



European guidelines on diagnosis and treatment of phenylketonuria: First revision[☆]

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ABSTRACT

Phenylketonuria (PKU) is an autosomal recessive inherited disorder of phenylalanine metabolism caused by deficiency of the enzyme phenylalanine hydroxylase that converts phenylalanine into tyrosine. Untreated, PKU results in elevated phenylalanine levels in blood and brain, which cause severe intellectual disability, epilepsy and behavioural problems.

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For this first revision of the European PKU Guidelines previous recommendations were re-evaluated and updated according to new research findings. Twenty-one professionals were divided across four working groups and supported by a coordinator and chair.

In addition to an update of the previous 70 recommendations, 20 new topics were included, resulting in a total of 87 statements in this first revision of the guidelines. Research publications were reviewed up until September 2022. Evidence was graded as high, moderate, low, very low or expert opinion and the recommendations were graded conditional or strong according to GRADE methodology. All recommendations were discussed during 14 plenary online or in person meetings. Recommendations were accepted if more than 75 % of the professionals were in agreement. When recommendations were not amended, the text reported in the European guidelines of 2017 remains valid.

1. Introduction

Phenylketonuria (PKU; McKusick #261600) is a rare autosomal recessive inherited disorder of phenylalanine (Phe) metabolism. Pathogenic variants in the gene encoding for the hepatic-based enzyme phenylalanine hydroxylase (PAH), cause a reduced ability to convert Phe to tyrosine (Tyr). Decreased PAH activity leads to increased blood Phe levels and crosses and competes with other large neutral amino acids at the blood brain barrier. The precise pathophysiology of PKU is not clear, but if untreated it leads to irreversible developmental problems, intellectual disability, motor deficits, skin rashes such as eczema, autism, seizures, challenging behaviour and psychiatric features.

In many countries, PKU is detected by newborn screening and treatment is initiated in early infancy. With early treatment, the majority of individuals achieve a normal intellectual ability with good quality of life, but suboptimal outcomes still exist [1–3]. The main treatment consists of a dietary Phe restriction, limiting natural protein intake, and it is supplemented with a Phe-free/low Phe protein substitute and special low protein manufactured foods. A subset of patients with PKU are responsive to the co-substrate tetrahydrobiopterin (BH4, prescribed as sapropterin dihydrochloride), which decreases blood Phe levels and/or allows (partly) liberalization of dietary restrictions. In Europe, Pegvaliase (phenylalanine ammonia lyase) is approved as a pharmaceutical treatment in patients aged from 16 years [4]. Pharmacological treatments are not available in all countries.

In 2017 the first European PKU guidelines were published [5,6], followed by a dietary handbook on PKU [7]. These projects were initiated by the patient organization, the European Society for Phenylketonuria and Allied Disorders Treated as Phenylketonuria (ESPKU) and health professionals, in response to variable PKU management practices across Europe [8–10]. These first European clinical practice guidelines gave recommendations, based on evidence and expert opinions, intended to optimize patient care and outcomes. They have led to discussions in many European countries regarding their national policies on PKU treatment targets and goals in Portugal, Turkey and United Kingdom [11–14].

The goal of this revision is to re-evaluate, update and extend the first European guidelines for the diagnosis and treatment of PKU. These guidelines are intended to be used by metabolic physicians, dietitians, biochemists, nurses, and other professionals such as obstetricians, midwives, neurologists, psychologists and social workers involved in the treatment of patients with PAH deficiency.

2. Methods

2.1. Members of the working groups

Some of the professionals involved in the working groups were different to the first publication. Three members resigned (associated with retirement and personal reasons) and eight new professionals were invited, one of whom declined. Professionals were invited according to their scientific and clinical expertise, using the same standards as for the first European PKU guidelines. The first of a series of 14 meetings took

place in 2018. Two adult metabolic physicians (E. Murphy and M. Langeveld) and 1 (neuro)psychologist (L. Aitkenhead) withdrew at the end of the process due to disagreement with recommendation 13 which had reached consensus >75 %. The remaining group consisted of 9 pediatric metabolic physicians (A.Be., S.B., A.Bo., J.C., T.C. F.F., M.G., A. Mu., F.T.), 2 pediatric neurologists (A.B., V.L.), 1 adult metabolic physician (F.M.), 3 metabolic dietitians (K.A., A.MacD, J.R.), 2 (neuro) psychologists (S.H., C.R.) and 1 biochemist (N.B.) divided across 4 working groups. The groups mainly focused on: dietary therapy (group A), outcomes in PKU and therapeutic target levels (group B), maternal PKU and late diagnosed PKU (group C), and (differential) diagnosis and pharmacological treatments (group D). The working groups were chaired by F.J. van Spronsen (FvS) and coordinated by A.M.J. van Wegberg (AvW), all together acting as authors (Supplemental Material 1). In total we had 14 plenary meetings, with 9 of them being held online (Supplemental Material 1).

At the end of the process, the ESPKU reviewed the manuscript.

2.2. Systematic literature review

The revision procedure consisted of two parts. In the first part, new and additional subjects/questions for consideration were identified, defined and assigned to one of the four working groups. In the second part, existing guidelines were updated.

Part 1: A complete list of new questions that were identified and addressed is added as Supplementary Material 1. Literature searches for new subjects did not include any date or language limits. The databases Pubmed, Embase and Cochrane were searched with the help of a librarian and titles and a total of 8024 abstracts were pre-screened independently by two working group members using the program Rayyan© (www.rayyan.ai). Publications were only included when written in English. A total of 735 full texts of potentially relevant papers were read and methodologically assessed using a checklist by two group members independently. The final included papers are discussed in this paper.

Part 2: To update existing guidelines and ensure any new references were identified for part 1, literature was searched from January 2013 until September 2022 from the databases Pubmed, Embase and Cochrane. For this part, the following search terms were used: phenylketonuria, PKU, phenylalanine hydroxylase deficiency, PAH deficiency, and hyperphenylalaninemia. After removing duplicates 3191 titles were pre-screened by FvS and AvW. Non-relevant (i.e. national prevalence studies on DNA variants in non-European countries and general reviews) and non-English papers were removed. The 1998 remaining papers were allocated to the working groups using Endnote (version X9.3.3.3). If there was any doubt about the relevance of any individual papers, the publications were accepted to go to the working groups. Some publications were assigned to more than one working group, and working groups could also re-assign publications to another working group. Two people from each working group thereafter screened titles and abstracts, read and methodologically assessed full texts of potentially relevant papers and re-evaluated the recommendations combining the included papers from the 2017 guidelines publication and the new selected

papers.

2.3. Grading the level of evidence and recommendations

Recommendations were based on evidence but if there was no or limited evidence, they were determined by expert opinion. All 20 new recommendations and the 70 recommendations of the first guidelines were discussed during 14 plenary online or in person meetings. Evidence was graded as high (A), moderate (B), low (C), very low (D) or expert opinion and the recommendations were graded conditional or strong according to GRADE (Grading of Recommendations Assessment, Development and Evaluation). A strong recommendation implies that most or all individuals with PKU will be best served by the recommended course of action (Grade handbook; <https://gdt.gradeapro.org/app/handbook/handbook.html>).

After plenary discussion, a consensus procedure was undertaken for each recommendation in which all participants had to state if they completely agreed, mostly agreed, partially agreed, mostly disagreed, or completely disagreed with the recommendation. When necessary, the recommendation was modified according to the results of the previous round(s). A cut-off of at least 75 % completely and mostly agreed was considered as consensus. The percentage of agreement is specified for each recommendation. Comments that were provided by any panellists who disagreed with a recommendation are included in Supplemental Material 1.

2.4. Amended recommendations and new topics

All 70 previous recommendations were subject to possible amendments. One of these 70 recommendations was removed and 2 recommendations merged (Supplemental Material 2). Amended recommendations are marked with an asterisk (*). In all recommendations “Phe-free L-amino acids supplements” are replaced by “Phe-free/low Phe protein substitutes”. This replacement and grammar or readability improvements are not marked with an asterisk. In Supplemental Material 2 an overview is available of all the previous reported recommendations and their revisions, including a list of the previous and newly included references. When recommendations are not amended, the text reported in the European guidelines of 2017 remains valid. New topics are marked with a double asterisk (**). Publications that were included in the 2017 guidelines have not been referenced again in this manuscript.

Definitions

Concurrent Phe level	The Phe level measured at or close to the day of outcome assessment.
Dried blood spot (DBS)	A practical method of collecting blood. A blood spot is collected on a blood card at home, which can then be posted or delivered to a metabolic laboratory for measurement of Phe (and Tyr).
ESPKU	The European Society for Phenylketonuria and Allied Disorders is the umbrella organization of 41 national and regional associations from 31 countries established by parents and where patients and health professionals work together.
Executive functioning	Cognitive processes that regulate behaviour. Examples include inhibitory control, working memory and cognitive flexibility.
Good treatment adherence	≥75 % of Phe levels in the recommended therapeutic target range
Hyperphenylalaninemia (HPA)	Phe levels >120 µmol/L
High risk pregnancy	A pregnancy that threatens the health or life of the mother or her foetus.
Late diagnosed	Late diagnosed refers to children diagnosed with PKU aged >1 month.

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Lifetime Phe levels, lifetime Tyr levels and lifetime Phe/Tyr ratio	Blood Phe levels from birth to the final age of testing. This is often expressed as the median blood Phe of annual mean Phe levels. Also referred to as historical blood Phe levels. This is similar for Tyr and subsequently Phe/Tyr ratio.
Maternal PKU syndrome	The teratogenic effects of elevated maternal Phe levels during pregnancy to the foetus.
Phe tolerance	The amount of Phe (mg/kg/day or mg/day) that maintains plasma Phe levels within the therapeutic target range. This may also be described as natural protein tolerance expressed as g/day calculating a Phe content in natural protein as 50 mg /1 g natural protein for most foods. In fruits and vegetables, the amount of food containing 1 g of natural protein usually contains a lower amount of Phe.
Protein requirement	Safe levels of protein requirement are the lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintain the body protein mass at energy balance with modest levels of physical activity.
Protein substitute	Protein replacements/substitutes are essential to prevent protein deficiency and optimize metabolic control. Protein substitutes are sourced from Phe-free/low Phe protein substitutes, including glycomacropeptide (cGMP).
Potential tetrahydrobiopterin (BH4) / sapropterin responsiveness	≥30 % reduction in blood Phe in a sapropterin loading test or 2 BH4 responsive PAH gene variants. Long-term sapropterin -responsiveness should be proven in a treatment trial adjusting the sapropterin dose, natural protein and Phe-free/low Phe protein substitute intake.
Tetrahydrobiopterin (BH4)	Co-substrate of phenylalanine hydroxylase enzyme. BH4 acts as a chaperone molecule in phenylalanine hydroxylase-deficient patients with BH4 responsive gene variants. BH4 is also a cofactor of Tyr and tryptophan hydroxylases and plays an important role in the conversion of L-arginine to nitric oxide (NO) by nitric oxide synthases (NOS).
Tetrahydrobiopterin (BH4) deficiencies	Defects in BH4 metabolism (either synthesis or regeneration) cause a deficiency of BH4. These include 3 known defects for synthesis and 2 for regeneration.
Tetrahydrobiopterin (BH4) / sapropterin responsiveness	For the purpose of these guidelines, it is defined as an increase of ≥100 % in natural protein or improved biochemical control (≥75 % of Phe levels in the recommended therapeutic target range) on a dose of sapropterin that ranges between 1 and 20 mg/kg of body weight.

3. Pathophysiology

The pathogenesis has been addressed by van Wegberg et al. 2017 and was further detailed in a recent review on PKU [4]. The specific cause of brain dysfunction in PKU is still unclear. Although it is hypothesized that low (cerebral) levels of Tyr and/or other amino acids and/or increased phenylpyruvate, phenyllactate and phenylethylamine may play a role, most studies suggest direct and indirect effects of high cerebral Phe.

High blood Phe is associated with low cerebral levels of large neutral amino acids and high cerebral amounts of Phe. Together, they are responsible for low levels of dopamine and in particular serotonin. Most studies have focused on white matter including imaging studies, functional studies on executive functioning and animal studies on

oligodendrocytes. However, some of the most severe manifestations such as intellectual disability and epilepsy may also have a grey matter component. Grey matter involvement has been found in old autopsy data and is acknowledged more recently in MRI data [4].

