment products incorporating it.	Acceptance of the treatment (usage of products), even when made more palatable, below clinical expectations; mean serum proteins and minerals, height and weight not significantly reduced during the study, supporting the safety of lowered intakes of amino acids and of nutritionally incomplete products. Significant increase in mean serum phenylalanine concentration ($p < 0.03$), but mean rise (0.1 mmol/L) during a corresponding mean age increase of 4.2 years lower than in other recent reports from longitudinal studies of outcomes during this age range with traditional products. Confirmed safety and efficacy of a more palatable and flexible approach to treatment.	High
Green B. et al., 2019	Clinical trial	28 days	33 g of a low-volume, nutrient-enriched, protein substitute (energy value 98 kcal; protein equivalent 20.0 g; carbohydrate 3.5 g; fat 0.3 g; DHA 100 mg)	12 (33.7 ± 2.6 years)	Eating behaviour, nutrient intake, and mood	Decreased natural protein, estimated phenylalanine intake, fat and saturated fat intakes with use of the experimental protein substitute; unchanged energy and carbohydrate intake; increased micronutrient intake to levels well within reference nutrient intake	Moderate

(*continued on next page*)

Table 1 (*continued*)

Author, year	Type of study	Study period	Supplementation (type of aminoacid mixture …)	Subjects	End point	Results	Strength of evidence
						recommendations; reductions in anxiety and confusion.	
Burlina A. et al., 2019	Clinical trial	12 months	Phe-restricted diet plus a slow-release LNAA product taken three times per day, at a dose of 1 g/kg body weight (nutritional information per 100 g: energy 399 kcal; fat 5.3 g, of which saturated fat 5.3 g; carbohydrates 12 g, of which sugars 0 g; fiber 5.8 g; equivalent protein 70.79 g; salt 1.6 g; L-arginine 1.92 g; aspartate 4.95 g; L-phenylalanine 0 g; L-isoleucine 10 g; L-histidine 3.36 g; L-leucine 12 g; L-lysine 5.44 g; L-methionine 2.72 g; L-tyrosine 24 g; L-threonine 2.56 g; L-tryptophan 8 g; L-valine 10 g)	12 (5 F–7 M; 19–38 years, mean ± standard deviation (SD) 29.6 ± 6.8 years)	Phe, tyrosine (Tyr), and Phe/Tyr ratio in a cohort of sub-optimally controlled adult patients with classical PKU	Unchanged Phe levels and increased Tyr levels; significantly decreased Phe/Tyr ratio in the majority of patients treated.	Moderate
Burlina A. et al., 2020	Clinical trial	12 months	Neutrafenil Micro R® (PIAM Farmaceutici S.P.A., Italy) (nutritional information per 100 g: energy 399 kcal; fat 5.3 g, of which saturated fat 5.3 g; carbohydrates 12 g, of which sugars 0 g; fiber 5.8 g; equivalent protein 70.79 g; salt 1.6 g; L-arginine 1.92 g; aspartate 4.95 g; L-phenylalanine 0 g; L-isoleucine 10 g; L-histidine 3.36 g; L-leucine 12 g; L-lysine 5.44 g; L-methionine 2.72 g; L-tyrosine 24 g; L-threonine 2.56 g; L-tryptophan 8 g; L-valine 10 g)	12 (5 F–7 M; 19–38 years, mean ± standard deviation (SD) 29.6 ± 6.8 years)	Adherence to therapy and quality of life (QoL) in a cohort of sub-optimally controlled adult PKU patients	In all patients self-reported higher adherence to medication, with 96% reporting a full adherence. Significant improvement in the QoL in all patients.	Moderate
Berry H.K. et al., 1990	Double-blind trial	12 months	A mixture of valine (150 mg/kg), isoleucine (150 mg/kg) and leucine (200 mg/kg) in addition to a low phenylalanine formula, compared to a control mixture (245 mg/kg of arginine and 245 mg/kg of aspartic acid)	16 patients (10–23 years)	Biochemical, neuropsychological tests and tests of motor and language ability	Improvement of cognitive functions (of attention in particular)	Moderate
Scala I. et al., 2020	Clinical study	12 months	LNAAs (MovisCom, 0.8–1 g/kg/day)	10 (6 F - 4 M, mean age 23.6 ± 4.5 years; range 18–32 years)	Plasma Phe and Tyr levels; neuropsychological assessment (American Psychological General Well-Being Index, Wisconsin Card Sorting Test, Test of Attentional Performance, 9-Hole Peg Test)	Significantly improved Tyr levels; improvement of distress and well-being rates, of executive functions, attention, and vigilance, no difference regarding hand dexterity	Moderate

Matalon R. et al., 2006	Multicenter study	1 week	0.5 g/kg/day or 1 g/kg/day of a formulation of LNAA (composition: tyrosine 195 mg, tryptophan 51 mg, methionine 32 mg, isoleucine 35 mg, threonine 32 mg, valine 35 mg, leucine 130 mg, histidine 30 mg, lysine 30 mg, arginine 30 mg)	8 patients receiving 0.5 g/kg/day of LNAAs (mean age: 20.5 years) + 3 patients receiving 1 g/kg/day of LNAAs (mean age: 16.5 years)	Blood Phe concentrations	Reduction of phenylalaninemia by 50% in both groups	Moderate
Kumru B. et al., 2019	Clinical study	2 years	Group I: Phe-free amino acid mixture containing vitamins, minerals and trace elements (L-phenylalanine 0 g; L-alanine 0.53 g; L -arginine 0.86 g; L-aspartic acid 1.36 g; L-cysteine 35 g; glycine 1.35 g; L-histidine 0.53 g; L-isoleucine 0.93 g; L-leucine 1.46 g; L-lysine 0.96 g; L- methionine 0.26 g; L-proline 0.97 g; L-serine 0.60 g; L-threonine 0.93 g; L-tryptophan 0.29 g; L-tyrosine 1.37 g; L-valine 1.07 g; L-carnitine 13 mg; taurine 25 mg). Group II: LNAA supplement (amino acids 73.5 g, including L-tyrosine, L-tryptophan, L-methionine, L-threonine, L-isoleucine, L-valine, L-leucine, L-histidine, L-lysine, L-arginine; 60 equivalent proteins g)	30 subjects: group I (10 patients, 4 F, mean age: 13.8 ± 2.8); group II (10 patients, 5 F, mean age: 14.8 ± 3.8); control group (10 subjects, 5 F, mean age: 13.6 ± 4.8)	Effect of LNAA supplements on oxidative stress (glutathione peroxidase and coenzyme Q10)	Lower glutathione peroxidase in patients taking LNAA supplements than the control group; lower coenzyme Q10 in patients using Phe-free amino acid mixtures than the control group and significantly higher in group II than group I.	Moderate
Daly A. et al., 2019	Randomized controlled crossover study	6 weeks	CGMP-AA vs. Phe-free L-amino acids (L-AA)	19 children (7 boys), median age of 10 years (6–16)	Blood Phe and tyrosine (Tyr) variability over 24 h	Increased blood Phe concentration and less blood Phe variability in children treated with CGMP-AA compared to L-AA.	High
Alfheeaid H. et al., 2018	Randomized cross-over study	300 min	A PKU type meal and protein substitute drink vs. an isocaloric and weight matched ordinary meal and protein-enriched milk	23 healthy subjects (11 F, mean age 24.3 ± 5.1 years)	Impact of a PKU type meal on appetite ratings, gut appetite hormones, thermic effect of feeding and fat oxidation	No detrimental impact on appetite and appetite hormones, and lower TEF and postprandial fat oxidation in consumption of a meal composed of special low protein foods than an ordinary meal.	Moderate

The intake of an adequate amount of protein substitutes, mainly in the form of Phe-free amino acid mixtures, is essential to ensure adequate growth, prevent deficiencies and provide a source of Tyr.

In Italy food for special medical purposes is reimbursed in full by the national health system on the indication of the accredited centers for rare genetic diseases. In Europe there are different approaches regulated by different Health Systems. Some countries such as Germany fully reimburse amino acid mixtures but not "low protein" products, others such as France impose a very limited list of reimbursable products, Great Britain adopts the same approach as Italy: total reimbursement on indication of accredited centers to diagnosis and therapy.

In patients with classic PKU, protein substitutes should represent at least 75% of the daily nitrogen requirement. Recent guidelines, moreover, suggest that total protein intake in PKU should exceed by almost 40% the FAO/WHO/UNU recommendations, in order to compensate given the lower bioavailability of employed Phe-free amino acids mixtures. The need for protein substitutes in obese patients, moreover, should be calculated based on ideal body weight; ideal body weight is considered the BMI equal to 25. The Phe-free amino acids substitutes should be assumed in 3–4 daily assumptions together with natural proteins and a source of carbohydrates [8].

Protein requirements in adults with PKU, however, have been generally extrapolated from estimates on the general healthy population through the nitrogen balance methodology. Recent evaluations by the more reliable Indicator Amino Acid Oxidation (IAAO) method have shown that protein requirements in adult are higher than previously published [24]. Consequently, the nitrogen balance method has been questioned, especially for elderly subjects, whose organism adapts to a low protein intake by consuming lean mass in order to maintain the nitrogen balance, and the current values of RDA (Recommended Daily Allowance, 0.8 g/kg body weight/day in the adult) may be inadequate to meet the metabolic demands of the adult to maintain lean mass and functional capacity [25]. However, since the anabolic response of muscle tissue to proteins introduced through the diet is altered in the elderly, a phenomenon known as anabolic resistance, a 25% increase in protein intake has been recommended in subjects aged> 50 years in order to limit the loss. Skeletal muscle mass; to date, however, only the Australian guidelines for PKU have implemented this recommendation [24].

In spite of the recent availability of new pharmacological treatments for selected PKU patients, nutritional intervention remains the cornerstone of therapy for PKU. It has been hypothesized that, by suitably modifying the absorption of free amino acids (aa) taken daily by patients, in particular by prolonging this absorption, it is possible to improve the nitrogen balance and reduce catabolic episodes, with benefits for growth in children and for maintenance. Muscle mass in adults.

The ideal protein source for PKU is "slowly absorbed", has no or very low Phe content and high of Tyr and large neutral amino acids (LNAA: tryptophan, threonine, isoleucine, leucine, valine, methionine, histidine) content, and a normal composition as regards the other amino acids. Glycomacropeptide (GMP), a 64 amino acid polar glycosylated peptide released by casein during the cheese making process, partially meets these characteristics, containing small amounts of Phe(2.0–5.0 mg Phe/g), and 2–3 times the amount of LNAA isoleucine, threonine and valine compared to other protein-based diet products [26,27].

For the formulation of GMP-based foods for special medical purposes, therefore, a highly purified molecule is required (containing <2.0 mg of Phe per g of protein), supplemented with arginine, histidine, leucine, tyrosine and tryptophan [28]. Unlike synthetic amino acids, GMP has, in fact, functional properties, such as good heat stability and solubility in an acid environment, which make it particularly suitable for use as an ingredient for food; GMP-based products (foods for special medical purposes such as beverages, puddings, puffed cereals, crackers, bars, salad dressings) could be easily integrated into a typical menu of a teenager with PKU, as they represent an improvement to how much it concerns flavor, convenience and variety of choices in dietary treatment [27].

GMP represents a dietary protein source with low Phe content that is more physiological than the commonly used synthetic amino acid mixtures (100% free aa), given that GMP-based foods mainly provide intact proteins (<25% added free amino acids). In fact, following the intake of intact proteins, greater protein synthesis and nitrogen retention are found compared to mixtures of single amino acids, and the consumption of GMP mimics the use of intact proteins thanks to a slower absorption and reduced degradation hepatic. A GMP-based protein substitution in PKU, moreover, was associated with lower postprandial plasma concentrations of urea nitrogen and higher insulin, and lower fasting phenylalaninemia levels than an amino acid-based diet; moreover, GMP seems to induce a marked sense of satiety in PKU patients, with a greater subjective feeling of fullness and a lower plasma concentration of ghrelin after meals [28].

As previously mentioned, patients with suboptimal adherence to nutritional therapy are at risk of developing deficiencies in terms of micronutrients, in particular those who do not take supplements based on amino acid mixtures (AAM), those who consume less than 0.5 g of protein/kg of body weight resulting from AAM and those who receive an overall protein supplement of less than 120% of the recommended values [29]. For years, protein substitutes for the nutritional therapy of people with PKU have usually been administered in the form of drinks or powders, typically characterized by an unpleasant taste and smell, often in association with other nutrients (vitamins and minerals); these characteristics, together with the large volume necessary to provide a sufficient amount of protein and the discomfort associated with their preparation, have always hindered good compliance by patients [30,31]. Recently, amino acid mixtures in tablets have been introduced, and a 2003 study investigated their effectiveness and acceptability compared to the classic powder and liquid formulations. In this randomized crossover study, 21 patients aged 8–25 years (mean age 15 years, 15 female subjects) took their usual source of protein substitutes for 12 weeks (mean daily amino acid dose of 66 g), and for another 12 weeks the amino acid blend in tablets as a source of at least 40% of their protein substitute requirement (each 1.3 g tablet contained 0.3 g of carbohydrates and 1 g of amino acids, with a tyrosine content of 66 mg/g of amino acids; average total daily dose of amino acids equal to 60 g). There was a better compliance with the new tablets (90% of subjects with complete adherence to the therapy vs. 65%), and the plasma levels of phenylalanine were lower and those of tyrosine higher than the use of the classic formulations, confirming the potential efficacy of these new formulations, which may represent valid alternatives in the treatment of PKU [30].

Another 24-week clinical study on 9 patients (8 females and 3 males, aged 10–32 years) evaluated an alternative product (Phlexy-10, SHS North America, Gaithersburg, MD, USA) available in three forms: pre-dispensed powder sachets to dissolve and consume as a beverage, fruit-flavored bars and capsules, with vitamins and minerals provided as a separate blend. One bar, one sachet and 20 capsules provided 10 g of amino acids (equivalent to 8.33 g of protein), with a smaller volume than traditional products, containing only amino acids. Compared to baseline, mean weekly blood phenylalanine levels were reduced by 40%, and blood concentrations of vitamins and minerals remained normal, with the exception of zinc (reduced in two subjects) and vitamin B12 (reduced in one patient) [31].

Again, with the aim of improving therapy compliance of patients with PKU, a randomized controlled study followed, for a period of 5 years, a total of 25 patients (age at the start of the study of 4–10 years). A new formulation of amino acids with improved palatability was administered: theoretically lower content of free amino acids in relation to energy (10 g of protein equivalent per 400 kcal/100 g of dry powder); 50% reduction in the content of amino acids containing sulfur (25 mg/g); elimination from the mixture of non-essential dicarboxylic amino acids aspartic acid and glutamic acid, also characterized by an unpleasant taste (composition of the experimental mixture: isoleucine 40 mg/g proteins, leucine 89 mg/g, lysine 91 mg/g, methionine plus cysteine 40 mg/g, phenylalanine plus tyrosine 93 mg/g, threonine 47 mg/g, tryptophan 15 mg/g, valine 67 mg/g; composition of the control mixture: isoleucine 54 mg/g protein, leucine 85 mg/g, lysine 93 mg/g, methionine plus cysteine 48 mg/g, phenylalanine plus tyrosine 47 mg/g, threonine 47 mg/g, tryptophan 14 mg/g, valine 62 mg/g). By means of specific intake registers compiled for four days, a reduction in the amino acid intake emerged in the experimental group from the beginning to the end of the study, with an acceptability of the new product, therefore, lower than expected.

However, serum proteins and minerals had not undergone significant changes in the two groups, and the levels of Phe following intake of the new product had increased to a lesser extent than that reported by other longitudinal studies on subjects treated with traditional products, confirming the safety and the efficacy of more flexible and palatable treatments [32]. The importance of improving compliance with dietary treatment based on the use of amino acid mixtures also emerged from the aforementioned study by Green et al., In which the reintroduction of a low-volume, nutrient-enriched protein substitute (nutritional information per serving, consisting of 33 g of product: energy 98 kcal; protein equivalent 20.0 g; carbohydrates 3.5 g; fats 0.3 g; DHA 100 mg; vitamins and minerals) after an average period of 4.5 years of poor adherence to the therapy, in fact, only nutritional benefits, but also a reduction in the levels of anxiety and confusion in participating patients [33].

In an attempt to find new alternative solutions for the treatment of adults with PKU, and to try to remedy the poor adherence to dietary therapy, a study on 12 patients aged between 19 and 38 years (5 females and 7 males), with sub-optimal control of phenylalaninemia, evaluated the effects of a new formulation based on LNAA on the blood levels of phenylalanine and tyrosine, often reduced in patients with PKU.

The subjects recruited took, for a period of 12 months, together with a diet low in Phe, a product in microgranules based on slow-release LNAA (containing all the essential amino acids in addition to arginine, aspartate, tyrosine, with sodium alginate as hydrophilic carrier), at a dose of 1 g/kg body weight three times a day (nutritional information of the LNAA mixture per 100 g: energy 399 kcal; fat 5.3 g, of which saturated fatty acids 5.3 g; carbohydrates 12 g, of which sugars 0 g; fiber 5.8 g; protein equivalent 70.79 g; salt 1.6 g; L-arginine 1.92 g; aspartate 4.95 g; L-phenylalanine 0 g; L-isoleucine 10 g; L-histidine 3.36 g; L-leucine 12 g; L-lysine 5.44 g; L-methionine 2.72 g; L-tyrosine 24 g; L-threonine 2.56 g; L-tryptophan 8 g; L-valine 10 g). This formulation alone provided 80% of the daily protein requirement, the remainder being taken through natural foods. Furthermore, coating the granules with a methylcellulose film limited the unpleasant taste that usually characterizes amino acid mixtures. Overall, adherence to this new therapy turned out to be very good, the blood levels of phenylalanine remained unchanged while those of tyrosine recorded a significant increase, with a consequent reduction in the Phe/Tyr ratio, and the quality of life, assessed by means of a specific questionnaire, was significantly improved in all participants [34,35]. As early as 1990 Berry et al. tested the effectiveness, in addition to a low phenylalanine formula, of a mixture of valine (150 mg/kg), isoleucine (150 mg/kg) and leucine (200 mg/kg) compared to a control mixture (245 mg/kg of arginine and 245 mg/kg of aspartic acid, which do not interfere with the transport of neutral amino acids), in a group of 16 patients aged between 10 and 23 years for four periods of three months each, showing a improvement of cognitive functions (of attention in particular), confirming the role played by LNAA in inhibiting the passage of phenylalanine through the blood–brain barrier and in reducing its toxicity on the central nervous system (Berry et al., 1990). This effect on cognitive functions was further confirmed by a recent Italian study on 10 adult patients (6 females and 4 males, mean age 23.6 ± 4.5 years, range 18–32 years), who were administered 0.8–1 g/kg/day, divided into 3 daily doses with main meals, of an LNAA-based product as the only supplementation of amino acids and vitamins (nutritional information per 100 g of product: energy 365 kcal; fat 1 g, of which saturated 0.7 g; carbohydrates 12 g, of which sugars 12 g; fibers 5 g; L-tyrosine 12.4 g, L-leucine 6.46 g, L-lysine 3.76 g, L-glutamine 2.32 g, L-proline 2.28 g, L-valine 2.2 g, L-isoleucine 2.2 g, L-tryptophan 2.2 g, L-threonine 2.08 g, L-arginine 1.84 g, aspartate 1.56 g, L-histidine 0.8 g, L-methionine 0.64 g; vitamins and minerals). After 12 months of such therapy, psychometric tests showed an improvement in levels of distress and well-being, and in executive functions, attention and alertness [36].

In a 2006 multicenter study involving Russia, Ukraine and USA, on the use of a formulation of LNAA (composition in amino acids: tyrosine 195 mg, tryptophan 51 mg, methionine 32 mg, isoleucine 35 mg, threonine 32 mg, valine 35 mg, leucine 130 mg, histidine 30 mg, lysine 30 mg, arginine 30 mg), 8 patients (mean age: 20.5 years) diagnosed with PKU had taken 0.5 g/kg/day (divided into three doses before meals) and 3 patients (mean age: 16.5 years) 1.0 g/kg/day of this product for one week. At the end of the study, the high levels of phenylalaninemia had been reduced by 50% in both groups, confirming the efficacy, at least in the short term, of LNAA in the treatment of PKU. In fact, the transport of neutral long-chain amino acids does not occur only at the blood–brain barrier level, but also at the blood-intestine barrier [37].

Over the years, different endpoints have been evaluated in groups of PKU patients treated with various amino acid mixtures. In a study on 20 subjects with a late diagnosis of PKU, in order to investigate the effect of different therapies on oxidative stress, a group treated with a phenylalanine-free amino acid mixture containing also vitamins, minerals and trace elements were compared with each other (information nutritional per 100 ml: 75 kcal; fats 0.9 g, of which saturated 0.2 g; carbohydrates 5.1 g, of which sugars 3.4 g; protein equivalent 11.5 g; salt 0.15 g; L-phenylalanine 0 g; L-alanine 0.53 g; L -arginine 0.86 g; L-aspartic acid 1.36 g; L-cysteine 35 g; glycine 1.35 g; L-histidine 0.53 g; L-isoleucine 0.93 g; L-leucine 1.46 g; L-lysine 0.96 g; L-methionine 0.26 g; L-proline 0.97 g; L-serine 0.60 g; L-threonine 0.93 g; L-tryptophan 0.29 g; L-tyrosine 1.37 g; L-valine 1.07 g; L-carnitine 13 mg; taurine 25 mg; or nutritional information for 100 g of neutral taste powder: 326 kcal; proteins 72 g; lipids 0.2 g, of which saturated 0.05 g; carbohydrates 9 g, of which sugars 0.82 g; fibers 0.8 g; gr group I, mean age 13.8 ± 2.8 years, range 10–19 years; 10 patients including 4 females), one group treated with an LNAA supplement (nutritional information per 100 g of powder: 315 kcal; fat 1 g, of which saturates 1 g; carbohydrates 22 g, of which sugars 18 g; fiber 4 g; amino acids 73.5 g, including L-tyrosine, L-tryptophan, L-methionine, L-threonine, L-isoleucine, L-valine, L-leucine, L-histidine, L-lysine, L-arginine; 60 equivalent proteins g; salt 0.1 g; biotin, vitamin B6, vitamin B12, folic acid; group II, mean age 14.8 ± 3.8, range 8–21 years; 10 patients including 5 females) and a control group (mean age 13.6 ± 4.8, range 8–21 years; 10 subjects including 5 females).

The study participants also followed a diet containing 200–1000 mg/day of Phe and 1.0–1.5 g/kg/day of protein or 1 g/kg/day (ideal body weight).

Glutathione peroxidase was reduced in patients taking the LNAA product compared to controls, while coenzyme Q10 was lower in those taking amino acid mixtures and significantly higher in group II than in group I, suggesting an influence of different treatments on these oxidative stress parameters and the opportunity to intervene with specific antioxidant adjuvant therapies for each patient [38].

Finally, a randomized controlled crossover study compared the effects on the variability of phenylalaninemia over 24 h of using casein-derived glycomacropeptide for 14 days with those of a Phe-free amino acid blend in a group of 18 children (of 7 of which males, average age 10 years). The use of glycomacropeptide as the only protein substitute was associated with less variability in blood Phe values, however the residual Phe contained in it was responsible for an increase in plasma concentrations of this amino acid [39].

Another randomized cross-over study evaluated the effects of different types of foods used in the dietary therapy of PKU on appetite, diet-induced thermogenesis (thermic effect of feeding, TEF) and fat oxidation in a group of 23 subjects. Healthy (including 11 women, mean age 24.3 ± 5.1 years). Eating meals consisting of low protein special medical purpose foods (including a drink; a cheese sandwich made with 2 slices of protein-free bread, 60 g, and 2.5 slices of cheese as a phenylalanine-free special medical purpose food, 50 g; protein-free mini crackers, 20 g; protein-free chocolate biscuits, 10 g) did not affect appetite or related hormones (GLP-1 and PYY), but resulted in thermogenesis and post-prandial fat oxidation lower than the consumption of a normal meal, an effect that could at least partially explain the increased prevalence of obesity in PKU patients on diet [40].

As for vegetable proteins, legumes are an important source of these proteins, but they also contain a share of carbohydrates and fiber. For the protein and Phe content, as reported in the Weetch study, legumes are foods to avoid in the low Phe diet [41].

The studies carried out so far suggest that there are valid alternatives to traditional powdered amino acid mixtures with large volumes, such as capsules, beverages or foods for special medical purposes substitutes for protein foods (with at least 70 g of protein/100 g of product), whose development and dissemination should therefore be promoted and encouraged.

2.2. Slow-release amino acids

This research was carried out based on the keywords: “phenylketonuria therapy” OR “PKU therapy”, AND “slow-release amino acid formulation” OR “slow-release large neutral amino acid”. 3 articles were sourced: 2 longitudinal studies and 1 narrative review (Table 2).

Since PKU is due to a defect in the enzyme Phenylalanine hydroxylase (PAH), responsible for the conversion of phenylalanine (Phe) into tyrosine (Tyr), in patients with PKU on a therapeutic regimen with a Phe-free diet, it is very important to ensure correct levels of tyrosine. For this, traditional Phe-free artificial amino acid formulas are generally enriched with Tyr [42]. Currently, data regarding the omeostasis of Tyr in patients with PKU are lacking.

The study by Porta et al. published in 2020, investigated the trend of tyrosine blood values in patients with PHA deficiency and the effect that a slow-release amino acid formulation therapy could have in PKU (Tyr content of 7.8 mg/100 g and absorption times similar to those of natural proteins vs Tyr content of 5.6 ± 1.5 mg/100 g of traditional formulas). The first investigation carried out was to monitor the levels of Tyr in the blood in patients treated with traditional aa formulas (n = 52) and in non-PKU patients with hyperphenylalaninemia on a free diet (n = 62); in addition, diurnal (trend of values in the day, n = 5) and absolute (n = 13) Tyr concentrations were studied in patients with PKU switched from traditional formulas to slow-release amino acid therapy. The results showed that Tyr values in PKU patients were subnormal and significantly lower than in non-PKU patients with hyperphenylalaninemia. The metabolic profile during the day in patients on slow-release amino acid therapy showed higher morning fasting and nocturnal Tyr concentrations than with traditional therapy. Finally, this picture was confirmed at follow-up, with normalization of morning fasting Tyr concentrations in patients on slow-release amino acid therapy and unchanged Phe controls. The authors conclude by stating that therapy with slow-release and therefore continuous amino acids improves the homeostasis of tyrosine in PKU compared to pulsatile absorption [9].

The study of Burlina et al. (2020) investigated the adherence of 12 adult patients affected by PKU (mean age 29,6 ± 6,8) with a persistent low-adherence to the Phe-restricted diet to a marked formulation (Neutrafenil Micro R®, PIAM Farmaceutici, Italy) of slow-release large neutral amino acid (LNAAs), in substitution of amino acids mixture (AAMs) along with Phe-restricted diet. The authors also investigated their quality of life with the phenylketonuria-specific phenylketonuria – quality of life (PKU-QoL) questionnaire. The study lasted 12 months and the patients were treated with 45,7 ± 9,8 g/day (mean ± SD) from the LNAA formulation. The supplement (dose 1 g/kg body weight, calculated every six months) was taken 3 times daily (breakfast, lunch and dinner time). No changes were made to their special low protein food (SLPF) supplementation. Due to the lack of vitamins and micronutrients in this formula, these supplements were given according to the European PKU guidelines. Subjective treatment adherence was assessed with the four-item Morisky Green Levin Medication Adherence Scale (MGLS). The authors also added three questions concerning the frequency of non-adherence as a report of subjective perception by the patient. After the 12-months study period, Tyr levels increased significantly in 11 out of 12 patients (92%) (mean 75 ± 16 μ mol/L; p = 0,0195). The mean Phe/Tyr ratio decreased significantly in 10 out of 12 patients (83%) (mean 12 ± 3 μ mol/L; p < 0,05). In two patients, such ratio showed a small increase (mean 17 ± 5 μ mol/L; p < 0,16). Neither weight nor BMI were significantly different when compared before to and after the introduction of LNAAs (p = 0,57 and p = 0,95 respectively). All patients reported a high level of medication adherence (mean of 96% reporting a full adherence). In concern to QoL, the analysis showed positive results, with significant difference (p < 0,05) in the domains of “your health”, “your PKU diet and supplements”, “your daily life with PKU”. The authors concluded that medication adherence should be assessed very carefully in PKU patients and LNAAs may give patients a further opportunity to improve medication adherence and QoL [35].

In a recent review by Daly et al. published in 2021, the authors traced the historical evolution of protein substitutes developed for patients with PKU. The review highlighted how the efficacy of protein substitutes in PKU is determined by various factors, including: nutritional profile of the protein substitute, its amino acid composition, dosage, and adequate energy intake. Finally, the authors conclude by emphasizing how important the use of protein substitutes is when managing PKU. Indeed, their application became pivotal thanks to their pharmacological properties and demonstrated clinical benefits [43].

2.3. Glycomacropeptide (GMP)

This research was carried out based on the keywords: “phenylketonuria” OR “PKU”, AND “glycomacropeptide” OR “GMP”. 7 articles were sourced: 1 prospective, self-controlled, clinical trial,

Table 2
Slow-release amino acids.

Author, year	Type of study	Study period	Methods	Subjects	End point	Results	Conclusion	Strength of evidence
Porta et al. (2020)	Longitudinal study	Duration of the tests was 24 h after Tyr administration	Tyr concentrations were monitored in 114 PKU patients on treatment with traditional amino acid formulations (n = 52) or non-PKU HPA on a free diet (n = 62); diurnal and long-term Tyr concentrations were evaluated in 5 and 13 patients with PKU, respectively, who switched from traditional to slow-release amino acids therapy. Duration of the tests was 24 h after Tyr administration; a normocaloric nonprotein diet was administered during the test to avoid additional Phe and Tyr intake; blood Tyr concentration was measured at 0, 1, 3, 6, 12, and 24 h after the oral loading.	114 patients (10.6 ± 11.9 years) with PKU on dietary treatment supplemented with traditional amino acid formulations (n = 52, 1175 samples) or non-PKU HPA on a free diet (n = 62, 430 samples)	To investigate Tyr homeostasis in patients with PAH deficiency and the effect of a slow-release amino acids therapy in PKU.	Tyr concentrations in PKU patients were subnormal and significantly lower than in non-PKU HPA ($p < 0.01$); the diurnal metabolic profile in patients on slow-release amino acids therapy revealed higher morning fasting and nocturnal Tyr concentrations then those with the traditional one ($p < 0.01$).	Slow-release amino acids therapy can improve Tyr homeostasis in PKU. If associated to optimized Phe control, may allow long-term clinical benefits in PKU patients.	Moderate
Burlina et al. (2020)	Longitudinal study	12 months	12 patients enrolled in a 12-month-trial of 1 g/kg/day slow-release LNAAs (Neutrafenil Micro R®, PIAM Farmaceutici, Italy, characterized by the incorporation of the amino acids in coated microgranules for prolonged release) plus a Phe-restricted diet. Over the 12 months of LNAA period the mean protein intake was unchanged for natural protein and 45,7 ± 9,8 g/day (mean ± SD) from the LNAA formulation. The supplement (dose 1 g/kg body weight, calculated every six months) was taken 3 times daily (breakfast, lunch and dinner time). Medication adherence was measured with the Morisky Green Levine Medication Adherence Scale; the QoL was measured using the phenylketonuria-quality of life (PKU-QoL) questionnaire. Phe, tyrosine (Tyr) levels, and Phe/Tyr ratios were measured fortnightly.	12 adult PKU patients (mean: 29,6 ± 6,8 y − 7 males and 5 females)	To evaluate adherence to therapy and quality of life (QoL) in a cohort of sub-optimally controlled adult PKU patients treated with a new LNAA formulation.	Before treatment, 3/12 patients self-reported a 'medium' adherence to medication and 9/12 reported a low adherence; 60% of patients reported a full adherence over the past four weeks. After 12 months of LNAA treatment, all patients self-reported a high adherence to medication, with 96% reporting a full adherence. Phe levels remained unchanged, while Tyr levels increased in most patients. The Phy/Tyr ratio decreased. All patients had a significant improvement in the QoL.	LNAAs may give patients a further opportunity to improve medication adherence and, consequently their QoL.	Moderate

2 randomized controlled cross-over studies, 1 retrospective study, 1 longitudinal prospective study, 1 systematic review with meta-analysis and 1 narrative review (Table 3).