Cerebral glucose consumption, representing brain activity, is decreased in animal and patient studies. Furthermore, high cerebral Phe causes amyloid-like fibrils (suggesting a resemblance with processes like Alzheimer-disease), affects the epigenome, and is a cause of oxidative stress (addressed in section 7.12). Finally, observed variations of the microbial diversity or abundance has been associated with an increased release of pro-inflammatory molecules [15].

Together, these data suggest that multiple mechanisms, potentially relevant throughout life, are involved.

4. Diagnosis

Most patients are diagnosed by newborn screening (NBS) rather than by clinical presentation. However, there are still countries where NBS is not or partially established. Historically patients with PKU have presented with severe delay in motor and intellectual development, but it is now established that there is a minority of patients who have little or no neurological impairment despite high blood Phe ($>1200 \mu\text{mol/L}$) [16–18]. Interestingly, some of these patients still develop psychological and psychiatric issues that may manifest early and later in life. Additionally, some patients may present in adulthood with dementia, visual problems and parkinsonism [6,19,20].

4.1. Newborn screening

PKU is identified through NBS, which should be available in every European country. NBS requires a robust infrastructure in which blood is taken from all newborn babies within a few days after birth, and the samples analysed by a well-equipped NBS laboratory that can manage and assess dried blood spots (DBS) efficiently. Less invasive techniques to diagnose PKU in urine are described, but not widely used as many diseases can be found in DBS [21,22].

Low-income and small countries might consider utilizing NBS laboratory facilities of another country. Infants with a positive NBS result should be referred to a specialized metabolic centre for diagnosis, assessment, treatment, and follow-up. Prenatal and pre-implementation diagnosis is feasible, and techniques have become less invasive with lower risks of miscarriage [23], although this is rarely conducted.

Recommendation 1

NBS for PKU should be considered a national requirement. Early detection is the prerequisite of early treatment and is cost effective.

Level of evidence: moderate (B)

Strength of recommendation: **strong**

Expert opinion: completely agree (100 %), mostly agree (0 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

4.2. Familial screening

There are recent reports of women who are identified with PKU through their newborn infants who had high blood Phe levels or presented with maternal PKU syndrome [24–26]. Familial screening has been included as a standard of routine care in many clinics and is especially important in women who were born in countries where the NBS program was not fully implemented or established at the time of their own birth. Identifying women with PKU through familial screening brings significant health benefit to the individual woman and her future

children.

Recommendation 2**

Screening for familial HPA/PKU in unscreened mothers of children with HPA or with signs of maternal PKU syndrome is recommended especially in countries where neonatal screening was unavailable when the mother was born.

Level of evidence: very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (90 %), mostly agree (10 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

4.3. Screening immigrants / refugees

Although many countries have implemented NBS for over 50 years, there are still several countries without NBS programs, no national coverage or with only recently implemented NBS [27–33]. Consequently, these countries may have undiagnosed or late diagnosed patients with PKU [34–38]. Immigrants or refugees from these countries are at risk of a missed PKU diagnosis as most receiving countries do not have a PKU screening program for immigrants [39,40].

A recent survey reported 259 diagnoses of untreated PKU patients between 2015 and 2020 in 77 centres from 36 countries [24]. More than half (56 %) were late diagnosed because of no or partial coverage or only had recent implementation of NBS. Within Europe, North America and Oceania, most missed cases were immigrants from countries without (national coverage of) NBS programs ($n = 52$). Almost all patients were symptomatic at diagnosis and the diagnostic age varied widely [24]. Timely diagnosis and treatment are crucial to improve the outcome of patients with PKU. Typical symptoms of untreated PKU, such as developmental or speech delay can easily be mistaken for effects of trauma, separation from family or unfamiliar language in refugee children [41,42].

As not all developmental signs and symptoms will be considered to be abnormal before school age, any immigrant originating from a country without NBS or with a non-universal NBS program is at risk of a missed diagnosis of PKU and other metabolic disorders.

Therefore, receiving countries should screen all immigrants/refugees systematically until at least 6 years of age (school age). Screening should be done at the first contact with the health care system of the country receiving this child. In addition, systematic screening and treatment is beneficial for immigrants/refugees of all ages diagnosed with PKU and treatment is essential to prevent maternal PKU syndrome.

Recommendation 3**

As a minimum, routine screening for PKU is strongly recommended for immigrant or refugee children younger than 6 years and selective (based upon specific clinical presentations) at any age.

Level of evidence: very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (90 %), mostly agree (5 %), partially agree (5 %), mostly disagree (0 %), completely disagree (0 %)

4.4. Differential diagnosis (defects in BH4 metabolism and DNAJC12 deficiency)

In any child referred with HPA (Phe $>120 \mu\text{mol/L}$), BH4 deficiencies, DNAJC12 and liver disease should be excluded, as these require different treatment strategies. BH4 deficiencies can present with any degree of HPA, but some may present with normal blood Phe levels with NBS [6].

In every case of a positive NBS test for HPA and before treatment commencement, samples for pterins in DBS or urine and

dihydropteridine reductase activity in a DBS should be taken. If the time until receipt of laboratory results of pterins and dihydropteridine reductase activity is a concern or delayed by more than 7 days, a 24 h sapropterin loading test can be performed, in addition to analysis of pterins and dihydropteridine reductase that would allow earlier diagnosis of BH4 deficiencies (and/or sapropterin responsive patients with PKU).

Recently, DNAJC12-deficiency was described as a cause of HPA [43–51]. Clinical manifestations of DNAJC12-deficiency are heterogeneous and range from asymptomatic to autism, attention deficit-hyperactivity disorder, intellectual disability and severe non-progressive parkinsonism and dystonia [52]. DNAJC12-deficiency clinically may resemble BH4 deficiencies and may need the same treatment strategy, although in cases without or milder neurological symptoms, sapropterin treatment may be the only treatment given [52].

DNAJC12 deficiency is diagnosed by performing DNA analysis. This is therefore indicated when pterins analysis and dihydropteridine reductase activity are normal and genotyping does not indicate 2 pathogenic variants in the PAH gene.

Defects in BH4 metabolism and DNAJC12 deficiency should be considered in any child with neurological problems of unknown origin, even with only slightly increased blood Phe levels. If pterin analysis is performed, this can be done in DBS, plasma, urine and CSF. If CSF analysis is performed, BH4 deficient patients usually have specific pterins and biogenic amine patterns while DNAJC12-deficient patients present only with low biogenic amine levels [53]. Still, especially for DNAJC12 deficiency, DNA analysis is the easiest option.

Recommendation 4*

In the differential diagnosis of all HPA, defects in BH4 metabolism and DNAJC12 deficiency should be excluded.

BH4 deficiencies should be investigated by measurement of pterins in DBS or urine and dihydropteridine reductase activity in dried blood spots.

Genotyping of DNAJC12 should be performed in the case of normal pterins and dihydropteridine reductase activity, and when PAH genotyping is not indicative of 2 pathogenic or likely pathogenic variants.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (90 %), mostly agree (10 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

4.5. Genotyping

The gene encoding PAH is located on chromosome 12 (region q22–24.1) consisting of 13 exons and 12 introns, covering a total of 100 kb of genetic data. Over 3300 PAH variants (PAHvdb database; <http://www.biopku.org/home/pah.asp>; last accessed 14–01–2024) are known to be associated with PAH deficiency. The majority of the variants (60 %) are missense, usually resulting in protein misfolding and/or impairment of catalytic functions [54].

Due to the difficulty in distinguishing between PAH and DNAJC12-deficiencies, patient genotyping is essential for the diagnosis of PKU. In addition, the genotype is helpful in determining the degree of protein dysfunction, residual PAH activity, responsiveness to pharmaceutical chaperones like sapropterin and consequently the metabolic phenotype.

Recommendation 5*

Patient genotyping is recommended as it is necessary to distinguish PAH deficiency from DNAJC12-deficiency. It may also help to predict sapropterin-responsiveness and assist in defining the metabolic phenotype.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (85 %), mostly agree (15 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

4.6. PKU classification

Historically, the most common phenotype classification was determined by the untreated Phe levels at diagnosis. Patients were classified as classical, moderate, and mild PKU or mild HPA. As patients commonly commence treatment before reaching their maximal blood Phe level, this method is no longer applicable. Therefore, an alternative classification was suggested in guideline 1.0 [5,6]. However, as with the availability of new treatments, this classification has been amended to include responsiveness to a co-factor. Those requiring treatment have a choice between dietary treatment, pharmacological treatment, or enzyme substitution, depending on the genotype and availability of the drug. If patients are co-factor responsive (i.e. sapropterin), they may be treated with a co-factor only or with the combination of a dietary Phe restriction and a co-factor.

Recommendation 6*

In order to maintain blood Phe levels in the recommended range, patients with PAH deficiency may be classified as:

- a) not requiring treatment (Phe <360 µmol/L), or
- b) requiring treatment, co-factor responsive, or
- c) requiring treatment, co-factor unresponsive.

Level of evidence: very low (D)

Strength of recommendation: **conditional**

Expert opinion: completely agree (75 %), mostly agree (15 %), partially agree (10 %), mostly disagree (0 %), completely disagree (0 %)

5. Initiation of treatment

5.1. Timing of initiation of treatment

In the first edition of the PKU guidelines, it was recommended that treatment should start as soon as possible and ideally before 10 days of age, acknowledging the absence of supporting evidence [6]. Table 1 outlines all studies that have addressed the influence of early treatment on outcome in PKU. Since 2015, only one research paper has addressed the age of treatment commencement in PKU [55]. Our initial recommendation has therefore not been amended.

Recommendation 7

Treatment should start as soon as possible, ideally before 10 days of age.

Level of evidence: expert opinion (for 10 days of age)

Strength of recommendation: **strong**

Expert opinion: completely agree (100 %), mostly agree (0 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Table 1
An overview of papers regarding timing of treatment initiation and development.

Author, year	Measure	Outcome
Smith 1990 [56]	IQ at 4 years (n = 657)	Decline of approximately 4 IQ points for every 4-week delay of treatment
Van der Schot 1994 [57]	IQ at 1–2 years (n = 33)	Mental development was inversely related to blood Phe (mean) >500 µmol/L during the first 30 days of life.
Zeman 1996 [58]	IQ at 15–19 years (n = 67)	Higher IQ scores in adolescents with PKU if treatment started within 3 weeks versus at between 4 and 5 weeks and 6–10 weeks of age.
Liemburg 2022 [55]	IQ at 10.8 ± 2.9 years (n = 64)	Phe levels within the first month of life >360 µmol/L negatively correlated with IQ in childhood, though a relationship to a specific time of exposure to higher Phe in the first month was not identified.

5.2. Phe level for treatment initiation

There is consensus that patients with PKU with untreated Phe levels $<360 \mu\text{mol/L}$ do not need treatment. The first European PKU guidelines suggested a follow-up period of 1 year in patients with blood Phe $<360 \mu\text{mol/L}$ to determine if blood Phe levels rise above $360 \mu\text{mol/L}$. This follow-up period is considered too short, as blood Phe can still increase after the age of 1 year when the dietary protein load increases and growth speed declines [59]. Clinical practice differs between clinics. Without any supporting clinical evidence, we suggest that follow-up should continue until at least 6 years of age as this is often defined as the critical period, essential for the structural development of the brain [60]. In girls and women, considering maternal PKU syndrome, a longer follow-up may be necessary.

In 2017, the evidence supporting the need for treatment in patients with untreated Phe levels between 360 and $600 \mu\text{mol/L}$ was contradictory and scarce. The recommendation given in 2017 [5,6] was not widely accepted [11]. Since then, three relevant additional papers have been published. De la Parra et al. compared the development and cognitive functioning in untreated children with Phe $120\text{--}360 \mu\text{mol/L}$ with treated children with PKU (no blood Phe given) at age 12 months, 36 months, 4 years ($n = 87$ treated and 53 untreated patients) and at 7 years ($n = 75$ treated and 26 untreated patients). Unexpectedly, the untreated children with Phe $120\text{--}360 \mu\text{mol/L}$ showed similar performance profiles and difficulties on WISC-R subtests (attention and working memory) compared to treated patients with PKU, which may indicate that even modest elevations in blood Phe affect specific functions [61].