An ideal protein source for PKU should be a slow-absorbing natural protein with no or very low levels of phenylalanine (Phe), high levels of tyrosine (Tyr) and other neutral long-chain amino acids (LNAA: tryptophan, threonine, isoleucine, leucine, valine, methionine, histidine), and a normal composition with regard to the other amino acids. No natural protein with these characteristics has been identified; however, Glycomacropeptide (GMP), a polar glycosylated peptide of 64 amino acids released by kappa-casein thanks to the action of chymosin during the cheese making process, has some, containing reduced amounts of aromatic amino acids (and therefore of Phe), and 2–3 times the amount of isoleucine, threonine and valine LNAAs compared to other protein-based dietary products. However, the commonly marketed product still contains small amounts of phenylalanine (2.0–5.0 mg Phe/g), while it does not contain the essential aa histidine and tryptophan, the semi-essential aa arginine and cysteine, and tyrosine, which should therefore be added as free amino acids to ensure their adequate daily intake [26,27,44].

For the formulation of GMP-based foods for special medical purposes, therefore, a highly purified molecule (containing <2.0 mg of Phe per g of protein) is required, supplemented with arginine, histidine, leucine, tyrosine and tryptophan [45]. Unlike synthetic amino acids, GMP has, in fact, functional properties, such as good heat stability and solubility in an acid environment, which make it particularly suitable for use as an ingredient for food; GMP-based products (foods for special medical purposes such as beverages, puddings, puffed cereals, crackers, bars, salad dressings) could easily be integrated into a typical menu of a teenager with PKU, as they represent an improvement as far as it concerns flavor, convenience and variety of choices in the context of dietary treatment [27].

From a 2014 review by Ney, it emerged that GMP represents a dietary protein source with low Phe content that is more physiological than the commonly used synthetic amino acid mixtures (100% free aa), given that GMP-based foods mainly provide intact proteins (<25% added free amino acids). In fact, following the intake of intact proteins, greater protein synthesis and nitrogen retention are found compared to mixtures of single amino acids, and the consumption of GMP mimics the use of intact proteins thanks to a slower absorption and reduced degradation hepatic. A GMP-based diet was also associated with lower postprandial plasma concentrations of urea nitrogen and higher insulin, and lower fasting phenylalanine levels than an amino acid-based diet; moreover, GMP seems to induce a marked sense of satiety in phenylketonuric patients, with a greater subjective feeling of fullness and a lower plasma concentration of ghrelin after meals [45].

In a randomized, controlled, crossover trial of 30 patients with early treated PKU aged 15–49 years, the efficacy and safety of a low-Phe diet in combination with edibles were compared. Special doctors based on traditional amino acid mixtures (amino acid medical foods, AA-MFs) with those of a similar diet but combined with glycomacropeptide-based foods (glycomacropeptide medical foods, GMP-MFs). The use of the latter was associated with a greater consumption of foods for special medical purposes, having been evaluated by patients as more acceptable than the mixtures of aa, with a reduction in gastrointestinal side effects (as it was demonstrated that the GMP acts as a prebiotic) and to a lower feeling of hunger, all with blood levels of Phe overlapping in the two groups [44,46]. A further recent three-year prospective longitudinal study by Daly et al., Starting from the observation that proteins, by increasing the release of gastrointestinal hormones and diet-induced thermogenesis, represent the macronutrient with the greatest satiating power, and that in phenylketonuric patients the intake of natural proteins is extremely reduced, with possible consequent alteration of the satiety mechanism, compared the use of amino acid formulas alone with that of GMP on satiety, body weight and BMI in 48 children with PKU (27 males and 21 females, ages 5–16). Adjusting the data for age and sex, however, no significant differences emerged in this sample regarding energy intake, weight, BMI and incidence of overweight/obesity, suggesting a correlation between GMP and satiety [47].

In 2018, a systematic review and meta-analysis by Pena et al., comprising respectively eight and two studies conducted between 2007 and 2018, did not reveal any difference between the use of amino acids alone and that of GMP added with essential aa for all outcomes considered (impact on the control of blood levels of Phe, changes in the control of tyrosine levels, nutritional biomarkers, acceptability and palatability of foods reported by patients). In particular, the meta-analysis conducted on the studies by Ahring et al. (8 patients, of which 7 females, aged 15–48 years) and by Ney et al. showed no significant differences in blood Phe and Tyr values, although a trend towards lower Phe and higher Tyr concentrations was observed in subjects treated with aa alone [46,48,49]. The authors concluded, therefore, that further better designed studies were necessary in order to provide more precise and reliable recommendations, given the scarcity of studies conducted up to that moment and their limitations (short duration, low sample size) [49].

GMP-based foods, unlike those consisting of free aa, improve the protein reserve, the use of Phe and, potentially, the long-term health of the bone; they also have an anti-inflammatory action on the intestine. GMPs can produce foods and beverages that provide 5–15 g of protein and only 15–25 mg of phenylalanine per serving, providing an alternative to amino acid blends for patients with PKU [44]. The study by Zaki and colleagues on 10 children with PKU (6 males and 4 females, aged between 4 and 16 years), who took their protein requirements exclusively in the form of amino acid formulas (for a period of 9 weeks) or by replacing 50% of the latter with GMP-based foods (for a further 9 weeks), he concluded that GMP can be used to provide 50% of the protein intake, allowing to improve nutritional power, palatability and therefore the acceptability of the diet, in the absence of toxicity or adverse effects reported [50]. Regarding the effects of diet on bone health, in the crossover study by Stroup et al. 8 subjects with PKU (aged 16–35 years) who were subjected to a reduced Phe content diet with the addition of amino acid mixtures or GMP-based foods were investigated for a period of 1–3 weeks. Intake of amino acid formulas was associated with 1.5–2.5 times greater renal acid potential load (PRAL) and 3 times greater net renal acid excretion compared to the use of GMP, while the latter significantly reduced urinary excretion. Calcium (40%) and magnesium (30%). Amino acid mixtures, therefore, seem to contribute to the onset of bone fragility in patients with PKU, an effect not observed with the use of GMP-based products [51].

Considering the lack of studies on the effects of long-term use of GMP, Pena and colleagues in 2021 developed a retrospective study to evaluate the impact of using GMP-based foods for an average period of 29 months in 11 patients with an initial mean age of 28 years (15–43 years). The results are encouraging since the use of GMP was not associated with any significant change in blood levels of Phe compared to baseline, while tyrosinemia was significantly increased [52].

2.4. Lipids

This research was conducted based on keywords: “phenylketonuria” OR “phenylalanine” OR “PKU” AND “omega 3” OR “lipids”

Table 3
Glycomacropeptide.

Author, year	Type of study	Study period	Supplementation	Subjects (age, sex, number …)	End point	Results	Strength of evidence
Nay D. et al., 2016	Randomized, controlled, crossover trial	Two periods of three weeks separated by a 3-week washout	Usual low-Phe diet combined with AA-MFs or GMP-MFs (formulation of glycomacropeptide comprising ~70% glycomacropeptide (cGMP-20) and 30% supplemental AAs	30 early-treated phenylketonuria subjects (18 F and 12 M, 15–49 y)	Efficacy and safety of a low-Phe diet combined with GMP-MFs or AA-MFs	Higher frequency of medical food intake with GMP-MFs than with AA-MFs. GMP-MFs rated by subjects as more acceptable than AA-MFs, with improved gastrointestinal symptoms and less hunger. No significant mean increase in plasma Phe despite a significant increase in Phe intake from GMP- MFs. Blood concentrations of Phe across time not significantly different.	High
Daly A. et al., 2020	Longitudinal prospective study	Three years	A protein substitute source: AA or two different amounts of CGMP-AA (CGMP-AA only, CGMP100, and a combination of CGMP-AA and AA, CGMP50)	48 children (21 F and 27 M), mean age at enrolment 9.3 years (5–16 y)	Effect of AA and two different amounts of CGMP-AA on satiety, weight and body mass index (BMI)	Similar macronutrient contribution to total energy intake from protein, carbohydrate and fat across the groups. Adjusting for age and gender, no differences in energy intake, weight, BMI, incidence of overweight or obesity between the groups.	Moderate
Zaki O. et al., 2016	Prospective, self-controlled, small-scale clinical trial	Two phases of nine weeks	Recommended daily allowances of protein in the form of amino acid formulae (AAF, phase I) or a combination of AAF (50%) and GMP (50%) (phase II)	10 patients (6 M and 4 F), 4–16 years (median age 6.73)	To evaluate the feasibility of use of GMP for partial replacement of artificial formula in treatment of children with PKU	Median and interquartiles of Phe levels not significantly different in phases I and II; no significant difference in Phe/Tyr ratio, amino acids, and other laboratory data between the two phases; no toxicity or side effects reported.	Moderate
Stroup B. et al., 2017	Two-stage, controlled, crossover intervention pilot study	1–3 weeks	Low-Phe diet in combination with AA-MF or GMP-MF	8 free-living participants with early treated PKU (16–35 y)	Impact of medical foods on skeletal fragility evaluated through dietary acid load, urinary excretion of renal net acid, calcium, and magnesium	1.5–2.5-fold higher potential renal acid load (PRAL) provided by AA-MF and 3-fold greater renal net acid excretion compared to GMP-MF; similar dietary protein, calcium, and magnesium intake; urinary excretion of calcium significantly reduced by 40% and of magnesium by 30% with GMP-MF; urinary calcium with AA-MF negatively correlated with L1–L4 BMD.	Moderate
Pena M. et al., 2021	Retrospective study	A mean period of 29 months	A low-Phe diet supplemented with L-AA and special low protein foods, with CGMP-AA providing 66% of protein equivalent intake from protein substitute	11 patients with a mean age at CGMP-AA onset of 28 years (range 15–43) (8 F-3 M)	Evaluation of metabolic control, anthropometry, body composition and biochemical parameters	No significant change in blood Phe with CGMP-AA compared with baseline; significantly increased blood Tyr on CGMP-AA.	Moderate

OR “PUFA”. 4 Articles were sourced: 1 observational study, 1 systematic literature review, 1 non-randomized controlled clinical study, 1 handbook (Table 4).

Within the food group of lipids we find many foods that are naturally very low in proteins and therefore can be eaten without having to be counted or measured. These include vegan cheese based on oils and starch (containing protein ≤0.5g/100 g or phenylalanine ≤25 mg/100 g), butter, margarine, vegetable oils [8].

Among these, however, butter and margarine contain more than 0.5g/100 g of protein but these are foods that are generally useable in small quantities and therefore should not cause concern in patients with phenylketonuria [53].

Regarding the intake of omega 3 fatty acids, a study showed that a fish-free diet does not induce early atherosclerotic changes and platelet activation in patients with PKU, this study conducted in 2015 involved 43 patients with PKU and 58 healthy controls. And prospectively examined the fatty acid profile, cimt (the thickening of the intima-media), the β stiffness index and platelet activation (flow cytometric determination of platelet activation markers). Despite the lower HDL cholesterol and higher triglyceride concentrations in the PKU patient group, there was no significant difference in the omega-6 or omega-3 fatty acid profile, CIMT, stiffness index β between both groups. Platelet activation was not improved in the PKU group. Surprisingly, the level of omega-3 fatty acids was no different in the two groups, implying that patients with PKU compensate for their lack of fish intake with vegetable fats rich in omega-3 fatty acids. There remains a need to conduct further interventional studies with omega-3 supplementation and to evaluate the long-term effect on atherosclerotic surrogate markers and platelet function in these patients [54].

A further double-blind, placebo-controlled clinical study analyzed 20 patients with classic PKU assigned to receive either LC-PUFA or placebo (olive oil) supplements for 12 months. The active capsules provided 26% fatty acids such as LC-PUFA and contained equivalent amounts of n-3 and n-6 fatty acids. Of the 20 children enrolled, 18 completed the study. Visual evoked potentials (VEPs) were measured. Initially, the latency times of the P100 wave before surgery were significantly prolonged in children with PKU compared to a healthy reference group. After the intervention period, P100 latencies were significantly improved in the group receiving LC-PUFA. The researchers then evaluated the effects of supplementing 38 PKU-affected children aged 1 year to 11 years for 3 months with encapsulated fish oil providing EPA (C20: 5n-3) and DHA (C22: 6n- 3) but no significant amount of n-6 LC-PUFA. At baseline, a clinical examination, routine blood tests (including phenylalanine concentration), and VEP were performed. PKU children were given fish oil capsules (Ameu; Omega-Pharma, Berlin, Germany) providing 15 mg of DHA/kg body weight per day. Each capsule contained 500 mg of fish oil (35% omega-3 fatty acids: 18% eicosapentaenoic, 12% DHA). The capsule shell (gelatin) contained 3 mg of phenylalanine. Otherwise, the dietary treatment remained unchanged. After 90 days, the clinical, laboratory and VEP tests were repeated. Plasma phenylalanine did not change in PKU children from baseline (266 ± 14 μmol/L) to the end of surgery (271 ± 21 μmol/L (not significant). Results were compared with 30 healthy control children. Aged 6.6 ± 0.5 years (not significant). Basically, absent dietary DHA intake in children with PKU is reflected in markedly reduced levels of DHA in plasma phospholipids and erythrocytes. In addition to the absence of dietary intake, a possible inhibitory effect of phenylalanine metabolites, in particular phenylpyruvate and phenylactate, on the endogenous synthesis of DHA has been discussed, but there is no firm evidence for this hypothesis. In children with PKU, blood levels of DHA are reduced to a greater extent than those of AA, compared to the values found in healthy omnivorous children. It is hypothesized that this lasting restriction in DHA supply could induce adverse effects on neural function in children with PKU.

These data indicate an increased speed of information processing in children with PKU after 3 months of supply of n-3 LC-PUFA. In contrast, there was no change in VEP latencies in healthy controls over 3 months. We also assessed body coordination and fine motor skills in patients and controls using Kurth's Rostock-Oseretzky (ROS) motor scale. The ROS test evaluates several specific motor functions, including static and dynamic balance, fine motor ability, and motor-rhythmic coordination. At baseline, children with PKU under good metabolic control showed significantly poorer motor skill performance on the ROS test than healthy controls. After 3 months of fish oil supplementation, PKU patients' ROS scores significantly improved and came much closer to healthy controls. It seems likely that a preformed DHA intake is generally required for optimal functional outcomes in child populations beyond infancy [55].

Given the scarce studies currently available, the advice is always to stick to a dietary scheme adhering to the WHO recommendations, i.e. consisting of an intake of lipids between 25 and 30% of the total calories of the diet, avoiding trans unsaturated fatty acids and containing acids. Saturated fat at less than 10% of the total calories of the diet.

There are no publications in the literature on the use of algae and derivatives as an protein-free source of omega 3, however there are publications on the rationale for the use of algae in the diet as a source of n3-PUFA and micronutrients, the study by Heitor Santos et al. demonstrated that to obtain about 2–3 g of total lipids from microalgae such as spirulina and chlorella it is necessary to ingest about 28 g of it in its powder form. The total lipid content can be practically zero in additional dosages (≈3 g/day). Chlorella minutissima UTEX 2219 and UTEX 2341 have 3.3% and 31.3% EPA of the total fatty acid content, respectively, but DHA was not detected in either strain. In an analysis of Spirulina platensis fatty acid profile from seven commercially available products, EPA and DHA were detected in just two samples, contributing 1.79% and 7.70% and 2.28% and 2.88%, respectively, of the total fatty acids. Spirulina and Chlorella contain not only macro and micronutrients, but also other compounds with antioxidant properties that can play a role in positive health outcomes [56]. Much more research is therefore needed in this field to characterize the biochemical content of microalgae to fully understand their benefits and possible concerns. Further studies will certainly be needed in the future on the use of algae and their derivatives in the diet of patients with phenylketonuria.

In conclusion, extra virgin olive oil, preferably raw, is the best choice as a condiment in PKU. Supplementation with omega 3, which cannot be found in nature from plant sources except algae, is recommended in patients with PKU.

2.5. Carbohydrates

This research was conducted based on the keywords: “phenylketonuria” OR “PKU” OR “phenylalanine” AND “Carbohydrates”. 2 articles were sourced: 1 systematic review and 1 multicenter cross-sectional observational study (Table 5).

Patients with PKU cannot consume traditional pasta, bread or flour but must stick to similar preparations belonging to the category of foods for special medical purposes. There are, pasta, rice, cous–cous, flours, bread, breakfast cereals, snacks, pizza bases, biscuits, bars, etc. Low protein foods that can be eaten without restrictions on a low phenylalanine diet include some starches such as cassava flour, arrowroot, sago, tapioca and corn starch which contain less than 0.5 g of protein/100 g (phenylalanine content ≤25 mg/100 g) [8].

Table 4
Lipids.

Author	Type of study	Study period	Methods	Subjects	End point	Results	Conclusion	Strength of evidence
Evans et al., 2020	This is not considered research as defined by the UK Policy Framework for Health and Social Care Research. Descriptive analysis	November 2015 to September 2019	112 consensus statements concerning the allocation of foods in a low phenylalanine diet for PKU were developed by the British Inherited Metabolic Disease Dietitians Group (BIMDG-DG) from 34 PKU treatment centres, utilising 10 rounds of Delphi consultation to gain a majority (≥75%) decision.	A mean of 29 UK dietitians (range: 18–40) and 18 treatment centres (range: 13–23) contributed in each round.	Overall dietary management is complex, and a high degree of patient/caregiver knowledge and application is required to effectively implement dietary care. Successful dietary treatment is hampered by inconsistent dietary advice associated with unclear and historical recommendations, lack of comprehensive food phenylalanine analysis, similar plant species containing a variable phenylalanine content per 100 g of food, and an overwhelming range of manufactured foods with unclear declarations of protein content. In the UK, national practical guidance about the implementation of dietary treatment in PKU has not been reviewed since 1993	The proposed guidance should be used in conjunction with individual tailored advice considering patient food likes, aversions, and level of understanding.	The development and publication of UK consensus statements for food labelling and protein allocation in the PKU diet is an important step in harmonising dietary advice and effecting consistency of care for patients with PKU.	Moderate
Htun et al., 2015	prospective observational study	–	In 43 PKU patients and in 58 healthy controls we prospectively examined the fatty acid profile, CIMT, β-stiffness index and platelet activation (flow cytometric determination of markers of platelet activation). CIMT was measured bilaterally by ultrasound. $CIMT_{mean}$ was defined as the mean value of the sum of $CIMT_{left}$ and $CIMT_{right}$.	43 PKU patients and 58 healthy controls	analyse the fatty acid profile of PKU patients and to correlate the results with surrogate markers of early atherosclerotic changes [enhanced carotid intima media thickness (CIMT) and β-stiffness index] and platelet activation.	The concentration of omega-6 fatty acids was 26.39 ± 1.29 amount % in the PKU group and 26.61 ± 1.08 amount % in the control group. Concerning the content of the omega-6 fatty acid chains 20:3 and 20:4 there was also no significant difference between both groups. The omega-3/omega-6 ratio was also not significant different between both groups (1:3.3 versus controls: 1:3.26; $p = 0.54$).	Despite of lower HDL-cholesterol and higher triglyceride concentrations in the PKU group, there was no significant difference in the omega-6 or omega-3 fatty acid profile, CIMT, β-stiffness index between both groups. Platelet activation was not enhanced in the PKU group.	Moderate
Koletzko et al., 2009	double-blind, placebo-controlled clinical trial	3 months	Supplemented children with PKU for 3 months with encapsulated fish oil providing a daily dose of 15 mg DHA/kg body weight.	36 children with PKU ages 1–11 years	the subtle functional deficits in early and well-treated patients with PKU cannot be fully explained by variations in plasma phenylalanine levels, it seems possible that metabolic imbalances induced by the strict diet, such as an absent DHA supply, might contribute to neurological abnormalities.	The score of PKU children improved in the subtests for fine motor skills, especially coin sorting ($P = 0.046$) and dynamic balance ($P = 0.004$).	DHA supplementation resulted in significantly faster visual evoked potential latencies, indicating more rapid central nervous system information processing. A supply of preformed n-3 LC-PUFA is required to achieve normal neural function in children with PKU.	High

Table 5
Carbohydrates.

Author, year	Type of study	Study period	Methods	Subjects	End point	Results	Conclusion	Strength of evidence
Couce et al., 2018	multicenter cross-sectional observational study	from February 2016 to April 2017	biochemical markers of basal and postprandial carbohydrate metabolism, Phe tolerance, waist circumference BMI, diet, tetrahydrobiopterin (BH4) supplementation, and adherence to treatment. Basal biomarkers and anthropometric parameters	83 patients (40.96% male; age range 4–52 years; 48.2% adults) were included in the study.	investigate biochemical markers of basal and postprandial carbohydrate metabolism in PKU patients	fasting insulin levels, with a mean of 12.74 ± 8.4 mlU/L, were altered in 15 PKU patients (26.3%) and markedly higher than in patients with MPHA ($p = 0.035$). The caloric intake in the form of carbohydrates was also higher in PKU than MHPA patients ($p = 0.038$) and it was correlated with basal insulin (rho = 0.468, $p = 0.006$), HOMA-IR (rho = 0.423, $p = 0.02$), BMI (rho 0.533, $p = 0.002$), and waist circumference (rho 0.584, $p = 0.0007$).	This study shows that PKU patients are at risk of carbohydrate intolerance and insulin resistance, more evident in adults and overweight patients, probably related to their higher caloric intake in form carbohydrate content. A higher dependency of AA mixtures was demonstrated in PKU patients.	Moderate

A 2018 cross-sectional observation study involved 83 pku patients, adults and children, in order to study biochemical markers of basal and postprandial carbohydrate metabolism in patients based on age, tolerance Phe, waist circumference, body mass index (BMI), diet, tetrahydrobiopterin (BH4) supplementation, and treatment adherence. These baseline biomarkers and anthropometric parameters were also evaluated in patients with mild hyperphenylalaninemia (MHPA) and in healthy controls. It has been shown that patients with PKU are at risk of glucose intolerance and insulin resistance, a situation more evident in adult patients and in overweight patients, probably this is due to their higher caloric intake in the form of carbohydrates, in fact carbohydrates represented 35.58% of the total energy content of the AA blends consumed by the patients who took part in the study [57].

Potatoes are an exception, in the study by Pimentel FB and colleagues boiled potatoes contained the highest levels of Phe (158.5 mg/100 g and 62.5 mg/100 g, respectively. Beets or parsnips have high Phe content and must be calculated in the daily dose of Phe [8,58].

In conclusion, naturally phenylalanine-free carbohydrates and specific protein-free analogue products, and potatoes (preferably boiled), are recommended.

2.6. Fruits and vegetables

This research was conducted based on keywords: “phenylketonuria” OR “PKU” OR “phenylalanine” AND “vegetables” OR “fruits”. 4 Articles were sourced: 1 narrative review, 1 open multicenter cross-sectional investigation, 1 experimental trial, 1 handbook (Table 6).

In the 2012 study by Rohde et al. 14 children (aged 2–10 years) were included in a cross-over study, the children were analyzed for two weeks while following conventional treatment (which takes into account the proteins provided by fruit and vegetables) and then they were analyzed over a period of another two weeks in which free consumption of fruit and vegetables was expected. Detailed daily dietary records were obtained during the study. Only vegetables with phenylalanine (phe) content above 75 mg per 100 g, such as peas, potatoes and dried fruit, had to be accounted for in the total amount of phe consumed.

The concentration of phe in the blood was monitored daily and it was found that it remained unchanged, thus demonstrating that the free consumption of fruits and vegetables has no negative effect on short-term metabolic control in the PKU patients involved in the study. According to the authors, further long-term multicenter studies are needed before full liberalization of fruit and vegetable intake can be recommended in the PKU diet [59].

Most fresh, frozen, dried, or canned fruit do not need to be counted on a low protein diet for PKU, as in normal quantities most fruit contains only a small amount of phenylalanine that can be retained. Negligible. The same goes for many types of vegetables which therefore do not need to be included in a low protein diet for PKU, if eaten in regular portions because they contain only a small amount of protein [60].

In conclusion, fruits and vegetables are allowed in the daily diet of these patients, in the quantity equal to 5 portions, without needing to be measured because they contain <75 mg of phe/100 g [8].

2.7. Fibers

This research was carried out based on the keywords: “phenylketonuria” OR “PKU”, AND “fiber”. Two articles were sourced: 1 prospective longitudinal study and 1 comparative study (Table 7).

Table 6
Fruit and vegetables.

Author, year	Type of study	Study period	Methods	Subjects	End point	Results	Conclusion	Strenght of evidence
Rohde et al., 2014	open multicenter cross-sectional investigation	3 days	From a 3-day diet record, protein supply as well as consumption of essential amino acids and several micronutrients were assessed and compared with the current recommendations and data for the healthy population. Statistical analyses	67 patients (6–45 years) with a phenylalanine tolerance⩾600 mg/day	To investigate micronutrient supply in phenylketonuria (PKU) patients on a relaxed diet.	Patients with a protein intake from AAM of at least 0.5 g per kg body weight (n = 34, mean 0.8 ± 0.2 g protein per kg) showed significantly higher intake of vitamin D (P < 0.001), calcium (P < 0.001), iron (P < 0.001), iodine (P < 0.001), folic acid (P < 0.001), vitamin B1 (P = 0.004) vitamin B2 (P < 0.001) andvitamin B12 (P < 0.001) than those patients with a protein intakeof less than 0.5 g from AAM per kg body weight (n = 20, mean0.3 ± 0.1). Moreover, the supply of all micronutrients exceeded therecommendations in those patients with at least 0.5 g protein perkg body weight from AAM: Vitamin C: 139% (P = 0.022), vitamin D:150% (Po0.001), vitamin B1:143% (P = 0.001), vitamin B2: 146% (P = 0.001), vitamin B12: 179% (P = 0.001), folic acid: 155% (P = 0.001),calcium: 151% (P = 0.001), iron: 182% (P < 0.001), zinc: 126% (P = 0.166), iodine: 130% (P < 0.001). Accordingly, the group with aprotein intake from AAM of less than 0.5 g protein per kg bodyweight did not reach the recommendations for some micronu-trients, especially folic acid (77%, P = 0.006) and iodine (79%,P ¼ 0.007).	Protein supply and consumption of all essential amino acids were sufficient in all patients. Supply of micronutrients depended on dietary regime. Patients with a total protein supply of 120% or more of the recommended amount and at least 0.5 g protein per kg body weight from amino-acid mixtures (AAM) were sufficiently supplied with all investigated micronutrients. All patients without AAM supplement showed severe micronutrient deficiencies in their diet records.	Moderate

Table 7
Fibers.

Author, year	Type of study	Study period	Supplementation	Subjects	End point	Results	Strength of evidence
Daly A. et al., 2020	Prospective longitudinal study	Three years	Special low protein foods (SLPFs)	48 children (27 M) with PKU (average age at enrolment: 9.3 years)	Contribution of the use of protein-free foods to the intake of macronutrients and fibers	Fibre intake = 83% of the Scientific Advisory Committee on Nutrition (SACN) reference value, with 50% coming from SLPFs with added gums and hydrocolloids.	Moderate
Schulz B. & Bremer H., 1995	Comparative study	Two food protocols (7 days and 4 days)	Phenylalanine-free amino acid mixture (AAM) vs. no supplementation	99 patients with PKU (12–29 years)	Food and nutrient intake	Low intake of fiber through the diet, equal to an average of 14 g/day (range 8–35).	Moderate

As regards the fibers intake, there are not many data in the literature. A recent three-year prospective longitudinal study, conducted on 48 children (including 27 males) with PKU, with an average age at enrollment of 9.3 years, investigated the contribution of the use of protein-free foods to the intake of macronutrients. And fibers, through the compilation of semi-quantitative dietary assessments and food frequency questionnaires (FFQ). It was found that these foods provide approximately 50% of the average daily fiber intake (provided more by low-protein bread and pasta than potatoes, vegetables and fruit), and that the overall average daily intake of fiber in these children reaches 82–83% of recommended levels (equal to 15 g/day for 2–5 years old, 20 g/day for 5–11 years old, 25 g/day for 11–16 years old and 30 g/day for adolescents aged 16–18 and for adults) [61,62]. However, it must be considered that the main source of fiber present in protein-free bread and pasta is represented by hydrocolloids, commonly used food additives whose effect on intestinal health is poorly known, while the intake of low-Phe fruit and vegetables, which can be taken ad libitum and represent an important source of fiber, in the children in the study was less than the 5 servings/day recommended by the WHO (World Health Organization) (Daly et al., 2020). Already in 1995, a comparative study on 99 patients with PKU, aged between 12 and 29 years, had shown, through the administration of food questionnaires, a low intake of fiber through the diet, equal to an average of 14 g/day (range 8–35) [63]. The intake of vegetables containing ≤75 mg of Phe/100 g should therefore be encouraged, also considering that the consumption of whole grains and fibers, associated with a reduction in the risk of cardio-metabolic diseases and colorectal cancer, is precluded in PKU patients [61].

2.8. Vitamins and minerals

2.8.1. Blood values

This research was conducted based on keywords: “phenylketonuria” OR “PKU” OR “phenylalanine” AND “micronutrients” AND “blood values”. 6 Articles were sourced: 1 cross-sectional observational study, 1 cross-sectional study with retrospective data, 1 retrospective, observational, single center study, 1 single-center case–control study, 1 systematic literature review, 1 literature review of papers (Table 8).