Evinc et al. studied 41 patients (aged 6–16y) with untreated blood Phe $<600 \mu\text{mol/L}$ and 29 controls. They completed a comprehensive neurocognitive attention and behavioural assessment, including IQ. Controls had significantly higher IQ scores and faster reaction times than patients with untreated blood Phe $<600 \mu\text{mol/L}$, but mothers of the control group also had higher IQs. When the subgroups with lifetime median blood Phe levels between 240 and $360 \mu\text{mol/L}$ and between 360 and $600 \mu\text{mol/L}$ were compared, it was concluded that when blood Phe increased, cognitive performance decreased, but the patients with a blood Phe of $240\text{--}360 \mu\text{mol/L}$ still did not perform like healthy controls [62].

Finally, a recent systematic review included 10 papers with individuals with blood Phe levels $<360 \mu\text{mol/L}$ and 7 papers with data with individuals with untreated Phe levels between 360 and $600 \mu\text{mol/L}$. The authors concluded that untreated blood Phe levels between 120 and $360 \mu\text{mol/L}$ are generally safe, but there is insufficient data to state that treatment is not required if untreated blood Phe is $>360 \mu\text{mol/L}$ [63].

Recommendation 8*

No treatment is recommended when untreated blood Phe levels are $<360 \mu\text{mol/L}$. Monitoring of blood Phe (at a lower frequency[#]) until a minimum of 6 years of age is recommended to determine if levels rise above $360 \mu\text{mol/L}$.

Level of evidence: low (C), however the age cut-off: expert opinion

Strength of recommendation: **strong**

Expert opinion: completely agree (90 %), mostly agree (10 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

[#]see recommendation 23

Recommendation 9*

In all children with untreated blood Phe levels $\geq 360 \mu\text{mol/L}$ treatment should be initiated.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (100 %), mostly agree (0 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

For late diagnosis see section 9.2.

6. Treatment goals

The primary goal of treatment is normal neurocognitive and psychosocial functioning. Blood Phe levels remain the best surrogate measure, and should be monitored regularly, aiming for blood Phe levels that stay within a given target therapeutic range, defined for a given age.

6.1. Recommended blood Phe target range for children

The first European PKU guidelines advised $120\text{--}360 \mu\text{mol/L}$ as the target therapeutic range for patients aged 0 to <12 years [5,6]. Although there are several new papers published addressing blood Phe levels and outcomes in children aged 0 to <12 years, few contained sufficient data to evaluate a cut-off target range. A consequence of the published European and USA guidelines could be that new studies often define patient groups according to blood Phe levels below or over $360 \mu\text{mol/L}$ and do not explore other cut-off levels in their analysis. The paper of van Vliet et al. and Thiele et al. reported no significant differences in mental health, executive functioning, behaviour and social functioning in children with PKU ($n = 31$ and $n = 27$ respectively) with a mean blood Phe level below $360 \mu\text{mol/L}$ compared to healthy controls, which confirms that maintaining Phe levels $<360 \mu\text{mol/L}$ is safe [64,65].

Some papers have reported a relationship between adult IQ, executive functioning, mental health, social skills and childhood blood Phe levels within or without the target range $120\text{--}360 \mu\text{mol/L}$, supporting Phe $<360 \mu\text{mol/L}$ for this age group [66–69]. Jahja et al. also reported that the quality of social cognitive functioning in 13 adolescents with PKU was poorer than in controls and negatively related to recent blood Phe levels as well as to Phe-levels when aged between 8 and 12 years [67].

Considering the overall body of evidence, a target blood Phe level between 120 and $360 \mu\text{mol/L}$ is recommended. Also, maintaining stable Phe levels appears important as Hood et al. and Romani et al. showed that blood Phe fluctuations throughout the life span may affect cognitive performance more than blood Phe levels per se [6,69]. However, more evidence is needed to determine the recommended targets for Phe fluctuation.

Recommendation 10

In treated PKU patients up to the age of 12 years, the recommended therapeutic target Phe levels are $120\text{--}360 \mu\text{mol/L}$.

Level of evidence: moderate (B)

Strength of recommendation: **strong**

Expert opinion: completely agree (100 %), mostly agree (0 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

6.2. Recommended blood Phe target range for adolescents

The first European PKU guidelines advised $120\text{--}600 \mu\text{mol/L}$ as the target therapeutic range for patients aged ≥ 12 to 18 years [5,6]. This recommendation remains unchanged. The amount and quality of data remains limited due to differences in study designs and outcome measures, mixed age groups, and large individual variability. A few studies have assessed the relationship between blood Phe and cognitive outcomes in adolescents while others have evaluated neuropsychological functioning in adulthood and retrospectively explored the impact of blood Phe exposure during adolescence.

In a follow-up of the original longitudinal study of Weglage et al., Feldmann et al. reported better IQ in adults who remained on diet during adolescence ($n = 17$; median blood Phe $496\text{--}707 \mu\text{mol/L}$) in comparison with adults who relaxed their diet after age 10 years ($n = 18$; median Phe $750\text{--}1038 \mu\text{mol/L}$) [6,70]. In a small study, Nardecchia et al. reported no IQ difference between controls (114.1 ± 8.9) and 4 young adults with PKU with lifetime blood Phe $<500 \mu\text{mol/L}$ (105.0 ± 9.9), but some differences between controls and 3 patients with Phe $500\text{--}600 \mu\text{mol/L}$.

(102.3 ± 14.2) [71]. Manti et al. found that in 65 young early-treated adults with PKU average blood Phe attained in the second decade of life affected IQ, but that this effect disappeared when IQ in the first decade of life was accounted for [72]. Jahja et al. found that within a cohort of 21 adults with PKU, blood Phe attained aged between 13 and 17 years (range 267–1069 µmol/L) correlated with two tasks measuring inhibitory control and cognitive flexibility (flanker task and shifting attentional set visual). Blood Phe increase after 12 years of age also correlated with the flanker task. However, adolescent blood Phe did not correlate with motor control (pursuit task), self-assessment reports of executive functions difficulties or mental health problems (assessed with the BRIEF and Adult-Self-Report questionnaire) [68].

Palermo et al. compared metabolic control in two groups of adults with PKU who either performed normally in an extensive battery of cognitive tasks ($n = 14$ with z -scores <0.5) or were impaired ($n = 9$ with z -scores $>-2SD$). The two groups did not differ in childhood blood Phe but differed between the ages of 11–16 years (659 µmol/L vs. 944 µmol/L respectively) and in adult blood Phe [73]. Romani et al. reported better cognitive performance and executive functions in 28 adult patients with PKU who maintained ($n = 14$) versus did not maintain ($n = 14$) metabolic control throughout life, and therefore had lower blood Phe in adolescence (432 µmol/L versus 964 µmol/L) [74]. In contrast, Aitkenhead et al. did not find a significant correlation between Phe when aged 12–18 years and cognitive functions in a group of 154 adults [75].

On reviewing studies that explored functioning during adolescence, Didycz et al. showed that, in 30 early treated PKU patients aged 13–18 years, attention and visual memory were strongly influenced by Phe in the past month, and that attention was better in patients with Phe <600 µmol/L [76]. Jahja et al. found that the quality of social cognitive functioning in 13 adolescents with PKU was poorer than in controls and negatively correlated with recent Phe levels (as well as to blood Phe levels between 8 and 12 years) [67].

Hood et al. and Romani et al. showed that blood Phe fluctuations throughout the life span may affect cognitive performance more than Phe levels per se [6,69]. As already described in the section on children, this suggests that maintaining stable blood Phe levels is important, but more evidence is needed to determine the recommended targets for Phe fluctuation.

Considering the primarily indirect evidence and the lower grade of evidence, an upper target Phe level of 600 µmol/L between the ages 12 and 18 years is recommended, and unchanged from 2017 [6].

Recommendation 11

In treated PKU patients aged 12–18 years, the recommended therapeutic target Phe levels are 120–600 µmol/L.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (100 %), mostly agree (0 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

6.3. Recommended Phe target range for adults

In the first European PKU guidelines, the recommendations for target therapeutic Phe range in adulthood was between 120 and 600 µmol/L, based on a limited number of papers with some outcomes considered [5,6]. Although the number of published studies has increased, data is incomplete and difficult to interpret due to low patient numbers, phenotypic variability, quality of metabolic control in childhood and absence of outcome data in patients aged >50 years with early treated patients with PKU. Studies on brain imaging by MRI, and brain function (EEG, PET studies on glucose consumption and protein synthesis, eye movement) and oxidative stress have not been considered. These tests are not routinely used in clinical practice though they may help to explain the relationship between brain function and metabolic control [6,77,78].

It is accepted that, as a group, adults with early treated PKU have impairments in neurocognitive testing [3]. It is also important to recognize that there is wide variability in neurocognitive performance, both between studies and within studies, and between different adults with PKU. Moreover, the group contains a considerable number of individuals without any impairment.

When considering the impact of Phe in adulthood, intervention studies to alter blood Phe in adulthood have also shown variable results, but there are indications that outcomes like attentional symptoms and mood status may improve with blood Phe lowering [6,79,80]. Many cross-sectional studies that found a relationship, did not correct for blood Phe during childhood and adolescence [78,81–83]. However, three different research groups have reported a significant relationship with blood Phe at the time of testing, after correcting for historical Phe levels, with processing speed ($n = 154$) [75], attention tasks ($n = 57$) [84], memory, sustained attention and visuo-motor tasks ($n = 37$) [74].

Furthermore, rare cases of acute neurological deterioration in adults with PKU, that partially or completely resolve after reintroduction of a Phe restricted diet and nutritional supplementation, suggest an impact of blood Phe levels, although deficiencies of vitamin B12, or other nutrients were not always considered [6,85].

The interindividual differences in neurocognitive outcomes have been more extensively reported in recent literature. Aitkenhead et al. reported normal scores (cut-off <1.5 SD) in 7 cognitive function tests in 53 % ($n = 79/147$) of patients with concurrent blood Phe 119–2135 µmol/L. Even though the study did show a significant relationship with concurrent Phe and processing speed, 78 % ($n = 60/77$) of patients with concurrent Phe >600 µmol/L scored normally [75]. Romani and Palermo similarly reported no abnormal (cut-off <2 SD) results in 62 % of individuals ($n = 23/37$) with concurrent blood Phe 720 ± 343 µmol/L in any of 36 cognitive measures, whilst again showing a relationship between blood Phe at the time of testing and memory, sustained attention and visuo-motor tasks [73,74].

Feldmann et al. reported no deterioration in IQ, information processing speed and sustained attention in a 10-year follow-up period in adults with yearly median blood Phe levels >700 µmol/L and <1000 µmol/L ($n = 35$) [70].

Studies report variable results regarding the impact of adult blood Phe levels on mental health (see section 7.7), but the quality of life is generally reported to be good (see section 7.6). The impact of the emotional burden of diet on the mental health of adults with PKU is increasingly recognized. Recent studies have considered anxiety in adults with PKU to be linked to the controlling style of their parents during childhood as they found higher anxiety scores in patients with the lowest blood Phe levels in childhood [75,86].

To summarise, the long-term effects of blood Phe levels during adulthood are still being determined. However, adult blood Phe does influence brain function and maintaining blood Phe levels <600 µmol/L during adulthood is considered to have less negative influence on brain functioning. There are no studies adequately addressing the question whether adults with Phe ≤ 360 µmol/L will function better than those with Phe ≤ 600 µmol/L; or if blood Phe levels in early and mid-adulthood will affect outcomes in elderly individuals with PKU.

Currently, we are unable to clearly differentiate the impact of blood Phe control at the time of testing in adulthood, the quality of blood Phe control achieved in childhood and adolescence and the impact of the burden of dietary treatment on neurocognitive and psychosocial functioning in adults. As more individuals with PKU are treated with pegvaliase, facilitating lower, more stable blood Phe levels, the answers to these questions may become clear.

The existing variation between individuals with clinical symptoms and the treatment burden should be acknowledged. When the psychological burden of (dietary) treatment far exceeds the clinical symptoms associated with Phe >600 µmol/L, it may be necessary to consider a personalised approach, agreeing an individualized Phe target. However, all patients should be fully aware of the potential clinical impact of

higher blood Phe levels, and that the long-term effects of high Phe concentrations during adulthood are unclear.

Recommendation 12*

Lifelong follow-up in a specialist metabolic centre is recommended for any individual with PKU, including adults not on Phe lowering treatment.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (100 %), mostly agree (0 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 13

In treated PKU patients aged >18 years, the suggested recommended therapeutic target Phe levels are 120–600 µmol/L.