A total of 112 patients (mean age 136.8 ± 82.1 months, range 18–377 months) with phenylketonuria and 36 healthy controls who had not used vitamin or mineral supplements were enrolled in the study conducted by Kose E. et al. in the last 6 months, the 112 patients in turn were divided into two groups based on their adherence to the diet according to the annual plasma Phe value (high dietary adherence was defined as mean annual plasma Phe level if lower 360 μmol/L for patients less than 6 years of age; <480 μmol/L for children between 6 and 10 years; ≤600 μmol/L for older patients).

The PKU patients involved in the study were taking the Phe-free amino acid formulas: Anamix Infant, PKU 2-first, 2-secunda, 3, PKU Lophlex LQ 20 (Nutricia Metabolics), PKU Cooler 10, 15 (Nestlé Health Science) and Comida PKU A, B, C (Comidamed). Analyzes were performed on blood samples to evaluate the following parameters: hemoglobin, vitamin B12, folic acid, iron, ferritin, transferrin saturation, copper, prealbumin, albumin, total protein, phosphorus, calcium, 25-hydroxy vitamin D, zinc, vitamin A and vitamin E levels.

In the analysis of the laboratory results of the PKU patients and the healthy control group, the median serum vitamin B12 level of the PKU patients (396 pg/mL) was higher than the healthy control group (260 pg/mL) ($p = 0.002$). Vitamin B12 deficiency was 15.2% and 30.6% in PKU patients and healthy controls, respectively ($p = 0.040$). The mean serum folic acid level was higher in PKU patients than in the control group ($p < 0.0001$). Folic acid deficiency was not detected in either group. Median serum ferritin and mean serum prealbumin concentrations were increased in patients with PKU compared with the control group (25.1 vs 13.4 ng/mL, $p = 0.007$; 24.1 ± 4.6 vs 21.9 ± 3.9 mg/dL, $p = 0.013$, respectively). The frequency of ferritin and prealbumin levels above the reference range was higher in PKU patients than in the control group. Mean serum phosphorus levels were lower in PKU patients than in the control group. In contrast, the median plasma level of vitamin A was higher in PKU patients than in the control group (55.0 vs 45.7 μg/dL, respectively, $p = 0.014$). There was no vitamin A or vitamin E deficiency in either group. Vitamin D 25-hydroxy deficiency was detected in 53.6% and 47.2% of patients with PKU and in the control group, respectively.

In evaluating laboratory findings, the mean plasma phenylalanine level was 943.2 ± 314.0 and 369.7 ± 102.8 μmol/L in the low diet adherence (LAD) and high diet adherence groups (HAD), respectively ($p < 0.0001$). The mean serum level of vitamin B12 was statistically higher in the HAD group than in the LAD group. The mean serum copper level was also higher in the HAD group than in the LAD. Although the frequency of copper deficiency was higher in LAD (7.0%) than in HAD (2.4%), this difference was not statistically significant. The serum prealbumin level was lower in the HAD group (22.5 ± 4.4 mg/dL) than in the LAD group (24.9 ± 4.6 mg/dL) ($p = 0.011$). Compared to the LAD group, higher serum phosphorus levels were observed in HAD. However, the median plasma levels of vitamin E did not differ between the LAD and HAD groups, and the frequency of plasma levels of vitamin E above the reference range was higher in the HAD than in the LAD group (21.9% vs 8.5%, $p = 0.030$). While mean serum folic acid and 25-hydroxy vitamin D levels were higher in the HAD group than in LAD, these differences were not statistically significant.

The study found that the Phe-free amino acid formulas used by these patients provide more folic acid, vitamin B12, copper and vitamin E than required by PKU patients, while the doses of vitamin A and zinc were found to be adequate for needs of PKU patients. A limitation of this study, however, is that it did not evaluate the dietary intake of vitamins and micronutrients in patients with PKU, which may have led to the overestimation of the effect of Phe-free amino acid formulas on the nutritional status of patients with phenylketonuria.

The results of this study also show that vitamin D deficiency is also frequent in patients with PKU compared to what has emerged in other studies, although this finding could be influenced by regional vitamin D deficiency caused by reduced sun exposure, typical in north of Spain. Kose's study shows how the nutritional status of PKU patients varies widely between regions and countries, and biochemical monitoring of these patients is important for identifying vitamin and mineral deficiencies [64].

In a further cross-sectional observational study by V. Crujeiras and colleagues, anthropometric and biochemical data were collected from 156 Spanish children and adolescents suffering from phenylketonuria and subjected to a naturally Phe-free diet, according to Spanish guidelines, with supplementation of Phe-free amino acids., it emerged that the analyzed prealbumin levels were decreased in 34.6% of patients, but the total protein level was in the physiological range, this data confirms that plasma Pre albumin is a more sensitive marker for monitoring the state of malnutrition compared to total protein. In addition, mean ferritin values were lower (41.4 mg/dL) in these patients than in patients with pre-albumin levels within the normal range (46.3 mg/dL). This result supports that a low plasma level of Pre albumin correlates significantly with impaired hematopoiesis [65].

In the retrospective cross-sectional study by Almeida et al., 84 children and adolescents affected by phenylketonuria were

Table 8
Blood values.

Author, year	Type of study	Study period	Methods	Subjects	End point	Results	Conclusion	Strength of evidence
Crujeiras et al., 2015	cross-sectional observational study	February to December 2014	Kolmogorov−Smirnov and the Shapiro−Wilk tests, Student t-test, Benjamini−Hochberg correction	156 patients (46.8% male; range age: 7 months−42 years old; 27.4% > 18 years)	Determine the vitamins and minerals status in PKU patients according to the Phe tolerance, age, diet, BH4 supplementation and adherence to diet and analyz potential risk factors involved in its deficiency or over supplementation.	Prealbumin was reduced in 34.6% of patients (74% with PKU phenotype and 94% below 18 years old), showing almost all (96.3%) an adequate adherence to diet. Selenium was diminished in 25% of patients (95% with PKU phenotype) and also 25-OHD in 14%. Surprisingly, folic acid levels were increased in 39% of patients, 66% with classic PKU.P and B_{12} were found significantly reduced in the patients with low adherence to diet (P: 3.3. vs 4.5 mg/dL, p: $1.27e^{-5}$; B_{12}: 628 vs 679 pg/mL, p: 0.03).	In this study 81.4% of patients presented biochemical markers out of recommended range but only Palb and Se have been found significantly affected because they were diminished in 34% and 25% respectively. High percentage of PKU patients with good adherence to diet with Palb deficiency and TP normal. Also a high percentage of PKU patients withSe deficiency as well as folic acid increase, should lead us to consider adjusting this micronutrient in the international standards supplements formulated milks. On the contrary, only 2.56% patients under BH4 treatment showed Se deficiency.	Moderate
de Almeida et al., 2020	cross-sectional study with retrospective data	–	anthropometric and biochemical data collection from patients with phenylketonuria in the age group 2−19.9 years. Nutritional status was classified according to the World Health Organization. Biochemical tests were compared to current recommendations.	84 patients (71.8%) median age of 10.7 years (2.4−19.9 years)	evaluate the anthropometric and biochemical characteristics of children and adolescents with this condition	hyperphosphatemia in 46 (55%), hypertriglyceridemia in 27 (50%), vitamin B12 elevated in 34 (41.2%), selenium deficiency in 10 (13.7%), insufficient zinc in 7 (8.9%), low globulin in 21 (26.9%), low HDL in 35 (59.3%) and elevated phenylalanine level in 28 (34.5%) patients in the sample	Most patients presented adequate according to anthropometric parameters and appropriate biochemical tests, except HDL, and moderate metabolic control of the disease. However, attention should be paid to the presence of overweight and need for biochemical monitoring of triglycerides, selenium, zinc, HDL, and phenylalanine.	Moderate
Kanufre et al., 2021	retrospective, observational, single centre study	12 month	three main treatment types used were defined in analysis: **PKU diet only**: Phe restricted diet supplemented with PS and SLPFs **BH4 + diet:**BH4 treated patients with Phe restriction and ±PS	87 patients (48% females) with a median age of 18 y (range from age 1 to 36 y). Nineteen patients were <12 y (median age of 8 y; range 1−11 y) and 68 patients ≥12 y (median age 22 y; range 12 −36 y). Of the 68 patients ≥12 y, 36	describe the metabolic control of patients with PKU in a single Portuguese centre comparing three different recommendations (European guidelines, US guidelines and Portuguese consensus)	the median blood Phe level was 300 μmol/L (range 168 −480); blood Tyr was 71 μmol/L (range 43−96). In patients aged ≥12 years, the median blood Phe level was 474 μmol/L (range 156 −1194) and Tyr was 67 μmol/L (range 40−94)	blood Phe levels were around 56% within therapeutic target according to the Portuguese consensus although there is a tendency for increasing median blood Phe levels with age. The number of blood Phe levels within target range according to the European guidelines	Moderate

(*continued on next page*)

Table 8 (*continued*)

Author, year	Type of study	Study period	Methods	Subjects	End point	Results	Conclusion	Strength of evidence
			Non-restricted diet:without PS or BH4 prescription	patients (53%) were >20 y.			and US guidelines blood Phe levels were around 83% and 26%, respectively. In consideration of the different Phe upper limits recommended, we must strive for safe levels that are associated with the best patient outcomes. There should be focus on improving alternative treatment options and clinical resources to enable patients to achieve lower blood Phe levels. More studies are needed comparing outcome of centres using different blood Phe targets, their frequency of monitoring and the resources that they have available to them to determine optimal blood Phe control.	
Kose, Arslan. 2019	single-center, case −control study	−	Biochemical and hematological markers were screened from fasting morning blood samples. Statical analysis	112 age- and sex-matched patients with PKU (53 females 59 males) and 36 controls (18 females, 18 males)	assess the nutritional parameters of patients with PKU on strict low-phenylalanine diet without vitamin and mineral supplementation compared to a healthy control group. Secondary objective was to identify the adequacy of vitamin/mineral supplementation in phenylalanine-free (Phe-free) amino acid formulas.	Median serum vitamin B12 level of patients with PKU was found to be higher than the control group ($p = 0.002$). Vitamin B12 deficiency was 15.2% and 30.6% in patients with PKU and healthy controls, respectively ($p = 0.040$). Mean serum folic acid level was higher in patients with PKU than the control group ($p < 0.0001$). In 55.4% of patients with PKU, and 2.8% of the control group, serum folic acid level was above the reference range ($p < 0.0001$). 25-Hydroxy vitamin D deficiency was detected in 53.6% and 47.2% of patients with PKU and the control group, respectively. Mean serum copper level was higher in the well-controlled (114.3 ± 26.7 μg/dL) group than the poorly controlled group (101.0 ± 29.1 μg/dL) ($p = 0.022$).	Phe-free amino acid formulas provide adequate vitamin A and zinc levels in patients with PKU, and result in excess folic acid, vitamin B12, copper and vitamin E values that are higher than required levels. Our results demonstrate a high percentage of vitamin D deficiency in patients with classical PKU and also in healthy controls in Turkey.	Moderate

analyzed by means of anthropometric and serum measurements. Of these patients. Serum calcium, total cholesterol and fractions, triglycerides, ferritin, phosphorus, total protein and fractions, selenium, vitamin B12, 25-OH-vitamin D and zinc collected in the last 24 months of these patients were examined. The values found were compared with the current literature, according to the age group. Results of biochemical tests showed that most patients had adequate serum levels of proteins, vitamins and minerals, however with low HDL in 59.3% (n = 50) of patients and insufficient globulin values in 26,9% (n = 22). Values above the reference values on the contrary were observed for phosphorus (55%, n = 46), triglycerides in 50% (n = 27), vitamin B12 (41.2%, n = 34) and Phe (34, 5%, n = 28).

Other deficiencies found less frequently, however, concerned calcium (females 9.9 ± 0.6 mg/dL, males 9.8 ± 0.6 mg/dL p0.94), selenium (females 82.0 (3.1–147.0) mg/L and males 77.0 (7.2–160.0) mg/L p0.252, vitamin B12 (females 858.0 (179.0–1909.0) pg/ml and males 688.0 (216.0–2000) pg/ml p0.562) and vitamin D (females 31.9 (17.2–83.0) ng/ml males 38.9 (18.8–70.0) ng/ml p0.01), with no significant differences between the sexes. Among the patients considered there was also an important frequency of overweight and obesity. In females, there was a higher frequency of adequate in the BMI/A index (p = 0.02) and a higher risk of overweight in the W/H index (p = 0.04). On the other hand, males showed a higher frequency of wasting and stunting in relation to females, according to the BMI/A index. The H/A index showed low/very low height for age in 11.9% (n = 11) of the study population and was similar between groups (p > 0.05) a predominance (n = 35, 87.5%) of age-appropriate weight with no gender difference (p > 0.05) and very low/low weight-for-age in 4 (24.9%) of patients male [20].

The previously illustrated study by Viviane Kanufre and colleagues showed that the Phe levels in the blood of the patients they considered were within the therapeutic target in 56% of cases, although there was a trend towards an increase in median levels of Phe in the blood with increasing age. In view of the higher values of Phe recorded, according to the authors it is necessary to strive to bring patients within the safe levels of Phe associated with the best outcomes for patients. Focus should be on improving alternative treatment options and clinical resources to enable patients to achieve lower blood Phe levels [66].

2.8.2. Intake of minerals and vitamins

This research was conducted based on the keywords: “micronutrient intake” OR “nutrient intake” OR “mineral and vitamin intake” AND “phenylketonuria” OR “PKU diet”. 8 articles were sourced: 2 multicenter cross-sectional studies, 3 prospective cross-sectional studies, 1 single-center case–control study, 2 narrative reviews (Table 9).

In the mini-review by Robert et al. published in 2013 [67] patients with PKU were defined as an “at risk” group for nutritional imbalances. Obtaining an optimal nutritional state is very difficult, in fact, especially when a large portion of the nutritional intake comes from non-natural sources. Micronutrient supplementation is essential in patients with PKU following dietotherapy treatment and these should be added to amino acid preparations without phenylalanine or taken in addition. The literature from 1990 to the publication of this review highlighted mainly zinc, selenium, iron, vitamin B12 and folate among the deficient nutrients. In particular, the studies on vitamin B12 present in this review are 6 for a total of more than 226 subjects; studies on selenium are 15, for a total of 783 subjects; those on zinc and copper 7 (373 subjects); those on iron 6 (411 subjects).

To follow, a study published in 2014 by Rohde et al. investigated micronutrient supplementation in PKU patients who followed a free diet. A total of 67 patients were aged between 6 and 45 years and with a phenylalanine tolerance> 600 mg/day. Patients were asked to report a 3-day food diary including food, drink and AAM to calculate the intake of phenylalanine, energy, protein, carbohydrate, fat, essential amino acid, calcium, iron, zinc, iodine, vitamins B1, B2, B12, C, D and folic acid. The intakes of macro and micronutrients were then converted into a percentage and related to age, weight, gender, according to the guidelines of the German Society of Nutrition. Group 1 did not use an AAM while group 2 that used it was divided into 2 subgroups: the 2nd consumed> 120% of the recommended protein intake, the 2 b < 120%. The results showed that the protein and essential amino acid intake was sufficient in all patients, while that of the micronutrients depended on the dietary regime. Patients with a total protein intake, provided by the amino acid blend (AAM), of 120% or more of the recommended amount and at least 0.5 g of protein per kg of body weight achieved sufficient levels of all the micronutrients studied. Patients without AAM supplement (group 1) instead showed severe deficiencies in all the micronutrients studied, when compared with current recommendations, except for vitamin B12. Even in comparison with the healthy population, group 1 consumed less of all micronutrients. In group 2, the intake of most of the micronutrients did not comply with the recommended one: in particular, subgroup 2a was sufficiently covered, while group 2 b had an insufficient intake of most of the micronutrients. Comparing the two groups, the intake of all micronutrients is higher in group 2a, except for vitamin C and zinc. On the other hand, it was significant for vitamin D, calcium, iron, iodine, folic acid, vitamins B1, B2 and B12. The authors conclude that non-dietary PKU subjects are at risk of insufficient vitamin and mineral intake compared to those who adhere and consume an AAM [29].

In the following year, 2015, a narrative review by Al Hafid was published [68] in which the importance of defining the solution to nutritional deficits, especially concerning vitamin D and vitamin B12.

That the intake of low phenylalanine protein substitutes represents the main source of micronutrients needed to combat the risk of nutritional insufficiency was also shown in the study by Hochuli et al., Published in 2018 [69]. This prospective cross-sectional study evaluated the effects of reducing the intake of an amino acid mixtures (AAM) in adult patients with PKU. Through the accurate collection of the food anamnesis (quality and quantity of food, hydration and AAM intake in 4 days of diet) and thanks to laboratory tests on completion, the nutrient intake was assessed in 20 adult patients with PKU. The patients were divided into two groups: group A (n = 15) who took the prescribed amount of AAM and group B (n = 5) with a lower than recommended AAM intake. The daily amount of AAM was 2.6 servings per day (range 2.0–3.0) in group A vs 1.4 (range 0–2.0) in group B. The results showed that group B consumed a proportionally higher amount of protein. Natural, but that in any case the total intake of these was lower than the recommended protein intake in 60% of group B patients, while it was lower than the recommended protein intake in only 7% of group A subjects. Caloric content was not significantly different between the two groups (group A: 2167 kcal/day, group B: 2272 kcal/day). Finally, in group B the intake of fats (especially saturated) was higher, while that of calcium, selenium, folate and vitamin B12 was lower than in the group with regular AAM intake, but only selenium, folate and vitamin B12 were results clearly below the recommended values. Despite this, the blood values of these micronutrients remained within the normal range in both groups, with the exception of vitamin B12 levels which were significantly lower in group B, but still within normal limits. In conclusion, relaxed AAM intake resulted in insufficient nutrient supply, despite a compensatory increase in consumption of natural protein. Care needs to be taken to ensure adequate nutrition in adults with PKU.

Table 9
Vitamins and minerals intake.

Author, year	Type of study	Study period	Methods	Subjects	End point	Results	Conclusion	Strength of evidence
Rohde et al., 2014	Multicenter cross-sectional study	3-day diet record	3-day diet record to calculate the intake of phe, energy, protein, carbohydrate, fat, essential amino acids, calcium, iron, zinc, iodine, vitamins B1, B2, B12, C, D, and folic acid. Protein supply and consumption of essential amino acids and micronutrientis were assessed and compared with current recommendation and data for the healthy population	67 patients/6−45 years) with a phe tolerance >600 mg/day. Group 1 did not use an AAM whereas group 2 that did use an AAM was divided into 2 subgroups: 2a consumed >120% of the recommended protein intake, 2 b < 120%.	To investigate micronutrient supply in PKU patients on a relaxed diet	Protein supply and consumption of all essential amino acids were sufficient in all patients. Supply of micronutrients depended on dietary regime. Patients with a total protein supply of 120% or more of the recommended amount and at least 0.5 g protein per kg body weight from amino-acid mixture (AAM) were sufficiently supplied with all investigated micronutrients. All patients without AAM supplement showed severe micronutrient deficiencies in their diet records.	PKU patients under a relaxed diet are at risk of an insufficient nutrient supply, if they have first no substitution with AAM, second a protein supply less than 0.5 g per kg body weight from AAM or third a total protein supply less than 120% of the recommendations.	Moderate
Hochuli et al., 2018	prospective cross-sectional study	4-day diet record	Nutrient intake was calculated in 20 adult PKU patients based on a structured food record and complemented by laboratory assessment of nutritional status.	20 adult PKU patients (aged >18 ys). Group A (n = 15) regular AAM intake; Group B (n = 5) AAM intake below calculated requirements.	To evaluate effects of reduced amino acid mixture (AAM) intake on nutrient supply	Group B consumed a higher proportion of natural protein (60 ± 23 vs. 33 ± 12%, p = 0.002); the total protein intake was < the recommended amounts in 60% of patients in group B vs 7% in group A (p = 0.03). Fat intake was > in group B (39 ± 9% of energy vs. 31 ± 6%, p = 0.03), mainly from saturated fats. Selenium, folate, and vitamin B12 intake was < the recommended intake in group B. Vitamin B12 levels were < in group B.	Relaxed AAM intake resulted in insufficient nutrient supply, despite a compensatory increase in consumption of natural protein. Care needs to be taken to ensure adequate nutrition in adults with PKU.	Moderate
Kose & Arslan. 2019	single-center case −control study		Biochemical and hematological markers including hemoglobin, serum vitamin B12, folic acid, iron, ferritin, transferrin saturation, copper, prealbumin, albumin, total protein, phosphorus, calcium, 25-hydroxy vitamin D, zinc, vitamin A and vitamin E levels were screened from fasting morning blood samples.	One hundred and twelve patients with classical PKU (53 females, 47.3%) and 36 healthy controls (18 females, 50.0%), who did not take vitamin or mineral supplementation at least for the last 6 months. The mean age of patients with PKU was 136.8 ± 82.1 months (18−377).	to assess the nutritional parameters of patients with PKU on strict low-phe diet without vitamin and mineral supplementation compared to a healthy control group. Secondary objective was to identify the adequacy of vitamin/ mineral supplementation in Phe-free amino acid formulas.	Median serum vitamin B12 level of PKU patients was > than the control group (p = 0.002). Vitamin B12 deficiency was 15.2% in PKU patients and 30.6% in healthy controls (p = 0.040). Mean serum folic acid level was > in PKU patients than the control group (p < 0.0001). 25-OH vitamin D deficiency was detected in 53.6% of PKU patients and 47.2% of the control group. Mean serum copper level was > in the well-controlled (114.3 ± 26.7 μg/dL) group than the poorly controlled	Phe-free amino acid formulas provide adequate vitamin A and zinc levels in patients with PKU, and result in excess folic acid, vitamin B12, copper and vitamin E values that are higher than required levels. There is a high percentage of vitamin D deficiency in patients with classical PKU and also in healthy controls in Turkey.	Moderate

						group (101.0 ± 29.1 μg/dL) (p = 0.022).		
Green et al., 2019	Multicenter study	3 + 28 + 3 days	20 PKU adults took 33 g of a low-volume, nutrient-enriched, protein substitute daily for 28 days. Outcomes of eating behaviour, nutrient intake and mood were assessed at entry (baseline, days 1−3) and after the intervention period (days 29−31).	20 PKU adults (33.7 ± 2.6 years), noncompliant for 4.5 years (range: 1−11 years)	To find strategies that ease the return to the PKU diet, while offering nutritional and cognitive advantages, because noncompliance is widespread in adults with PKU and is associated with adverse metabolic, nutritional and cognitive abnormalities.	At baseline, intakes of natural protein and estimated phe were high (66.4 g and 3318.5 mg, respectively) and intakes of calcium, magnesium, iron, zinc, iodine and vitamin D were below country-specific recommendations. With use of the experimental protein substitute, natural protein, estimated phe intake ($p = 0.043$ for both), fat and saturated fat intakes declined ($p = 0.019$ and $p = 0.041$, respectively), while energy and carbohydrate intake remained unchanged. Micronutrient intake increased ($p \leq 0.05$ for all aforementioned, B_{12} and vitamin D increased by 19.8% and 10.4%) to levels well within reference recommendations.	This study demonstrates that reintroducing a low-volume, nutrient-enriched protein substitute delivers favourable nutritional and possible mood benefits in noncompliant PKU patients. This preliminary knowledge should be used in the design of new strategies to better facilitate patients' return to the PKU diet, with the approach described here as a foundation.	Moderate
Akış et al., 2020	cross-sectional study	Blood phe concentrations in the preceding 12 months were considered.	Serum vitamin B12 and folate concentrations were analysed by chemiluminescence immunoassay. Plasma methymalonic acid (MMA) and total homocysteine (Hcy) concentrations were measured by liquid chromatography-tandem mass spectrometry and liquid chromatography, respectively. cB12 was calculated by using a formula involving blood parameters.	53 children and adolescents (aged 5−18 years, median age of 9.5 years, 20 females and 33 males) with PKU under dietary treatment (initiated after diagnosis in the neonatal period) and 30 healthy controls (aged 5 −17.5 years, 16 females and 14 males). PKU diet was restricted in natural protein and supplemented with a Phe-free aa mixture enriched in vitamins and minerals.	to evaluate vitamin B12 status in PKU patients by using combined indicator of vitamin B12 status (cB12) as well as MMA and Hcy, in comparison with healthy controls.	MMA and folate concentrations in PKU group were higher compared with controls. MMA concentrations were high in 56.5% of the patients and 26.7% of the controls with normal vitamin B12 concentrations. Based on cB12, a significant difference within the normal values was detected between the groups. However, although 24.5% of PKU patients and 13.3% of controls had decreased vitamin B12 status according to cB12, there was no significant difference.	Children and adolescents with PKU having a strict diet can be at risk of functional vitamin B12 deficiency, that can be accurately determined by measuring MMA concentrations.	Moderate

(*continued on next page*)

Table 9 (*continued*)

Author, year	Type of study	Study period	Methods	Subjects	End point	Results	Conclusion	Strength of evidence
Mozrzymas et al., 2020)	cross-sectional study	Blood phe concentrations in the preceding 12 months were considered.	The dietary and PKU formula intake of vitamin K was calculated in 34 PKU patients. Vitamin K status determined by the measurement of prothrombin induced by vitamin K absence (PIVKA-II).	34 patients with PKU, 11 male (32.3%) and 23 female (67.7%). Patients with classical PKU: at diagnosis, required a low Phe diet to maintain plasma Phe levels within 2–6 mg/dL (120 −360 mmol/L) and whose Phe levels without diet exceeded 20 mg/dL (1200 mmol/L)	to evaluate vitamin K status in relation to dietary intake and phenylalanine dietary compliance in patients with phenylketonuria (PKU).	A higher total intake of vitamin K and dietary vitamin intake expressed as μg/day ($p = 0.033$ for both) and %RDA ($p = 0.0002$ and $p = 0.003$, respectively) was observed in patients with normal PIVKA-II levels. Abnormal PIVKA-II concentrations were associated with a lower OR (0.1607; 95%CI: 0.0273 −0.9445, $p = 0.043$) of having a median Phe concentration > than 6 mg/dL.	vitamin K deficiency is not uncommon in PKU and may also occur in patients with adequate vitamin K intake. PKU patients with better dietary compliance have a higher risk of vitamin K deficiency.	Moderate

The case–control study by Kose et al., in 2019 [64] had as its primary objective to define the nutritional parameters of patients with PKU on a strict diet without phenylalanine and without vitamin supplementation, in comparison with a group of healthy subjects, as well as to identify the adequacy of such supplementation in these patients. The subjects enrolled were 112 (including 53 women) with PKU and 36 (including 18 women) healthy controls. None of them were supposed to have taken supplements in the previous 6 months. Biochemical and hematological markers including hemoglobin, serum vitamin B12, folic acid, iron, ferritin, transferrin saturation, copper, prealbumin, albumin, total protein, phosphorus, calcium, 25-hydroxy vitamin D, zinc, vitamin A and vitamin E levels were screened from fasting morning blood samples. The results showed that the mean (median) serum level of B12 was higher in patients with PKU than in controls, in fact, the B12 deficiency was 15.2% in patients with PKU and 30.6% in controls. Folic acid levels (mean) were higher in PKU patients and in 55.4% of the latter and in 2.8% of controls the folate level was below reference ranges. The frequency of below-range ferritin and prealbumin values was higher among PKU patients, and vitamin D deficiency was found in 53.6% of PKU patients and 47.2% of controls. The authors conclude by stating that the Phe-free amino acid formula, in sick subjects, guarantees adequate levels of vitamin A and zinc and that it results in an excess of folic acid, B12, copper and vitamin E, which are greater than the required levels. In addition, the study shows a greater vitamin D deficiency among affected patients than among healthy ones.

Given the importance of diet therapy in patients with PKU and given the poor adherence to this therapy in adults, it is important to find strategies and new methods to facilitate their involvement and avoid metabolic disorders and nutritional and cognitive deficits. For this purpose, a multicenter study was conducted, published in 2019 by Green et al. [33]. Twelve non-compliant adults with PKU took 33 g of a protein substitute consisting of a blend of essential and non-essential amino acids each day for 28 days. Eating behavior, nutrient intake and their mood were evaluated in the 3 days prior to taking the supplement and again at the end of the supplementation period (days 29–31). During this period, the subjects maintained their lifestyle (nutrition and physical activity). Between time zero and the end of the intervention it was found that: the protein intake remained stable, the contribution of natural proteins to the total protein intake decreased, while the proteins deriving from substitutes increased significantly. Consequently, the estimated Phe intake has also decreased significantly. The intake of fat (and saturated fat) on the other hand was reduced, while the calorie and carbohydrate intake remained stable between the two moments. At time zero, the intake of natural proteins and therefore phenylalanine was high (66.4 g proteins and 3318.5 mg phenylalanine), while with the help of the protein substitute the intake of natural proteins and phenylalanine decreased. The intake of calcium, magnesium, iron, zinc, iodine and vitamin D were below the values recommended by the UK RNI. With the intake of the protein substitute, the intakes of calcium, magnesium, iron, zinc, iodine, vitamin B12 and vitamin D are increased at the final time, reaching the values recommended by the UK RNI (for example vitamin B12 and vitamin D). D increased by 19.8% and 10.4% respectively). Thiamine, riboflavin and vitamin C were already reaching recommended values, but they all increased by the end of the study. Behavioral and mood-related aspects have also undergone an improvement. The study suggests that, thanks to a low-volume nutrient-enriched protein substitute, it is actually possible to re-involve patients with non-compliant PKU in their dietary management, with immediate benefits on nutritional and psychological status.

Regarding vitamin B12, in the cross-sectional study conducted by Akış et al. and published in 2020 [70], 53 patients between 5 and

18 years of age, including 20 females and 33 males, were enrolled to evaluate for possible vitamin B12 deficiencies in adolescents diagnosed with PKU and on a diet poor of Phe. Vitamin B12 (functional vitamin B12) status was analyzed using a combination of vitamin B12 indicators (cB12) and other biomarkers such as methymalonic acid (MMA) and homocysteine (Hcy). The patient group was further divided into two categories, "high adherence" and "low adherence", according to their blood Phe levels in the last 12 months and patients with levels of Phe were included in the "high adherence" category. of Phe lower. The study found that methylamalonic acid and folate levels were higher in PKU patients. The analysis of vitamin B12 levels in serum did not show significant differences with the control group. On the other hand, considering the levels of functional vitamin B12 (i.e. the metabolically active and estimated starting from the metabolites MMA and Hcy), in particular, a higher value of plasma MMA in the group of patients with PKU, while the value of total Hcy showed no major differences between the two groups. The serum folate concentration was also higher in the PKU group than in the controls. Although B12 was lower in the PKU group, both groups had sufficient B12 levels. In conclusion, adolescent PKU patients on a strict diet are at risk of functional vitamin B12 deficiency which can be easily determined by measuring, for example, the concentration of methylamalonic acid.