Level of evidence: very low (D)

Strength of recommendation: **conditional**

Expert opinion: completely agree (75 %), mostly agree (20 %), partially agree (0 %), mostly disagree (5 %), completely disagree (0 %)

6.4. Recommended target Phe to prevent maternal PKU syndrome

High blood Phe during pregnancy has a teratogenic effect on the developing foetus, causing growth retardation, microcephaly, intellectual disabilities, and birth defects, including congenital heart defects (CHD) [6].

Women with untreated blood Phe levels ≤ 360 µmol/L do not require treatment during pregnancy for their unborn child, but should be monitored for adequate nutritional intake and noting increasing Phe concentrations. As discussed in the first PKU guidelines, earlier studies examined different blood Phe cut-offs, but showed no effect on birth measurements and cognitive development of the offspring when untreated blood Phe levels were on average ≤ 360 µmol/L [6].

Women with blood Phe levels >360 µmol/L should commence a Phe-restricted diet before conception. Many, if not all, features of the maternal PKU syndrome are preventable by starting a Phe restricted diet before conception. Data indicates that patients already pregnant (with blood Phe >360 µmol/L) should commence treatment immediately. The MPKUCS study indicated that the major factors associated with good child outcome were normal maternal intelligence and well-treated pregnancies with mean blood Phe control ≤ 360 µmol/L [6]. Some clinics aim for stricter Phe levels during pregnancy to create a safety buffer or because amino acids in the foetus are approximately 1.5 times higher than the amino acids in the mother [87]. However, the outcome in relation to lower target Phe recommendations has not been evaluated in studies. Therefore, the recommendation remains unchanged.

See section 9.1 for more details on maternal PKU.

Recommendation 14

No treatment is recommended when untreated blood Phe levels are consistently <360 µmol/L before or during pregnancy.

Level of evidence: moderate (B)

Strength of recommendation: **strong**

Expert opinion: completely agree (85 %), mostly agree (15 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 15

In pregnancy, the recommended therapeutic target Phe levels are 120–360 µmol/L.

Level of evidence: moderate (B)

Strength of recommendation: **strong**

Expert opinion: completely agree (100 %), mostly agree (0 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 16*

The recommended therapeutic target Phe levels should be achieved pre-conception. If the patient is already pregnant on presentation, all measures should be taken to aim for therapeutic target Phe levels to be reached within 7 days.

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Level of evidence: moderate (B)

Strength of recommendation: **strong**

Expert opinion: completely agree (90 %), mostly agree (10 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7. Monitoring of PKU

In Table 2 necessary follow-up and investigations are outlined, and these are discussed in more detail in this section.

7.1. Biochemical markers used for the assessment of metabolic control

7.1.1. Dried blood spots versus plasma

Blood Phe is currently the most important and most widely used biomarker available to monitor treatment, as blood Phe levels correlate strongly with Phe in the brain and cerebrospinal fluid (CSF) and with neurocognitive and behavioural outcome. Past studies were largely based on plasma Phe levels (gold standard), where it is now routine practice to perform DBS measurements. It is critical that results obtained from different methods are reliable. The difference between results obtained from tandem mass spectrometry and those from ion exchange chromatography is usually less than 10 % [88,89].

It is known that plasma and DBS results are not completely comparable, and plasma has been reported to be from 15 % lower to 30 % higher than DBS, depending on pre-analytical and analytical differences that also may be laboratory dependent [88–91].

Pre-analytical factors contributing to variation include the type of filtration paper, bloodspot volume, punch size and location, tube anti-coagulants and extraction efficiency [6,90]. Too much squeezing the fingertip might cause leakage of lymphatic fluid and thus sample dilution.

Another contributing factor to the difference between plasma and DBS results is that DBS consists of dried whole blood, a mixture of plasma and erythrocytes [88,90]. Erythrocytes are reported to contain less than one-third Phe compared to plasma [88]. So, as the hematocrit value (proportion of erythrocytes in whole blood) is a determinant factor in the differences between DBS and plasma, some authors suggest using a correction factor [88,89,91].

The measurement in the matrix of DBS has been disputed for some time. The volumetric absorptive microsampling using Mitra(TM) 4-sampler clamshells, as described in Bratkovic et al. can make a difference suggesting that that method outperforms using the DBS matrix especially due to less variation in sampling volume [92].

It is important for laboratories to be aware of pre-analytical and analytical differences between plasma and DBS results and to ensure high quality within their laboratory. In addition, it should be mandatory that patients and families receive training on how to take good-quality blood spots and that training is updated at regular intervals.

If tandem mass spectrometry is unavailable, it is important to note that high-performance liquid chromatography and fluorometric enzyme studies are generally comparable and offer greater precision than the Guthrie method.

7.1.2. Phe variation

Variation or fluctuation of blood Phe (expressed as standard deviation (SD) or standard error of the estimate (SEE)) may also be relevant in determining cognitive outcome [6,69,74,93–96]. In some papers, blood Phe variation correlated better to cognitive performance test than average blood Phe [95,96]. However, as there is no definition or recommendation for an acceptable blood Phe variation, and it is difficult

Table 2

Minimum requirements for the management and follow-up of patients with PKU.

	Childhood (<12 y)	Adolescence (12–18 y)	Adulthood (≥18 y) excluding maternal PKU	Maternal PKU
Outpatient visit ^a	Given good clinical and metabolic control: Age 0–1 years: every 2–3 months* Age 1–3 years: three times per year Age 3–12 years: twice per year Extra clinic visit as indicated * after initial training	Given good clinical and metabolic control: twice per year Extra clinic visit as indicated	Given good clinical and metabolic control: once to twice per year Extra clinic visit as indicated	Given good clinical and metabolic control: once pre-conception/first trimester, once second (optional) and once third trimester (optional) Extra clinic visit as indicated
Clinical nutritional assessment	Every outpatient visit: dietary assessment (3-day food record/24 h recall), anthropometric parameters (weight, height, BMI), clinical features of micronutrient, and Phe deficiency (especially anorexia, listlessness, alopecia, perineal rash), and gastrointestinal symptoms	Every outpatient visit: dietary assessment (3-day food record/24 h recall), anthropometric parameters (weight, height, BMI), clinical features of micronutrient and Phe deficiency, and gastrointestinal symptoms	Every 12–24 months: dietary assessment (3-day food record/24 h recall), anthropometric parameters (weight, height, BMI), clinical features of micronutrient and Phe deficiency, and gastrointestinal symptoms	Every outpatient visit: dietary assessment (3-day food record/24 h recall) and weight
Metabolic control ^a	Age 0–1 year weekly Phe Age 1–12 years fortnightly Phe Increased frequency as indicated Annually: plasma amino acids	Monthly Phe Increased frequency as indicated Annually: plasma amino acids	Monthly Phe Increased frequency as indicated Annually: plasma amino acids	Pre-conceptionally: at least weekly Pregnancy: at least twice weekly Increased frequency as indicated
Biochemical nutritional assessment	Annual measurement of vitamin B12 (homocysteine or methylmalonic acid, haemogram, MCV and ferritin (iron status), and vitamin D. Other markers of vitamins and mineral status or lipids can be considered if clinically indicated.		Annual measurement of vitamin B12 (homocysteine or methylmalonic acid, haemogram, MCV and ferritin (iron status), and vitamin D. Other markers of kidney function and bone health status (creatinine and alkaline phosphatase, calcium/creatinine excretion), vitamins and mineral status or lipids can be considered if clinically indicated.	Pre-conceptionally and at the start of pregnancy: plasma amino acids Pre-conception and at the start of pregnancy: folic acid, vitamin B12 (homocysteine or methylmalonic acid), MCV and ferritin, full blood count Pregnancy: when indicated
Bone Density	BMD measurement only indicated when there are specific clinical reasons or when patients are known to be at particular risk of metabolic bone disease	The first measurement of BMD should be undertaken during early adolescence - When BMD is abnormal, the measurement (with or without change of treatment) should be repeated after 1 year. If the results are still low but stable, yearly BMD measurement is unnecessary. - When BMD is normal, no repeat measurement is necessary. Further DXA need only be considered when there are clinical reasons to do so.	BMD measurement is only indicated when there are specific clinical reasons or when patients are known to be at particular risk of metabolic bone disease	Not indicated
Neurocognitive functions	Testing should be offered around 5 years Testing should include IQ and the domains of executive functioning (especially reasoning, cognitive flexibility and working memory) and sustained attention. Extra neurocognitive tests as indicated.	Testing should be offered around 12 years Testing should include IQ and the domains of executive functioning (especially reasoning, cognitive flexibility and working memory) and sustained attention. Extra neurocognitive tests as indicated.	Testing should be offered around 18 years and thereafter every (5 to) 10 years. Testing should include IQ and the domains of executive functioning (especially reasoning, cognitive flexibility and working memory) and sustained attention. Extra neurocognitive tests as indicated.	Not indicated
Mental health and behavioural problems	Every outpatient visit: clinical assessment / discussion	Every outpatient visit: clinical assessment / discussion	Every outpatient visit: clinical assessment / discussion	Not indicated
Psychosocial functioning, general wellbeing and QOL	Annually: Clinical assessment / discussion Use of a questionnaire may be considered to support this.	Annually: Clinical assessment / discussion Use of a questionnaire may be considered to support this.	Annually: Clinical assessment / discussion Use of a questionnaire may be considered to support this.	Women may need extra support if they unable to conceive.
Psychiatric examination	At onset of symptoms of psychiatric disturbances	At onset of symptoms of psychiatric disturbances	At onset of symptoms of psychiatric disturbances	Not indicated
Neurological complications	If neurodegeneration occurs	If neurodegeneration occurs	Annually: clinical examination	Not indicated

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Table 2 (continued)

	Childhood (<12 y)	Adolescence (12–18 y)	Adulthood (≥18 y) excluding maternal PKU	Maternal PKU
White matter abnormalities (MRI)	When there is an unexpected clinical course and/or unexpected neurological deficits	When there is an unexpected clinical course and/or unexpected neurological deficits	When there is an unexpected clinical course and/or unexpected neurological deficits	Not indicated
Other organs (eye, heart, kidney)	If clinically indicated	If clinically indicated	If clinically indicated	Not indicated
Age group specific investigations	/	/	/	Ultrasound at 18–22 weeks of pregnancy with screening for organ development (especially if there is lack of optimal metabolic control) Echocardiogram should be considered in all infants who are conceived by women with either high blood Phe levels or poor maternal blood Phe control during pregnancy

For patients with untreated Phe 120–360 µmol/L and untreated Phe between 360 and 600 µmol/L, see section 7.2.3.

to distinguish the effect of blood Phe variation itself from poor metabolic control, there is no cogency to support its use in current practice.

7.1.3. Phe/Tyr ratio

As reported in the first European PKU guidelines, Phe/Tyr ratio has been correlated to the patient outcome and, on occasion, even to a greater extent than blood Phe control [6]. Sharman et al. reported a significant correlation between lifetime blood Tyr and lifetime Phe/Tyr ratio and Global Executive Composite of the BRIEF ($r = -0.546$, $p = .027$ and $r = 0.478$, $p = .049$ respectively) in 13 children with PKU (mean age 13.95 ± 1.8 years), whereas this correlation was not present for lifetime Phe [97]. McGinnity et al. investigated the relationship between markers and cerebral glucose metabolism in 8 PKU and 3 HPA adults with memory problems. They reported that 8 of 11 patients had cortical deficits of cerebral glucose metabolism, and both mean, and SD historical Phe/Tyr ratio was more indicative than absolute blood Phe levels [77].

Blood Tyr has a wide diurnal variation associated with Phe-free/low Phe protein substitute supplemented with Tyr. A reliable Phe/Tyr ratio should be measured after an overnight fast [6]. Furthermore, the blood Phe/Tyr ratio is difficult to use in clinical practice, similar to Phe variation, as there is no clear recommended target range.

7.1.4. Other metabolic biomarkers

Recent studies have evaluated other potential markers such as large neutral amino acids, neurotransmitter metabolites such as homovanillic acid [98], 5-hydroxyindoleacetic acid [98], melatonin [99] and prolactin [100], and potential new biomarkers detected by untargeted metabolomics [101,102]. However, the clinical relevance of these novel markers is unknown.

Recommendation 17

Blood Phe (either DBS and/or plasma) should be measured to monitor metabolic control, as this is the most clinically relevant biomarker.

Level of evidence: moderate (B)

Strength of recommendation: **strong**

Expert opinion: completely agree (100 %), mostly agree (0 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 18**

Considering the necessity for frequent follow-up and immediate clinical care, analyses of DBS by MS/MS should be used in PKU monitoring on condition that:

- adequate sample quality is assured by regular training of patients and caregivers
- the accuracy of the results is assured by the laboratory through accredited/certified pre-analytical and analytical procedures.

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(continued)

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (85 %), mostly agree (15 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 19

Phe variation and Phe/Tyr ratio are not recommended as biomarkers for routine care.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (75 %), mostly agree (25 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7.2. Frequency of blood spot monitoring and outpatient clinic visits

7.2.1. Time to receipt of laboratory results

Blood Phe is usually monitored by home blood sampling or in outpatient clinics. The primary method for monitoring involves patients/family caregivers sending blood Phe samples via postal services to the laboratory for analysis and timely reporting of the blood results to the patients/family caregivers is essential. As postal routines in countries differ, and some countries are dependent on plasma samples only, the recommendation focuses on the time between receiving the sample in the laboratory and reporting the result to the patients/caregivers in a routine situation. This should be a maximum of 2 working days. This is particularly relevant for pregnant women and children. However, even for adults a short roundabout time can serve as a motivating factor for sending in blood samples.

PKU teams may need to instruct patients to send their samples on specific days if laboratories measure samples only once weekly.

Consideration can be given to centralizing Phe measurements to a smaller number of laboratories to minimize between-laboratory variation [91].

Recommendation 20*

The time between receipt of a blood Phe sample by the laboratory and the patient/caregivers receiving the result should be a maximum of 2 working days.

Level of evidence: expert opinion

Strength of recommendation: **strong**

Expert opinion: completely agree (65 %), mostly agree (35 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7.2.2. Follow-up of patients with untreated Phe >360 µmol/L

A **minimum** frequency of blood Phe measurements and outpatient clinic visits is suggested per age group. Newly diagnosed infants and women during the preconception period and pregnancy may require more frequent (at least once weekly) contact with the metabolic team to provide education, reassurance and close support to patients/family caregivers. As appropriate, contact may be in person, by telephone or by video consultation. Video consultations do have limitations and can only partly replace some outpatient visits, but are useful for patient education and may be helpful if patients are logistically unable to attend a clinic in person [103].

Recommendation 21

Suggested minimum frequency of blood Phe measurements:

Age Frequency

0–1 year weekly

1–12 years fortnightly

>12 years monthly

Pregnancy pre-conception weekly, pregnancy twice weekly

Increased frequency of blood Phe measurements may be required following treatment changes and clinical or adherence concerns.

Level of evidence: expert opinion

Strength of recommendation: conditional

Expert opinion: completely agree (75 %), mostly agree (25 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 22*

Suggested minimum frequency of outpatient clinic visits (assuming good clinical and metabolic control):

Age Frequency

0–1 years after initial training, every 2–3 months

1–3 years three times per year

3–18 years twice per year

>18 years once to twice per year

Pregnancy once pre-conception/first trimester[#], once second (optional) and once third trimester (optional)

Increased frequency of outpatient visits may be required following treatment changes, changes in social circumstances (such as change of school, leaving home), clinical or adherence concerns.

[#] to discuss issues such as the maternal PKU syndrome, management of nausea and vomiting of pregnancy, genetic counselling, and follow-up of the child after delivery

Level of evidence: expert opinion

Strength of recommendation: conditional

Expert opinion: completely agree (75 %), mostly agree (20 %), partially agree (5 %), mostly disagree (0 %), completely disagree (0 %)

It is important that blood Phe samples are collected under the same conditions and at the same time of the day in order to compare the results. Although it was always assumed that fasting Phe levels were the highest throughout the day, recent literature shows that this is not necessarily the case [104,105]. Several studies confirm earlier research [6] that unevenly divided intake of natural protein does not significantly influence Phe fluctuation during the day but that unevenly divided intake of protein substitutes does [104,105].

Blood Tyr levels are known to fluctuate widely (up to 300 %) during the day which could be explained by the ingestion of the Tyr-enriched protein substitute [6,105]. To estimate reliable Tyr levels, blood samples should be collected in the morning after fasting overnight.

7.2.3. Follow-up of patients with untreated Phe ≤360 µmol/L and untreated Phe between 360 and 600 µmol/L

As discussed in section 5.2 patients with untreated blood Phe levels between 120 and 360 µmol/L do not need treatment but should receive clinical follow-up for at least the first 6 years to evaluate if blood Phe increases with a declining growth speed and an increasing protein load.

Acknowledging that Viall et al. recommends at least monthly monitoring for the first 2 years [59] there is no evidence to support regularity or maximum patient age of follow-up, and practice varies

among centres.

Of course, in these patients, there is a risk of Phe levels being marginally above 360 µmol/L. Since we aim for Phe levels <360 µmol/L preconceptionally and during pregnancy, it is essential to keep female patients in follow-up, in contrast to male patients.

We recommend the following minimum follow-up:

Recommendation 23**

Follow-up of patients with untreated Phe 120–360 µmol/L

Suggested minimum frequency of blood Phe measurements:

Age Frequency

0–1 year monthly

1–6 years once every three to six months

> 6 years determined individually, especially in women

Suggested minimum frequency of outpatient clinic visits (assuming good clinical and metabolic control):

Age Frequency

0–6 years annual

> 6 years determined individually

Level of evidence: expert opinion

Strength of recommendation: conditional

Expert opinion: completely agree (65 %), mostly agree (35 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Similarly discussed in section 5.2, patients with untreated blood Phe levels between 360 and 600 µmol/L require blood Phe lowering treatment. Patients with milder phenotypes are commonly responsive to sapropterin treatment and/or tolerate more natural protein, although heterogeneity exists between patients. Children on sapropterin treatment, with stable blood Phe levels towards the lower end of the range (e.g. < 240 µmol/L) may require less frequent follow up than those with Phe towards the upper end (e.g. > 300 µmol/L).

The target Phe level for patients ≥12 years of age is 120–600 µmol/L, meaning that some patients might not require any treatment ≥12 years. When women are planning pregnancy, their blood Phe levels may need to be lowered (120–360 µmol/L), so females remain in regular follow up.

7.3. Metabolic centre and team

All patients should be treated in a specialized metabolic centre with a specialized metabolic laboratory. The absolute minimum of health professionals within the metabolic team should be a metabolic physician and a dietician with experience in IMD. Access to a (neuro)psychologist and/or social worker is strongly recommended to support patients with acceptance of their disorder, treatment and for neurocognitive assessments [6]. A neuropsychologist often performs neurocognitive assessments, while a clinical psychologist focuses on assessing and helping patients with emotional or mental difficulties throughout life. Access to both is recommended.

Recommendation 24

All patients with PKU should have access to a metabolic physician, dietician and (neuro)psychologist

Level of evidence: very low (D)

Strength of recommendation: strong

Expert opinion: completely agree (90 %), mostly agree (10 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7.3.1. Centres of excellence

In Europe, centres of excellence for metabolic diseases, including PKU, have been established in some countries. The decision to endorse a centre as an expert facility for PKU or another rare disease (prevalence of <1:2000 people) lies with national governments/health services. This process differs across countries. In some countries, governments have designated all centres treating metabolic diseases as expert facilities for PKU, while in other countries only a few metabolic centres have been endorsed as centres of expertise for PKU.

The aim is to ensure every patient with any rare disease is connected to a centre of excellence. This connection allows for the collection and sharing of knowledge, thereby ensuring optimal care for patients. The list of European expertise centers for rare diseases, and thereby for PKU can be found at Orphanet (<https://www.orpha.net>). Centres officially endorsed by their governments for a centre of excellence can be a member of MetabERN (the European Reference Network for Metabolic Diseases). The primary goal of ERNs is to identify where in Europe high-level PKU care can be sought, especially in countries or regions where PKU care is not well established. It is based on the principle that any patient with a rare disease within Europe should have free access to excellent care.

7.4. Transition and patients lost to follow-up

Transition is a continuous process by which the primary responsibility for treatment is forwarded from parent/family caregiver to the patient, and from pediatric to adult medical services. This is a long-term process in which children gradually learn about their condition and treatment from an early age and then start to take the lead in their care. At the same time, parents should be guided to step down from their leading and controlling role. Acknowledging that this is a very individual process, some patients will have completed this process before the age of 18 years, while others continue to be supported by their parents/caregivers well into young adulthood. During adolescence and/or young adulthood, it is not uncommon for patients to struggle with maintaining their treatment regimen. The transition process therefore needs to be well planned and individualized with involvement of both patient and parents/caregivers. There are various toolkits available that can help guide this process like the “Ready Steady Go” programme [106].

All patients should be transferred from pediatric to adult care. The timing of this transfer commonly occurs between 16 and 18 years of age, although some flexibility is required depending on the maturity and circumstances of the patient. Biasucci et al. recently proposed a pathway to set up a successful PKU transition process [107].

Ernst et al. reported in a group of patients 2 years after participating in an education programme, a positive influence on self-efficacy and an active role in their own treatment [108]. Peres et al. reported that 2 years after transfer, patient attendance remained high and blood Phe levels remained stable [109]. Mutze et al. reported that of 96 patients who were transferred to an adult team in the last 10 years, 81 % still maintained contact with their medical/dietetic teams and that metabolic control was stable [110]. In addition, most patients (83 %) were satisfied with the transition process and adult care [110].

In contrast, in Austria, Beghini et al. identified a high number of patients “lost to follow-up” (LTFU) using the NBS data between 1966 and 2000 and follow-up data of the metabolic centres. As many as 177 of 279 patients were LTFU, defined as no recorded contact and no blood Phe measurement for 2 years [111].

The impact of neuropsychological and neurocognitive deficits on patient ability and organization skills, inadequate knowledge of treatment recommendations, lack of insurance coverage, inadequate transition to adult care and fear can also contribute to LTFU [112–114]. A focus group of 8 PKU patients who were LTFU for over 2 years reported that they felt unprepared to continue treatment as adults and feared being judged by clinicians for allowing treatment to lapse [114]. Clinicians should be aware of the reasons for disengagement and contact patients to notify them that they can always return to the clinic regardless of their treatment status. If possible, without a new clinician referral.

Transitioning to a dedicated metabolic adult team can also be challenging for various organizational and financial reasons, as well as

inexperience with maternal PKU care. Specific adult services may not be available in some countries or may be geographically distant from patients [115,116]. Video consultations may help. If a pediatric clinic is still involved, it should be acknowledged that adults should be managed differently.

Recommendation 25*

Young adults should be transferred from pediatric services to a specialized adult metabolic team to ensure lifelong specialized follow-up.
Transition should be performed in a structured manner. This process should ideally be started in early adolescence.

Level of evidence: low (C)

Strength of recommendation: strong

Expert opinion: completely agree (80 %), mostly agree (20 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 26**

Clinicians should be aware of the reasons for disengagement from the metabolic clinic and adult patients should be offered an open invitation to return to the clinic if they request further follow up.

Level of evidence: low (D)

Strength of recommendation: strong

Expert opinion: completely agree (80 %), mostly agree (20 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7.5. Neurocognitive functioning

Cognitive impairment should be monitored in PKU. Neuropsychological assessment is important to evaluate the effects of new treatments and possible changes occurring across the lifespan. To monitor cognition in clinical practice, there is a need to establish which measures are most appropriate for the PKU population.

In children with PKU, studies have frequently shown impairment compared to controls in working memory, inhibitory control, sustained attention, and abstract reasoning and the level of impairment has been associated with blood Phe [6,62,117]. In early-treated adults with PKU, meta-analysis has shown that reasoning, visual-spatial attention speed, sustained attention, visuo-motor control, and flexibility are the most strongly affected cognitive functions [3].

Studies from the literature have used different tasks to evaluate cognitive impairment in PKU, with multiple options being available to assess a given cognitive function. Results have been variable across studies, but there are also enough communalities to allow us to put forward some suggestions of the type of tasks to include in an assessment which should be sensitive to PKU impairment and to the level of metabolic control. Cognitive tasks generally require a combination of skills, even when they are categorized, for convenience, under a single label. In PKU, the tasks that are most sensitive require a combination of executive functions, processing speed, and fluid (processing new information) rather than crystallized knowledge (accumulated knowledge), particularly in adults. Examples include:

- **Digit symbol coding, trial-making test B-A** [69,75], assessing flexibility/planning, visuo-motor coordination, and processing speed;
- **Visual search tasks** assessing flexibility/planning, working memory, visuo-spatial attention, and processing speed: [66,69];
- **Verbal fluency tasks** assessing flexibility/planning and lexical retrieval;
- **Dot tasks** [66,76], **rapid visual information processing task (RIVP)** [69] and **continuous performance test** [62], assessing sustained attention
- **Go-no-go tasks** assessing inhibitory control, processing speed: [117,118];

Table 3
Recommended tasks which have shown high sensitivity to PKU-related cognitive impairment and/or changes in Phe levels and which could be used for neuro-cognitive assessment.