Regarding vitamin K, the first study to evaluate the status of this vitamin in relation to food intake and dietary compliance in patients with phenylketonuria (PKU) was published in 2020 by Mozrzymas et al. [71]. The study included 34 subjects with PKU (11 males and 23 females, born between 1980 and 2015), treated with a restrictive diet in terms of natural proteins and supplemented with a Phe-free amino acid mixture enriched in vitamins and minerals. Dietary vitamin K intake was calculated by measuring vitamin K-induced prothrombin (PIVKA-II) and compared with the American recommendations of recommended daily allowance (RDA). The PIVKA-II cut-off was set at 3 ng/mL, higher values were considered abnormal (vitamin K deficiency), while lower values were considered normal. The vitamin K status thus calculated was normal in 25 patients (73.5%) and 32 subjects (94.1%) satisfied the recommendations for vitamin K intake. The analyzes took into account the blood concentrations of Phe in the previous 12 months. It was found that there are significantly more results showing Phe values > 6 mg/dL in patients with normal prothrombin concentration induced by the absence of vitamin K (PIVKA-II) than in those with abnormal levels. Similarly, a higher dietary vitamin K intake was observed in patients with normal PIVKA-II levels. The study found that the main differences in vitamin K intake between non-adherent and dietary patients are related to dietary intake. In addition, the main source of vitamin K in food for adherent patients is vitamin K1 found in green leafy vegetables, broccoli, cabbage, vegetable oils and fats. The authors conclude by stating that vitamin K deficiency is not uncommon in patients with phenylketonuria and can also occur in patients with adequate dietary vitamin K intake. It is interesting that PKU patients with better dietary compliance have a greater risk of vitamin K deficiency. These results suggest reviewing the dietary recommendations on vitamin K intake for both formulations and the diet based on natural products.

In conclusion, from the data obtained from the studies conducted so far it would seem that subjects with PKU who do not adhere to the diet are more at risk of an insufficient intake of nutrients, compared to those who adhere and consume protein substitutes, as these are enriched with vitamins, minerals and, often, long-chain fatty acids, as reported in the review by MacDonald et al. [8]. This is true for most nutrients with the exception of some vitamins, which seem to be more deficient in those on a strict diet: in particular, from the studies conducted so far, vitamin K has been found to be deficient in these patients, as demonstrated by Mozrzymas et al., and the metabolically active form of vitamin B12, as emerged from the study by Akış et al. The vitamins that in particular have been found to be most frequently deficient remain vitamin B12, folate and vitamin D, while among the minerals we find selenium, zinc and iron. This is likely due to the fact that these micronutrients are found mainly in animal products and protein foods, which are restricted or excluded from the diet of PKU patients. It is therefore good that, in the event that protein substitutes are missing, they are guaranteed through supplementation and that the blood values of vitamin D, vitamin B12, folic acid, iron are monitored through routine blood tests, at least annually.

2.9. Calcium intake

This research was conducted based on the keywords: "calcium intake" OR "calcium supplementation" OR "osteoporosis" AND "phenylketonuria" OR "PKU diet". 4 articles were sourced: 1 prospective case–control study, 1 cross-sectional retrospective study, cross-sectional study and the European guidelines on phenylketonuria (Table 10).

A prospective study published in 1994 by Allen et al. [72] investigated the correlation between PKU and BMD, evaluating the latter not with ultrasound but with dual-energy x-ray absorptiometry (DXA). Whole body bone mineral density (TBMD) was measured in 32 prepubertal children with PKU on diet and compared with that of a control group consisting of 95 healthy subjects of the same age. The density of the column (SBMD), on the other hand, was measured only in 24 subjects with PKU and in 55 subjects of the control group. The food intakes at the time of the DXA measurement were assessed using a 4-day food diary. Results showed that TBMD was lower in PKU subjects, with no sex differences. SBMD was also lower in sick subjects than in controls and similar in males and females. These results were also confirmed after correcting the data for height and weight. As regards the intakes, the group with PKU assumed similar quantities of energy, proteins and fibers compared to the controls, while the intakes of calcium, phosphate and magnesium were higher in the sick subjects. This result suggests that their low BMD results regardless of the inadequate calcium intake with the diet. Finally, no correlation emerged between TBMD and SBMD and diet.

The retrospective study by Geiger et al., of 2016 [73] evaluated the vitamin D status in 88 children, from the Northwest Pacific, with metabolic error-related diseases (IEM), including PKU which is the most common of these diseases (20 out of 88 patients), as a high incidence of osteopenia was observed in these patients. The authors' hypothesis was that children with HAI would have lower levels of vitamin D than healthy controls and that children with PKU would also have reduced BMD. BMD of the hip and lumbar spine was measured by DEXA in 19 of the 20 patients with PKU who were enrolled in the study (male, 10 [53%]; female, 9 [47%]). 16 patients had normal BMD in both the hip and spine ($-2 <$z score <2). 2 patients had reduced hip BMD (z score $= -2.4$ and -3.6), and 1 patient had reduced lumbar spine BMD (z score $= -2.1$), but no patient had reduced both hip and hip BMD. to the lumbar spine. Three-day food diaries were returned by 12 of 20 PKU patients (age range, 11–17 years). Due to the wide age range in the study subjects, dietary intake was normalized and expressed as energy and protein per kg of body weight and calcium and phosphorus as mg per 4186 J (1000 kcal) consumed. From the results on these 12 patients, the dietary intake of vitamin D and phenylalanine was respectively 11.3 ± 1.2 mg/dL and 610 ± 396 mg/d, while the normalized energy and protein intake was respectively 50 ± 27. kcal/kg of body weight and 1.2 ± 1.2 g/kg of body weight, while finally that of calcium and phosphorus of 601 ± 326 mg/1000 kcal and 610 ± 302 mg/1000 kcal

Table 10
Calcium intake.

Author, year	Type of study	Study period	Methods	Subjects	End point	Results	Conclusion	Strength of evidence
Allen et al., 1994	prospective case −control study		BMD of the total body (TBMD) was measured in 32 prepubertal children with PKU and in 95 age-matched control subjects. Spine bone mineral density (SBMD) was also recorded in a subset, 24 with PKU and 55 control subjects. The effect of dietary intake on bone mass was assessed in 30 of the children with PKU and in 12 control subjects.	32 prepubertal children with PKU (20 M, 12 F) and 95 age-matched control subjects (57 M, 38 F). Females aged <10 y and males <12 y.	To investigate if children with PKU have a reduction in bone mineralization compared with control subjects,	In PKU children, TBMD and SBMD were significantly lower than in the control subjects after adjustment for height and weight ($P = 0.03$ and $P = 0.003$, respectively). The PKU children had a higher intake of Ca ($P < 0.0001$), P ($P = -0.0002$), and Mg ($P < 0.0001$),	These results suggest that PKU patients' lower BMD occurred despite an adequate diet based on current recommendations.	Moderate
Geiger et al., 2016	cross-sectional retrospective record review	Patients recruited from January 2012 through June 2012.	BMD of thelumbar spine and hip determined by DEXA, compared with age- and sex-matched normative values and expressed as a *z* score. Vitamin D concentration was measured as the biologically inactive form of 25(OH)D2 and 25(OH)D3. Intact PTH levels and plasma calcium and alkaline phosphataseconcentrations were also measured. Data were compared with established normal ranges for each laboratory measurement. 3-day diet records were given to each patient to record all foods, beverages, and supplements. Food intake was analyzed for content of vitamin D, calcium, phosphorous, calories, and total protein divided into natural and synthetic protein, and Phe.	88 patients with IEMs (45 F, 43 M), and 445 children (247 F, 198 M) on unrestricted diets (controls). The age range of patients in both groups was 8−20 years. PKU patients are in total 20 (11 M, 9 F, ages ranged from 9 to 19 years, with an average age of 12.6 ± 2.8 years. All patients were diagnosed as having PKU vianewborn screeningand had been started on a low-phe diet at the time of diagnosis.	To evaluate the vitamin D status of children with IEMs who live in the Pacific Northwest with limited sun exposure and determined whether BMD in children with PKU, correlated with diet or biochemical markers of bone metabolism.	the 25-hydroxyvitamin D concentrations were normal and not significantly different between groups (IEM patients, 27.1 ± 10.9; controls, 27.6 ± 11.2). Normal BMD at the hip or spine (−2 <z score <2) was measured in 20 patients with PKU. There was a correlation between lumbar spine BMD and dietary calcium intake. We saw no evidence of low serum vitamin D in our population of children with IEMs compared with control children. We also found no evidence for reduced BMD in children with PKU on specialized diets, but BMD was associated with calcium intake.	Dietary intake of essential nutrients in medical food-based diets supports normal 25-hydroxyvitamin D levels and BMD in children with IEMs, including PKU. The risk of vitamin D deficiency among patients consuming a medical food-based diet is similar to the general population.	Moderate
Yamada et al., 2018	cross sectional study	34 days	Evaluation of food intake, anthropometry, and biochemical and phalangeal quantitative ultrasound were performed before (phase 1) and after (phase 2) calcium supplementation (1000 mg/d) for 34 d. Phalangeal quantitative ultrasound measured with amplitude-dependent speed of sound [AD-SoS]).	The study included 18 patients with PKU aged 5 −18 yr (61% male) under clinical and nutritional treatment.	To evaluate the short-term effects of calcium supplementation in PKU children and adolescents, because reduction of BMD and the risk of osteopenia have been reported to occur in PKU patients.	There was an inadequate intake of P and vitamin D, the same occurring with serum concentrations of these nutrients. About 50% of the patients had an accumulation of adipose tissue measures, with a negative correlation between Z-score, BMI, and phalangeal quantitative ultrasound. There was a significant difference in	The reduction in P excretion associated with increased AD-SoS between the two phases suggested increased bone formation and showed no negative effects in relation to short-term calcium supplementation in children and in adolescents with PKU.	Moderate

urinary P excretion with higher values before supplementation. Comparison of the two phases revealed significantly higher AD-SoS values after the supplementation ($p = 0.017$).

Statistical analysis was performed using t test for paired samples, Wilcoxon's test, and McNemar's test ($p < 0.05$).

respectively. In the same study, the authors found no evidence of reduced BMD in PKU children on specialized diets, but BMD was associated with calcium intake. Intake of essential nutrients in medical food-based diets supports normal 25-hydroxyvitamin D and BMD levels in children with MEI, including PKU. The risk of vitamin D deficiency among patients who consume a diet based on medical foods is similar to the general population.

Also the chapter on 'osteopenia and PKU' within the European guidelines published in 2017 by Wegberg et al. [74] reports the results of numerous studies on the subject. In particular, 3 systematic reviews have been published on bone density in PKU: Enns et al. (9 studies published after 2000), Hansen et al. (16 studies) and Demirdas et al. (13 studies). The 9 papers of the review by Enns et al. agree that there is a sub-optimal outcome for bone health in PKU; Hansen et al. with the meta-analysis of 3 papers they found a significantly lower spinal BMD in 67 subjects with PKU (both treated since childhood and in adulthood) compared to 161 healthy controls; finally, Demirdas et al. with their meta-analysis, they demonstrated that in patients with PKU the BMD (Z-scores), compared to their healthy peers, is lower for the whole body (in 3 studies, $n = 133$), for the lumbar spine (7 studies, $n = 247$) and for the femur (2 studies, $n = 78$).

In the work of Wegberg et al. [74] various studies are also reported that investigate the nutritional factors related to osteopenia in PKU. Some studies [75,76] speak of calcium and vitamin D deficiency, but of a good content of these two in the various Phe-free amino acid supplements. For example, the study by Perez-Duenas et al. shows a positive correlation between BMD and mineral intake concluding that a correct intake of amino acid supplement is necessary for bone mineralization [77]. Furthermore, it was found that supplementation with vitamin D improved BMD in a cohort of patients with inadequate intake [75]. At the same time, other studies show a reduced BMD even in those who are on a strict diet (with Phe-free L-amino acid supplements containing adequate levels of calcium and vitamin D) and have good metabolic control [78]. Osteopenia has been shown to be associated with increased parathyroid hormone (PTH) and alkaline phosphatase activity in patients with classic PKU and a poor diet, both of which are related to calcium and vitamin D deficiency [79,80].

However, the intake of micronutrients is not the only factor involved in the pathogenesis of bone disease in PKU, although the role of calcium in bone metabolism appears to be identical to that observed in classical osteoporosis [74]. Bone health also depends on the quality of its structural proteins. The impact on the general protein state, including the biological value of the various proteins and the percentage of proteins deriving from natural sources, is often not considered in studies. In the study by Miras et al. comparing subjects with mineral bone disease (MBD) and healthy subjects, the former had a lower intake of natural proteins than the healthy ones. Furthermore, the patients treated with BH4 (which allows a higher intake of natural proteins) were those who did not have bone problems [78]. In conclusion, Van Wegberg et al. affirm that in these patients it is important to ensure a good intake of calcium and vitamin D, regular physical activity and optimization of protein intake from natural sources [74]. Furthermore, the suggestion is to set up a correct follow-up of BMD throughout adolescence.

Finally, the recent study by Tanaka et al. published in 2018 [81] aimed to evaluate the short-term effect of calcium supplementation in children and adolescents with PKU (18 patients, aged 5–18 years) under clinical treatment and dietary. The calcium supplementation consisted of calcium carbonate tablets to be taken 3 times a day with meals for a total of 1000 mg of calcium per day. All patients used a specific Phe-free protein substitute formula

containing vitamin D. They took calcium for 34 days and before and after this period dietary intake, anthropometric parameters, plasma markers (including Phe, calcium) were evaluated, phosphate, vitamin D …) and urinary (calcium and inorganic phosphate) and an ultrasound analysis of the phalanges (quantitative ultrasound - QUS) was performed. As for micronutrients, the results showed that calcium intake reached at least 90% of the recommendations, while 50% of patients had an inadequate intake of phosphate and 67% an intake of vitamin D below the required values. The results obtained from the ultrasound evaluations instead showed a significant increase in AD-SoS (amplitude-dependent speed of sound) values after calcium supplementation. The authors conclude by stating that a short treatment with calcium supplementation in children and adolescents with PKU can improve bone quality, assessed with ultrasound on the phalanges. At the start of the study, 22% of patients had an AD-SoS value that was broken from the range, a value that was reduced to 11% after treatment.

To conclude, it is not clear whether this mineralization deficit is due to a primary defect in bone turnover inherent in the disease or is the result of long-term dietary treatment [81], but it is desirable that to ensure good bone health and quality of life in adulthood it is important to achieve good bone peak in adolescence and to maintain adequate calcium and vitamin D intakes in adulthood. For this purpose, in adults it is essential to monitor bone metabolism through annual MOC and blood monitoring of total calcium, ionized calcium and vitamin D values in order to implement, in case of inadequate blood values, supplementation personalized with calcium and at the same time with vitamin D.

2.10. Nuts

This research was carried out based on the keywords: “phenylketonuria” OR “PKU”, AND “nuts”. Only one article, a crossover study, was sourced.

To date, there are no studies in the literature aimed at specifically investigating the effects of a possible consumption of dried fruit in patients with PKU. In 2012, however, Rohde C. et al. evaluated in 14 children with PKU (aged 2–10 years) the metabolic impact of a free intake of fruits and vegetables containing less than 75 mg Phe/100 g over a period of two weeks, showing how, despite a mean increase of 58 mg of the daily Phe intake, the blood levels of this amino acid remained unchanged [29].

In general, only fruits and vegetables containing less than 50 mg/100 g of phenylalanine can be freely included in the diet of patients [41]; it is therefore necessary to keep in mind that dried fruit, containing quantities of Phe well above this limit (for example, dried sweet almonds: 1063 mg/100 g of product; hazelnuts: 595 mg/100 g; dried walnuts: 642 mg/100 g) (IEO, 2015), must be consumed by patients with extreme caution, based on individual tolerance to phenylalanine.

In conclusion, a 20 g serving of hazelnuts and walnuts can be taken daily.

2.11. Water intake

This research was conducted based on the keywords: “mineral water” OR “calcium mineral water” AND “phenylketonuria” OR “PKU diet”. 1 article was sourced: PKU dietary handbook to accompany PKU guidelines.

As for the water supply, there are no studies on the matter or particular guidelines on the quantity and type of mineral water recommended in the person suffering from PKU.

In the guidelines published by MacDonald in 2020, a list of permitted “drinks” is shown in the table of low protein foods allowed without restrictions. The list shows the following list: water, squash, lemonade, cola drinks, fruit juice, black tea, green tea, coffee, tonic water, soda water and mineral water. All of these drinks are freely permitted as long as they do not contain aspartame [8].

It is good that these patients follow the general guidelines for the population and maintain proper hydration, preferably choosing calcium-rich waters to ensure a good intake of this mineral at the same time. The calcium contained in water is absorbable like the calcium contained in dairy products [82,83].

2.12. Aspartame

This research was conducted based on keywords: “phenylketonuria” OR “PKU”, AND “aspartame”. 2 articles were sourced: 1 cohort study and 1 observational study (Table 11).

Aspartame is a methylenester of the dipeptide aspartate and Phe, and therefore a source of Phe, is added to a wide variety of foods: low calorie sweeteners, soft drinks (including sodas and fruit juices), iced tea, flavored mineral water, energy drinks, dessert mixes, frozen desserts, dessert syrups/sauces, mint, jelly, chewing gum, fruit yogurt, popsicles and ice cream. Aspartame is also added to about 600 pharmaceutical products (these are both over-the-counter and physician-prescribed drugs) including chewable multivitamins and cough medications [84].

Aspartame is completely hydrolyzed to phenylalanine (50%), aspartic acid (40%) and methanol (10%) in the intestinal lumen. It is estimated to be added to more than 6000 foods and drinks.

The amount of phenylalanine in aspartame-containing foods and beverages is not declared on ingredient labels and its impact on metabolic control in PKU patients is not well established. It is not mandatory for manufacturers to declare the amount of aspartame added to foods, thus making it impossible for people with PKU to estimate their intake of phenylalanine from this source.

80% of PKU patients need to take less than 500 mg per day of Phe (10 g of natural protein per day) to avoid its elevated blood levels.

Patients with PKU and/or parents/caregivers were invited to participate in this study.

The questionnaire was created on the Online Surveys platform (https://www.onlinesurveys.ac.uk) and placed on the UK National Society for Phenylketonuria (NSPKU).

Respondents answered questions about any consumption of aspartame-containing foods, drinks and medications, how often this had occurred, the reason for this accidental ingestion, and any symptoms it caused [84].

Several authors agree in strongly recommending national and international patient societies and advocacy groups to ask for the responsibility of companies to declare the amount of Aspartame in their drinks and products [84,85]. In conclusion, aspartame should be avoided.

2.13. Alcohol

This research was conducted based on keywords: “phenylketonuria” OR “PKU”, AND “alcohol”. Only 1 article was sourced: 1 cohort study, the same study analyzed in the chapter “Aspartame”.

Some alcoholic beverages may contain sources of protein (such as milk, egg or cream) and therefore phenylalanine. Beer (alcoholic beverage) also contains protein (0.9 g in a 285 ml glass - standard size).

Alcoholic beverages that do not contain protein or phenylalanine will not affect the blood levels of phenylalanine. In any case, the effects of excessive alcohol consumption are the same for people with and without PKU.

We can find aspartame, a food prohibited for patients suffering from phenylketonuria that we have dealt with in a special chapter,

Table 11
Aspartame.

Author, year	Type of study	Study period	Methods	Subjects	End point	Results	Conclusion	Strength of evidence
Newbould et al., 2021	Cohort Study	between April and July 2020	Cross sectional online survey	206 wholly or partially completed questionnaires. Fifty-five per cent ($n = 114$) of respondents were adults (18 or over) with PKU or parent/carers of adults with PKU and 45% ($n = 92$) were the parent or carers of children with PKU. All respondents were normally residents in the UK. The PKU population described by the respondents were: 58% ($n = 119$) female; 41% ($n = 85$) male, 1 respondent was 'non-binary' and 1 preferred not to say.	examine the accidental aspartame consumption in PKU	The aspartame containing food/drinks accidently consumed were fizzy drinks (68%,$n = 103/152$), fruit squash (40%, $n = 61/152$), chewing gum (30%, $n = 46/152$), flavoured water (25%, $n = 38/152$), ready to drink fruit squash cartons (23%, $n = 35/152$) and sports drinks (21%, $n = 32/152$).	Repeated accidental aspartame consumption is common, particularly in adults with PKU. It is important that health care professionals and policy makers understand the impact of aspartame and policies affecting the increased use of aspartame such as the sugar tax on the lives of people with PKU.	Moderate
van Vliet et al., 2020	Observational Study	7 days	people in 10 European countries were approached to send us bottles or cans of soft drinks, obtained from local supermarkets. To study within- and between-batch variation, three samples from three batches of Coca Cola zero and Fanta Orange zero (i.e., 9 samples, 3 batches per soft drink) were bought in The Netherlands and were analyzed in duplicate. Statistical analyses	A total of 111 soft drinks in original cans or bottles were obtained from Belgium (n = 15), Denmark (n = 2), Finland (n = 21), France (n = 4), Germany (n = 7), Spain (n = 7), Sweden (n = 4), the Netherlands (n = 38), Turkey (n = 4) and the United Kingdom (n = 9).	developed and validated a method for measuring APM and its degradation products, including Phe, in soft drinks using LC–MS/MS.	Large between-country variations were observed.	There is a large variation in the amount of APM and its degradation products in the measured soft drinks. Also, substantial differences were observed in the concentrations measured in similar soft drinks bought in different countries	Moderate

in alcoholic drinks mixed with energy drinks that contain it (eg rum and cola). Since aspartame contains phenylalanine, this should be avoided in people with PKU.

Some types of beers (lager) may contain aspartame; in any case, it is always good to check the label on the drink. Furthermore, some alcoholic drinks containing cream (Baileys, liqueurs or creamy cocktails …) contain proteins (therefore also Phenylalanine), therefore, it is good to avoid these drinks.

The amount of phenylalanine contained in aspartame cannot be identified from food labels. An online survey was carried out to look at accidental consumption of aspartame in people with PKU.

55% of respondents (n = 114) were adults with PKU or their parents/guardians and 45% (n = 92) were parents/guardians of children with PKU [85]. In conclusion, alcohol should be limited or avoided.

2.14. Diet schemes

The dietary intake of PHE varies greatly according to the calorific value of the diet, since all foods, including substitute products, may contain small amounts of PHE (see Appendix 1). According to the latest systematic review conducted in the literature, many of the patients affected by PKU can take, without incurring variations in phenylalaninemia higher than that established by the competent scientific societies, up to a maximum of 500 mg/day of dietary Phe [8]. For these reasons, we have drawn up three typically Mediterranean diets, with caloric values of 1500 Kcal, 2000 Kcal and 2500 Kcal respectively; which correspond to the caloric ranges sufficient to satisfy the needs of the majority of the adult population. In each of these diets, we determined whether the consumption of specific portions of some foods usually "excluded" from free consumption (in particular plant foods with a PHE content greater than 75 mg/100 g of product and potatoes) could induce an increase in the overall dietary intake of PHE such as to exceed the limit of 500 mg/day.

From the calculations performed (reported in full in Appendix 2 and Appendix 3) it is possible to draw the following conclusions: in all the diets analyzed, it is always possible to consume a 150 g portion of potatoes, to be accompanied by a smaller portion of bread or other similar substitute product (depending on the diet in question), in order to provide an amount of carbohydrates in the meal equivalent to that produced by the consumption of substitute products alone. Furthermore, in the case of the 2000 kcal regimen it is possible to consume a portion of 20 g of hazelnuts and walnuts; while in the 1500 Kcal one it is also possible to consume in addition a portion of 20 g of almonds and cashews, which, on the other hand, can induce an increase in the overall PHE intake of over 500 mg in the case of the 2000 Kcal regime. All the other food groups and/or consumer products, on the other hand, are at risk of exceeding this threshold and are therefore to be consumed exclusively according to portions that can be defined using the most widespread methods in the literature [8].

Even the data regarding the protein content or PHE reported in the bromatological composition databases may present some inaccuracies: for example, the protein content present in some international databases (eg, FDA and Ciqual) is not always obtained from HPLC analyzes, but it is often estimated using elementary analyzes (Dumas method, in which the protein content derives from the nitrogen content multiplied by the coefficient 6.25, a coefficient considered by definition equal for each cell regardless of type, species and even kingdom [ISO 16634–1: 2008]; moreover, according to a recent study, the real protein content of some plants could be better described using methods based on elementary analyzes of the nitrogen content and using a coefficient of 5.75 [86].

Furthermore, even considering these values as "reliable", the amount of PHE varies in the same food depending on various factors such as the storage temperature of some foods [87], environmental factors related to the cultivation of materials prime [8] and also other nutritional factors, still little considered today, such as cooking.

The cooking methods of some specific foods can significantly influence the Phe content of the diet. It is possible that PHE, despite being a hydrophobic amino acid, can be solubilized in the cooking broth by being transported by some proteins. In fact, there is some evidence that boiling induces a significant decrease in PHE content, as reported in the study by Ito and collaborators [88], in many foods analyzed. The results of this and other studies conducted on the effect of transformations induced by temperature and industrial transformations on food [87–90] are potentially relevant in PKU subjects, especially in the case of subjects with a caloric requirement higher than 2000 Kcal, in which the quantity of Phe deriving from the consumption of substitute products increases considerably.

This finding has potentially important implications. In fact, the consumption of some foods prepared by boiling could decrease the overall intake of dietary PHE with the diet by a value such as to allow a more "safe" use of some plant foods with a Phe content greater than 75 mg per 100 g of product such as certain processed plant products, such as dried fruit and coconut or almond milk, as well as allow for even safer use of some vegetables above 50 mg, which affect the overall PHE intake of the diet in not negligible (see Appendix 2) especially for caloric ranges from 2000 Kcal upwards or for subjects with a tolerable dose of PHE lower than 500 mg/day. Therefore, the use of some boiled foods could be an excellent tool to stimulate an increase in the variety of the diet. This could have important consequences, both on the physiological level, ensuring a higher weekly intake of micronutrients in the long term, and on the psychological one.

It is necessary to carry out further studies, in particular on substitute products to be boiled, such as pasta or rice, in order to assess whether there is also a substantial difference in the PHE content between raw and cooked food for these products. In the case of the standard versions of these products, the data published in the bromatological composition databases considered do not report significant changes in the Phe content following cooking. Finally, it would be interesting to evaluate how the content of this amino acid can possibly decrease even in the case of cooked fruit.

In summary, we recommend the use of the following portions: 150 g of potatoes, preferentially boiled or roasted [88–90], and 20 g of walnuts and hazelnuts, can be a good way to provide reasonably safe consumption portions for the general population of these foods, simultaneously stimulating the person with phenylketonuria to adopt a more varied and balanced diet.

The dietary regimes with a calorific value of 1500, 2000 and 2500 Kcal were drawn up according to the official recommendations of the LARN 2014 [91] and CREA 2019 [92]. For each diet, protein-free, low-protein products were used to replace carbohydrate and protein sources. PHE and bromatological composition similar to that of the main products on the market for the treatment of this pathology and protein substitutes with low PHE content, resorting to isocaloric substitutions in the case of substitutes for carbohydrates, given the great heterogeneity of the most used products.

With regard to protein substitutes without phenylalanine, only protein powders with a content of at least 70 g of protein per 100 g of product were considered, in order to draw up dietary regimes that are structurally homogeneous and realistic in portions and in the overall setting.

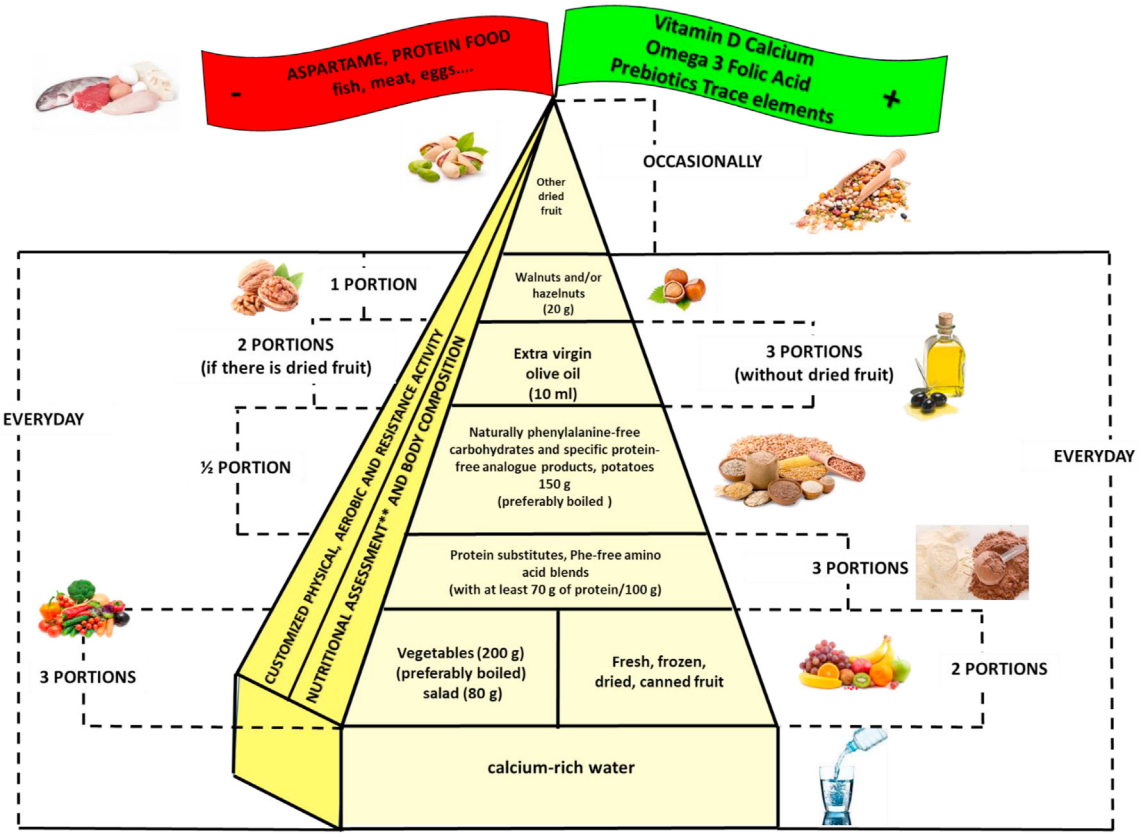

Fig. 2. The food pyramid for adult patients with PKU.

For each food source with a PHE content greater than 75 mg/100 g of product, and in the case of potatoes, all the PHE values reported in each of the following food composition databases were taken into consideration: CREA, IEO, CIQUAL, USDA and McCance and Widdowson, in order to verify that each diet prepared did not bring a quantity of PHE higher than 500 mg/day.

As regards the consumption of fruit and vegetables, only foods with a PHE content of less than 75 mg/100 g of product were used [8].

3. Conclusions

The built food pyramid for adult patients with PKU can be useful for the life-long management of this disease (Fig. 2). Artificial phenylalanine-free formulations are essential to integrate the dietary intake of natural protein, which should be tailored to individual metabolic phenotype to optimize optimal metabolic control of PKU also during adolescence and adulthood.

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Author contributions

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Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

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