Domain	Time (minutes)
Flexibility/visuo-motor control	
(digit-symbol coding/Symbol Digit Modalities Test, TMT-B)	5
Sustained attention (choosing from dot tests, CPT, RVP)	5
IQ – e.g., WASI (similarities plus matrix reasoning)	5
Working memory: Digit span	5
Flexibility/lexical retrieval: verbal fluency	5
Depending on resources	
Visual search measuring accuracy and RTs	5
Word learning (choosing from CWLT, RAVLT)	7

- **Pegboard tasks, motor screening task, motor performance tasks** [69,119,120] assessing visuo-motor control;
- **California verbal learning task (CVLT)** [75,83], **rey auditory verbal learning test (RAVLT)** [74], assessing verbal memory and learning;

Some tasks where no impairment is expected could be included as control tasks to better evaluate specific effects of changes in metabolic control (e.g., tests examining vocabulary and spelling in adults).

The following neuropsychological assessment for patients is suggested although the limited evidence on which this is based is acknowledged (Table 3).

For the frequency of assessment, recommendations are based on knowledge from developmental psychology of sensitive windows of cognitive and social development and clinical practice in PKU care. It is recommended to offer neurocognitive assessment as a minimum:

- 1) at the start of formal education around 4–6 years to ensure that the child is ready for school and there are no issues that need attention,
- 2) at 12 years of age as target blood Phe levels change, adherence to dietary treatment may decline, and there is often a transition to a new education environment,
- 3) at 18(–20) years when there will be increasing independence and a transfer to adult clinical services,
- 4) every (5-)10 years thereafter to ensure that cognition is stable and to provide a reference point in case of any subjective cognitive change with aging.

Recommendation 27*

Neuropsychological assessment should be offered in all patients around age 5, 12, 18 years and thereafter every (5 to) 10 years.
Testing should include IQ and the domains executive functioning (especially reasoning, cognitive flexibility and working memory) and sustained attention. Tests should preferably involve visuo-spatial stimuli and a processing speed component. Tasks tapping a combination of functions would be most sensitive.
Perform (additional) assessments, for specific clinical indications, including:

- Non-optimal metabolic control in children and adolescents; >50 % of the Phe levels are out of target range over a period of 6–12 months (depending on age < 12 or 12–18 years)
- Reported problems in education or in the workplace; not showing progress in school for 6 months
- Concerns reported by patients or parents / caregivers or family
- Concerns of the metabolic team

Level of evidence: moderate (B) for domains of testing, expert opinion for the frequency and clinical indication of testing
Strength of recommendation: strong
Expert opinion: completely agree (85 %), mostly agree (15 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7.6. Psychosocial functioning

Health-related quality of life (HRQoL) has been widely researched. Results of generic HRQoL questionnaires completed by patients/family caregivers indicate a good quality of life. The PKU-specific QoL-questionnaire (developed with assistance from industry) can address the specific problems encountered in PKU [6] and is a valid tool to evaluate before and after an intervention [121]. Unfortunately, this questionnaire is not free to access. A PKU impact score (PKU SSIS) has also been developed (by industry) to address the burden of illness [122].

Recent papers have confirmed an overall normal HRQoL in patients with PKU [75,123,124]. Aitkenhead et al. reported no difference between 149 adults with PKU and 74 controls in educational, occupational, quality of life or emotional outcomes, or on a measure of family functioning, and showed only minor differences in relationship style [75]. Huijbregts et al. however demonstrated adults had lower scores on cognition and domains of depressive mood and anger. A negative association with lifetime Phe was demonstrated for sleep, pain, anger, and sexuality [124]. Vieira et al. reported a low HRQoL in a cohort of Brazilian early treated PKU children, probably resulting from the harmful intellectual consequences due to poor adherence and challenges within the healthcare system [125].

Using the PKU-specific QoL Questionnaire, excellent HRQoL was demonstrated in adolescents and adults, but suboptimal metabolic control was negatively associated with HRQoL [126,127]. Anxiety about blood Phe levels (during pregnancy) received the highest scores in 21 Turkish adults [128]. Using Time Trade- Trade-Off and Discrete Choice Experiment surveys, dietary Phe restriction and symptoms negatively associated with HRQoL [129].

To conclude, although the overall HRQoL is normal in patients with PKU, certain domains of wellbeing may be impacted.

Recommendation 28*

Health-Related Quality of Life, psychosocial functioning and general wellbeing should be discussed at least annually in the outpatient clinic. For all patients, anxiety regarding blood levels and guilt about non-adherence should be discussed. The use of a questionnaire may be considered to support this.

Level of evidence: moderate (B)
Strength of recommendation: strong
Expert opinion: completely agree (80 %), mostly agree (20 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7.7. Mental health

The impact of early treated PKU on mental health is most likely to be multifactorial, associated with the experience of a long-term health condition, elevated blood Phe associated with executive function deficits, and individual and family coping strategies, as demonstrated in earlier, mainly pediatric, studies [6]. Mental health is a state of mental well-being that enables people to cope with the stresses of life, realize their abilities, learn well and work satisfactorily, build relationships and contribute to their community (<https://www.who.int/news-room/fact-sheets/detail/mental-health-strengthening-our-response>). Mental health conditions include mood disorders, anxiety disorders, personality disorders and psychotic disorders, however mental health is more than the absence of mental disorders. (<https://www.who.int/news-room/fact-sheets/detail/mental-health-strengthening-our-response>).

Recently, da Silva et al. reported (predominantly hyperactive/

impulsivity clinical presentation) ADHD in 5/27 patients <17 years, associated with blood Phe levels [130].

Internalizing symptoms such as anxiety, withdrawal, low mood, depression, and distress have also been reported in some adult patients [131–134]. Currently it is not fully understood to what extent factors such as disease burden and increased blood Phe levels influence mental health. Some studies report a relationship with concurrent blood Phe [78,83], blood Phe of the preceding two years [81,131], lifetime blood Phe [135] or childhood blood Phe [66,136], while other studies reported no relationship between blood Phe levels and internalizing problems [134]. Anxiety may be associated with a controlling style of parenting during childhood as authors report higher anxiety scores in patients with the lowest blood Phe levels in childhood [75,86].

As reported in the first European PKU guidelines, an intervention Phe loading study showed worse mood with high Phe levels and an intervention sapropterin study, reported improved ADHD symptoms with lower blood Phe levels [6]. A recent intervention study reported improvement on depression scales with better dietary control, but without blood Phe correlation [79]. In trials where blood Phe levels were decreased using pegvaliase, no relationship between Profile of Mood States (POMS) scores and Phe was found [137].

Regardless of the cause of mental health symptoms, it is clear that they are present in a subset of patients with PKU and therefore assessment and support should be part of every routine clinic consultation, especially from adolescence.

Recommendation 29*

Issues with mental health and behavioural problems (i.e. concerns related to age-appropriate functioning in the community, at home, in education or the workplace) should be discussed as part of every clinical consultation and during neuropsychological assessments.

Subjective or objective adaptive issues, emotional, behavioural or social concerns should prompt further exploration of difficulties (possibly with ad-hoc instruments such as validated questionnaires) and may include psychological referral.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7.8. Brain MRI

PKU is associated with white matter (WM) abnormalities and grey matter (GM) abnormalities on magnetic resonance imaging (MRI). GM is responsible for processing and interpreting information, while WM is crucial for transmitting signals across different areas of the nervous system. However, due to the unclear clinical impact of WM and GM abnormalities, neuroimaging is not advocated in routine care. Therefore, this recommendation remains unchanged.

Recent MRI Diffusion Weighted Imaging (DWI) studies confirm earlier findings and report reduced water diffusion in cerebral white matter and a negative correlation between DWI-related measures and blood and brain Phe levels [6,138,139]. Both reduction of whole and regional brain volumetry and swelling of cerebral areas have been reported in MRI studies [98,140–142], but their clinical significance is uncertain. A prospective study involving 37 early treated patients with PKU and 71 controls (7 to 21 years) undergoing neuropsychological and brain MRI assessments at 1.5–1.6-year time intervals detected a more rapid deterioration of DWI-derived markers with age in subjects with PKU than in controls. The authors propose the decline of mean diffusivity as the anatomical background of the effect of lifetime exposure to blood Phe on executive functions [141].

Published cases without WM abnormalities suggest the limited impact of blood Phe under 600 $\mu\text{mol/L}$ on MRI WM signal [6]. Over this threshold, the severity of signal changes is influenced by lifetime and/or concurrent blood Phe although with large interindividual variability [6,78]. The contribution of blood Phe to the pathogenesis of these

alterations is further supported by white matter signal improvement following lowering of blood Phe [6,138].

More recent MRI studies also pointed to the involvement of GM in adult early treated PKU patients. Those studies reported decreased GM volume [143,144] and a correlation between the regional pattern of grey matter atrophy and the profile of serotonin and dopamine metabolite depletion in CSF [98]. As for WM abnormalities, the clinical impact of these alterations is not clear.

Recommendation 30

Neuroimaging should not be included among the tools for routine monitoring of the disease in healthy individuals with early treated PKU but should be reserved for patients exhibiting an unexpected clinical course and/or unexpected neurological deficits or for research purposes.

Level of evidence: low (C) / very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (80 %), mostly agree (20 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7.9. Other organs

Recent data suggest that involvement of other organs like eye, heart and kidney might be included in the spectrum of PKU involvement.

7.9.1. Eye

In PKU, there are some studies indicating the possibility of loss of vision that may have a relation with earlier studies that highlighted decreased visual evoked potential (VEP) latencies in early (as well as late) treated patients with PKU. It was suggested that long chain polyunsaturated fatty acids (LC-PUFA) may be influential as both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) status and VEP latencies improved in PKU patients receiving LC-PUFA supplementation [145–147].

Gramer et al. studied LC-PUFA status and the dopamine hypothesis (i.e. increased blood Phe may cause decreased dopamine levels in the brain and eye associated with inadequate Tyr in the central nervous system) in 36 patients with PKU (17.2 ± 10.1 years) and 18 healthy controls (17.9 ± 9.9 years). They reported significant differences in contrast sensitivity, colour vision, and electroretinography, mainly explained by the Phe /Tyr imbalance (dopamine hypothesis) rather than the LC-PUFA status [148].

Using optical coherence tomography (OCT) Nowak et al. reported a correlation in the retinal nerve fibre layer thickness and both lifetime blood Phe levels and IQ in 15 early treated patients with PKU (15.2 ± 5.2 years), although the mean thickness was similar to controls in other studies [149]. Servozzo et al. reported lower thickness of parafoveal and perifoveal retinal layers and macular ganglion cell complex in 50 early treated adults with PKU (30.7 ± 8.0 years) compared to 50 controls (30.45 ± 7.18 years), using OCT angiography [150].

Finally, five adult patients who discontinued dietary treatment, were reported to have developed acute visual loss, which partly ($n = 2$) or completely ($n = 3$) resolved when dietary treatment was re-initiated [6,85].

Overall, there seems to be a retinal effect which might be a marker of neurological damage rather than impairment of the eye. However, a nutritional component, such as vitamin B12 deficiency, cannot be completely excluded. At this point, routine eye follow-up is not recommended.

7.9.2. Heart

In the context of PKU, discussion about the heart focuses on cardiac function, potentially related to arterial stiffness. Recently, several papers have been published about arterial and cardiac function. Hermida-Ameijeiras et al. evaluated pulse wave velocity as an arterial stiffness marker in 41 patients with PKU (aged 6 to 50 years) and matched

controls. They reported increased aortic stiffness in patients with PKU and an association with blood Phe levels. However, the PKU group included more obese patients than the control group and this may have explained the differences found [151]. Nonetheless, high blood Phe may be a risk factor for cardiac aging as suggested in a mouse study [152] and for reduction of thickness of ventricular wall and consequently reducing ventricular ejection capacity [153].

Currently there is insufficient data to support routine evaluation of heart function.

7.9.3. Kidney

Although there are no reports of chronic renal failure in PKU, literature suggests some risk of renal glomerular dysfunction. Two large studies highlighted a higher adjusted prevalence ratio for renal insufficiency with hypertension and for chronic kidney disease compared to the general population, in patients with PKU in the USA and Germany, respectively [154,155]. These observational studies followed the report by Hennermann et al. who described in 67 patients with early and late diagnosed PKU aged 15–43 years that glomerular filtration rate decreased in 11/59 (19 %) in addition to proteinuria, defined as a protein excretion of >150 mg/24 h, in 19/62 patients (30.6 %). BMI was increased in 25 patients, while 10 patients did not adhere to the prescribed diet [156]. However, these results are in contrast to the finding of normal renal function markers and absence of arterial hypertension in 52 treated patients with PKU compared to controls [157]. They also found significantly lower plasma levels of asymmetric dimethylarginine (ADMA) when compared with controls, a finding partly confirmed by Andrade et al. [158]. ADMA is an endogenous inhibitor of nitric oxide synthase and is linked to kidney dysfunction [157]. Considering that renal response to protein/amino acid infusions is multifactorial [159] it is recommended that all evidence is carefully interpreted and all potential risk factors are considered.

Commonly, studies do not describe renal function in patients with PKU and give limited dietary information, particularly about the type of protein substitutes prescribed and dosage of protein equivalent consumed. It is currently unclear if there is a negative impact of dietary treatment of PKU on renal function.

Recommendation 31**

Routine follow-up is not recommended for organs like the kidney, heart and eye, but can be considered if clinically indicated.

Level of evidence: very low (D)

Strength of recommendation: **conditional**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7.9.4. Gut / microbiome

Gut symptoms in PKU include both feeding issues, oesophageal dysfunction, flatulence, and constipation. Feeding problems have been described in children and adults with PKU [133,160–162], but without apparent increased risk of gut mucosa inflammation [163]. When describing 21 cases of co-existing conditions in patients with PKU, 6 were gastrointestinal [164]. In a study from the USA, the adjusted prevalence ratio of gastritis and esophagitis, oesophageal disorders and gastroesophageal reflux disease were higher for patients with PKU than the general population [154] and in a German insurance study, infectious gastroenteritis and colitis were described as one of the comorbidities with a prevalence higher than 10 % among patients with PKU [155]. When compared with controls, constipation was higher in infants up to 6 months of age [161] and young children [162], with almost one

in 5 caregivers reporting that their children with PKU were taking laxatives [133]. Issues such as abdominal pain, constipation, flatulence, retching and regurgitation have been associated with the high osmolality of the lower volume amino acid based protein substitutes [133,161].

It was first suggested in 2011 that the nutritional composition and characteristics of a Phe restricted diet might change the gut microbial ecology and affect host physiology [165]. Evidence suggests that gut microbiota is characterized by a lower microbial diversity [166,167] with potentially different taxonomic groups, which may impact the metabolomic profile. Verducci et al. described a lower degree of microbial diversity and a decrease in faecal butyrate content in patients with PKU [168], related with depletion of *F. prausnitzii* [169]. However, it is not clear if the presence of distinct taxonomic groups within the gut microbiome of patients with PKU is a consequence of the disease or its treatment [170]. More studies are needed to investigate the impact of pharmacological treatments and change in dietary practices or cGMP protein substitutes on microbiota in patients with PKU [171,172], and their influence on metabolomic profile [173].

Recommendation 32**

Any kind of gastrointestinal symptoms can potentially be problematic and routine screening in the clinical setting may be considered.

Level of evidence: low (C)

Strength of recommendation: **conditional**

Expert opinion: completely agree (70 %), mostly agree (30 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7.10. Bone

Osteopenia and osteoporosis have been described in PKU for many years (van Wegberg 2017). Some studies describe a high rate of osteopenia (41 %) and osteoporosis (10 %) in adults with PKU [174] but recent metanalysis suggests that although bone density is lower than in normal population it remains within the normal range for the majority of patients [175].

Many factors have been implicated in the pathophysiology of low bone density observed in PKU, where an increase in osteoclast activity may have a central role [176,177]. Factors that have been suggested include inadequate calcium and vitamin D intakes [178,179], limited natural protein intake [6], energy deficiency [180,181], oxidative stress [177], fatty acid profile [182], and Phe-free protein substitutes.

Patients with PKU have an increase in calcinuria, which is not usually associated with calcium deficiency [6]. Furthermore, the Phe-free amino acid supplements provide a higher dietary acid load, potentially increasing urinary excretion of renal net acid, calcium and magnesium [183].

Some studies have shown that male patients with PKU have a lower BMD than females [184]. A protective effect of cGMP has been proposed [6,185], but this was not confirmed by a 3-year longitudinal study in children [186]. Finally, no data is available evaluating if liberalization of a Phe-restricted diet with sapropterin treatment improves bone density [187].

Practically, good blood Phe control, an adequate intake of calcium and vitamin D, regular exercise and optimization of natural protein intake is important. We suggest follow-up of BMD during early adolescence which, if sub-optimal, can be improved before the peak bone mass is reached. A follow-up by DXA or other methods is indicated in patients who have a low BMD at the first evaluation. In adulthood bone health status can be monitored by measuring alkaline phosphatase [188]. If it is

increased, and not associated with liver abnormalities and recent bone fracture, further investigations are warranted.

Recommendation 33

Adequate intake of calcium and vitamin D, physical activity and optimization of natural protein intake should be ensured for bone mineral density in PKU.

Level of evidence: low (C) / very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 34*

Measurement of Bone Mass Density (BMD) by DXA is indicated in PKU.

The first measurement of BMD should be undertaken during early adolescence before the peak bone mass is reached.

- When BMD is abnormal, the measurement (with or without change of treatment) should be repeated after 1 year. If the results are still low but stable, yearly BMD measurement is unnecessary.
- When BMD is normal, no repeat measurement is necessary. Further DXA need only be considered when there are clinical reasons to do so.

Level of evidence: very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (65 %), mostly agree (35 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7.11. Nutritional follow up

Optimal nutritional status is achieved when nutritional intake matches nutritional needs at any moment in the life cycle. A Phe-restricted diet alone or in combination with pharmaceutical treatments may alter the quantity and quality of the nutrition consumed [189].

A careful dietary assessment is an important tool to determine food and nutrient intake, food patterns, and detect any disordered eating. It is important to collect accurate dietary data since this information is critical to help interpret all other components of nutritional status. The nutritional status evaluation should also include data on anthropometry, physical examination, and biochemical markers [190].

There are no publications in PKU that specifically address the best biochemical markers for nutritional monitoring. A nutritional assessment that includes biochemical markers should be done annually from one year of age. It is important to recognize the usefulness of combining static assays with some functional parameters. While the former mainly quantify nutrient status, the functional markers may give important information about cellular physiology (ferritin, hemoglobin, MCV for iron; methylmalonic acid or total homocysteine in serum for cellular vitamin B12) [6]. A minimum number of biochemical markers is suggested, although the frequency of monitoring and range of markers may vary according to the clinical status of individual patients. In adult patients or with overweight or obesity, additional markers may be considered, e.g. total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, and insulin [6,191–193].

Recommendation 35*

In PKU, it is recommended that nutritional status is routinely assessed by anthropometry, physical examination, dietary assessment, and biochemical markers.

Level of evidence: moderate (B)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 36*

An annual nutritional review is required for any patient who is on a Phe-restricted diet or is self-restricting high protein foods. It is suggested to include:

- a clinical examination including anthropometric parameters (weight, height, BMI).
- plasma amino acids, vitamin B12 (homocysteine)[#], hemogram, MCV and ferritin (iron status), and vitamin D.

(continued on next column)

(continued)

- other markers of kidney function and bone health status (creatinine and alkaline phosphatase, calcium/creatinine excretion), vitamins and mineral status or lipids can be considered if clinically indicated.

[#] When homocysteine is not available, methylmalonic acid can be used as an alternative.

Level of evidence: low (C)

Strength of recommendation: **conditional**

Expert opinion: completely agree (70 %), mostly agree (20 %), partially agree (10 %), mostly disagree (0 %), completely disagree (0 %)

7.12. Oxidative stress

Oxidative stress is observed in PKU as in many other (neurodegenerative) disorders. In PKU it may help the understanding of cerebral PKU pathophysiology. Oxidative stress is evaluated mainly by global markers i.e. reactive oxygen species (ROS), total antioxidant species (TAR), total antioxidant status (TAS), total oxidant status (TOS), and coenzyme Q10, glutathione peroxidase activity, and L-carnitine [194,195].

In PKU, there is clear data suggesting increased oxidative stress is related to poor metabolic control [6,196,197], in addition to micronutrient deficiencies (selenium, zinc, co-enzyme Q10 and perhaps L-carnitine) [6].

There is only one study describing the influence of antioxidant status and the clinical status of patients with PKU that found a lower Conner's continuous performance test in patients with lower selenium status [198]. Due to a lack of clinical data associated with anti-oxidant status, no specific biochemical monitoring is advocated. Good metabolic control and a healthy nutritional status appear to be the key factors in reducing oxidative stress.

Recommendation 37

- No routine monitoring of the anti-oxidant status is currently recommended due to insufficient data regarding the clinical consequences of oxidative stress in PKU.
- Blood Phe control within target ranges should be maintained and micronutrient deficiencies should be avoided as these are important factors in reducing oxidative stress and its possible consequences.

Level of evidence: low (C) / very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (85 %), mostly agree (15 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

7.13. Support when chronic poor metabolic control is observed in children

As reported in the previous European PKU guidelines, there are some cases of children with poor metabolic control whose parents or caregivers consistently decline to engage with healthcare professionals (e.g. non-attendance at clinic appointments, non-response to telephone calls, sporadic return of blood Phe spots). Health care providers have a legal obligation to protect and care for each child in their clinic and when a child has chronic poor metabolic control. Suggested actions are given in recommendation 38.

Recommendation 38*

In patients up to 12 years

when >50 % of the Phe levels are out of target range over a period of 6 months, consider:

- 1) increased frequency of blood Phe monitoring and outpatient visits and re-education
- 2) psychology consultation / social worker intervention
- 3) hospital admission
- 4) alternative therapies (such as sapropterin, if not been given before)

When >75 % of blood Phe levels are out of target range over a period of 6 months and there are other signs of failure of adherence such as lack of co-operation, clinic non-attendance, unresolved issues outside PKU, consider consultation with social services and child safeguarding measures.

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Level of evidence: expert opinion

Strength of recommendation: **strong**

Expert opinion: completely agree (80 %), mostly agree (15 %), partially agree (5 %), mostly disagree (0 %), completely disagree (0 %)

8. Dietary treatment

8.1. Natural protein restriction (requirements/deficiency)

The Phe tolerance is defined as the amount of Phe (mg per kg of body weight or mg/day) that maintains blood Phe levels within the recommended therapeutic target range. This may also be described as natural protein tolerance expressed as g/day. Little is known about how Phe tolerance changes throughout life with PKU, although from the age of 3 years, natural protein tolerance is consistently <0.5 g/kg/day protein in patients on a Phe restricted diet only, not helped by chaperone treatment [7].

Phe tolerance is influenced by PKU phenotype, pharmaceutical treatments, target therapeutic blood Phe, growth, energy intake, protein equivalent dose from protein substitute and pregnancy. Actual tolerance of Phe from natural protein may be higher than the amounts prescribed by health professionals and accurate data on Phe from natural protein intake are commonly difficult to establish. The few studies that have explored Phe/natural protein intake in adulthood have shown that patients commonly tolerate more than the amounts prescribed during childhood years [199,200].

Phe deficiency should be avoided. Symptoms are anorexia, listlessness, alopecia, perineal rash and poor and variable growth in children [6]. In adults, hypophenylalaninemia has been associated with alopecia [201].

Recommendation 39*

Phe intake should be methodically titrated until blood Phe is consistently maintained within the Phe target range.

The actual Phe intake of all patients (on dietary and/or pharmacological treatment) should be re-assessed annually. If blood Phe levels are consistently maintained within therapeutic target range, a challenge with extra dietary Phe should be considered to avoid unnecessary restrictions.

Phe deficiency should be avoided.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (90 %), mostly agree (10 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.2. Protein requirements

In PKU, adequate nitrogen intake is necessary for linear growth, adequate muscle and skeletal mass, and to support immune function. In addition, there is evidence that Phe-free/low Phe protein substitutes will help optimize blood Phe control and improve Phe tolerance. Most international recommendations for PKU advocate an additional protein equivalent amount of 20 to 40 % compared with WHO/FAO/UNU 2007 safe levels of protein intake [6,202,203].

Many surveys describing total protein prescription report a higher amount than suggested in the first European PKU guidelines, most of them published before 2017 (van Wegberg et al. 2017). Since the late 1990's, studies from the US and Europe describe a mean/median total protein intake ranging between 2.7 and 3.1 g/kg/day (17–24 g/day) in infants at 6 months [204–206]. By 3 years of age, mean total protein intake was between 2.0 and 2.2 g/kg/day [206,207]. In late childhood (4–10 years), studies from Europe [207–215], and Australia [216] reported a minimum and maximum average protein intake of 1.2 and 2.8

g/kg/day, whilst the mean/median intakes mostly ranged between 1.5 and 2.0 g/kg/day.

In adolescents (aged 11–18 years) from Europe, data from longitudinal studies report a mean/median total protein intake of 1.1–1.6 g/kg/day [1.1–1.2 g/kg/day in Spain and Germany; 1.6 g/kg/day in Portugal] [207,208,215,217]. Data describing the protein intakes of adult PKU patients is limited. Two studies from Germany [218] and Portugal [215,217], report a mean total protein intake ranging between 1.2 and 1.4 g/kg/day. In the UK, one study described a mean total protein intake of 1.1 g/kg/day, around 33 % more than WHO/FAO/UNU 2007 requirement of 0.83 g/kg/day [219].

Overall, the evidence underpinning protein requirements is limited and a “one size fits all” approach may not adequately reflect changes in protein requirements across a lifespan [220]. WHO/FAO/UNU 2007 safe levels of protein intake are used as a reference to define minimal protein requirements for the healthy population. They are age specific until the age of 19 years but then remain static through adulthood.

In the non-PKU population, several metabolic and physiological factors modulate protein requirements including the protein digestibility of protein sources consumed, the dose ingested per meal/serving, co-ingestion of carbohydrate, energy intake, growth rate, physical activity status, and gender [220]. In PKU, most of the nitrogen is supplied by Phe-free amino acids or low-Phe cGMP. In PKU, the amount of natural vs protein substitute, residual PAH activity, and the patient's protein status (influenced by adherence with protein substitute) all affect protein requirements. Phe-free amino acids have a lower biological efficiency compared with natural protein sources [6]. They are associated with increased oxidation, lower protein retention and higher nitrogen loss especially when taken in large single doses rather than small frequent amounts. Amino acid availability is also influenced by energy/protein ratio, amino acid ratios, genetic influences, age, and physiological stage.

Evidence for protein requirements has been extrapolated from nitrogen balance studies evaluating the minimum protein (i.e., nitrogen) requirements to balance nitrogen losses in healthy adult populations. This does not consider long-term physical and functional outcomes. Results from the indicator amino acid oxidation (IAAO) method, suggests that protein requirements are higher than previously estimated both in the healthy and PKU populations, but research is still limited, and participant numbers studied are small [221,222]. In contrast, Van Rijn et al. found no difference in protein metabolism between adults with PKU and matched healthy volunteers using a L-[1-13C] valine tracer method. In this study, six patients with PKU received a dose of Phe-free amino acids equivalent to 0.96 g protein/kg per d (120 % of the WHO safe levels of protein intake) while the six healthy volunteers received 0.8 g intact protein/kg per d (100 % of the recommended daily allowance) [223].

Some studies indicate sub-optimal growth in PKU which may reflect sub-optimal protein intake. In a meta-analysis, growth impairment was reported in eight of thirteen studies [201]. Length/height was shorter, particularly during the first three years of life, in puberty, and on reaching adulthood [201]. In children, studies that reported a higher total protein intake had optimal growth [201]. Although children are more sensitive to growth impairment, utilization and digestibility of protein decreases with age [202]. Little is known about protein status in elderly people with PKU, but a lifetime of consuming synthetic protein and low intake of protein substitute intake (associated with low adherence) may increase the risk of frailty and sarcopenia (loss of skeletal muscle mass, strength and function) in later life [203].

More research is necessary to understand protein requirements in PKU in both children and adults. There is insufficient new evidence to change existing recommendations, although a separate recommendation is given for children and adults with PKU. Individual nutritional needs, health status and clinical condition should always be considered. Examples for calculation are provided in the previous European PKU

guidelines and the accompanied dietary handbook on PKU [6,7].

Recommendation 40*

In PKU, protein intake should optimize health and functional outcomes. Many factors affect individual protein requirements. Total protein intake should supply at least the age-related safe levels of protein intake [WHO/UNU/FAO 2007, but for patients on dietary treatment additional protein is required as follows:

For children (≤ 17 y), an additional 40 % of Phe-free/low Phe protein equivalent from protein substitutes is suggested.

For adults (≥ 18 y), an additional 20 % to 40 % of Phe-free/low Phe protein equivalent from protein substitutes is suggested.

Level of evidence: low (C) / very low (D)

Strength of recommendation: **conditional**

Expert opinion: completely agree (75 %), mostly agree (25 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.3. Protein substitutes

Protein substitutes are an essential source of Phe-free/low Phe nitrogen for all patients on dietary management. Amino acid derived nitrogen is crucial for synthesizing precursors of major energy molecules, nucleic acids and making compounds that can regulate major biochemical signalling pathways, such as nitric oxide [224]. They also provide a source of Tyr and other large neutral amino acids (LNAA, see section 8.3.3), and usually are supplemented with micro and macronutrients. They contain a variable amount of added carbohydrate, fat and energy. They are commonly based on free L-amino acids or low Phe cGMP. Commercial cGMP always contains some residual Phe and it is supplemented with free rate limiting amino acids (section 8.3.1) although its optimal amino acid profile still remains to be determined. Plasma amino acid availability is influenced by the efficiency of dietary protein digestion and absorption rates in the intestine. A assessment of digestibility of protein sources is limited in PKU.

Compared to natural protein, free amino acids bypass degradation by proteases in the digestive process, resulting in blood levels that are higher, peak faster and decrease more quickly than when compared with natural protein [225]. To help minimize the effect of this, they should be given in equally distributed doses, 3 to 4 times throughout the day.

Sustained-release Phe-free amino acid formulations aim to prolong the absorption of free amino acids and provide a physiological absorption profile similar to that of natural proteins. Prolonged release amino acids either have a sodium alginate matrix or are small granules coated with ethyl cellulose and sodium alginate, produced by physiologic technology. Animal and non PKU human studies suggest benefit but data in patients with PKU is still limited [226–228].

Several new age-targeted proteins-substitute formulations have been developed with the aim of improving palatability and acceptance, and ultimately patient adherence. They are commonly in the form of powders, liquids, gels, granules, and tablets [205,229–231]. Pre-packaged and pre-measured products have helped accuracy, adherence, and ease of protein substitute prescription for clinicians [232]. Their palatability, smell, texture and after taste remain challenging to patients.

There is only basic and under developed guidance for the formulation, evaluation, and patient access to marketed protein substitutes, designed for PKU. European legislation states that protein substitutes are considered as foods for special medical purposes (FSMPs). This legislation allows flexibility in their formulation providing it is based on sound medical and nutritional principles to meet their intended use and are subject in principle to general food legislation, and novel foods, contaminants additives and packaging legislation. The amino acid and nutritional composition of protein substitutes may vary, and overall stricter guidance is necessary to regulate their nutritional composition

and scientific evaluation to ensure clinical safety.

Recommendation 41*

- A Phe-free/low Phe protein substitute should be prescribed to any patient with PKU treated with a Phe restricted diet consuming less than the FAO/WHO/UNU 2007 safe levels of natural protein intake.
- All countries should assure access to a wide variety of protein substitutes to ensure individualized patient care, accommodating for patient developmental age, preferences and natural protein tolerance.
- Phe-free/low Phe protein substitutes should be evenly administered and given at least 3 times throughout the day.
- Ongoing patient adherence with protein substitute should be monitored by examining blood Phe control, patient growth, protein and nutritional status, and prescription usage.
- All manufacturers should provide clinical evidence to support the nutritional composition, efficacy and patient tolerance of any new protein substitutes based on any Phe-free/Low Phe protein source.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (85 %), mostly agree (10 %), partially agree (5 %), mostly disagree (0 %), completely disagree (0 %)

8.3.1. Glycomacropeptide

Casein glycomacropeptide (cGMP) is a low Phe bioactive 64-amino acid glycomacropeptide derived from kappa casein in bovine milk. It contains sialic acid (N-acetylneuraminic acid), galactosamine and galactose. This protein is high in threonine, isoleucine and valine but low in some essential or semi-essential amino acids i.e., histidine, leucine, tryptophan, methionine, Tyr and arginine. It is usually supplemented with rate limiting amino acids (except Phe). To utilize cGMP as an alternative protein substitute to L-amino acids, cGMP should be supplemented with any deficient amino acids to meet WHO requirements. It should be noted that currently not all available products meet these requirements, so the amino acid profile of cGMP based protein substitutes should be checked before use to ensure they meet age appropriate requirements. cGMP has many functional and physiological properties. It acts as a prebiotic, increases the production of short-chain fatty acids (SCFA), and has anti-inflammatory properties [233]. It is associated with improved taste and palatability [234–236]. Currently however, there is no clear evidence that cGMP has any beneficial impact on growth, body composition, nutritional biochemistry, kinetics, blood Phe variability, microbiota, bone and satiety in PKU [172,237–247].

With cGMP usage, blood Phe levels may increase as it does contain some residual Phe, with most formulations containing around 36 mg Phe per 20 g protein equivalent. Six published studies from 4 patient cohorts, in 3 countries have investigated the use of cGMP on blood Phe control for ≥ 6 months [172,234,240,241,244,248]. Fifty percent ($n = 4$) of studies include patients aged from 9 to 43 years (total number of subjects = 38). Only three studies included children below the age of 10 years, but they were from the same patient cohort and centre. Five of six studies reported an increase in blood Phe with cGMP, and this reached statistical significance in 3 of 6 studies in children but blood Phe was still maintained within the expected therapeutic target range of 120 to 360 $\mu\text{mol/L}$. In 2 of 3 studies in older patients (teenagers and adults), the blood Phe level increased but did not reach statistical significance, possibly due to low patient numbers in each study ($n = \leq 18$ or less). The protein equivalent provided by cGMP was variable between studies and provided from 33 % to 100 % of protein substitute intake. If cGMP is prescribed in children, it should be carefully introduced (its Phe content should be calculated and measured as part of the daily Phe allocation) and close monitoring of blood Phe control is essential. No studies have reported using cGMP in children under 4 years of age. Experience with only one pregnancy has been published [249].

There may be advantage in using cGMP in adolescents or adults who are not adherent with their Phe-free amino acid supplements. The improved palatability of cGMP's may enable patients to take all their protein substitute as prescribed but potential impact on blood levels should be considered.

Recommendation 42**

cGMP can be prescribed as a protein substitute in patients with PKU aged ≥ 4 years provided that its Phe content is considered in daily Phe allocation, and that the profile of all amino acids meets age-appropriate requirements.

Level of evidence: moderate (B)

Strength of recommendation: **strong**

Expert opinion: completely agree (75 %), mostly agree (20 %), partially agree (5 %), mostly disagree (0 %), completely disagree (0 %)

8.3.2. Tyrosine

Tyr is an important precursor of the catecholamines dopamine and norepinephrine. Fasting blood Tyr levels are commonly low but peak post protein substitute administration. A Cochrane systematic review reported that there is no new evidence to demonstrate the effectiveness of Tyr supplementation in patients on dietary treatment, supplemented with protein substitute in PKU [250].

Recommendation 43

Additional tyrosine supplementation is not advised for patients on dietary treatment as tyrosine is already added to all Phe-free/Low Phe protein substitutes suitable for PKU.

Level of evidence: moderate (B)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.3.3. Large neutral amino acids

As described in the first edition of the European PKU guidelines there is insufficient evidence to support routine use of LNAA [6]. LNAA includes tryptophan, Phe, Tyr, histidine, methionine, threonine, leucine, isoleucine, and valine. The non-Phe LNAA's are suggested to compete with Phe for transport across the blood gut-blood barrier, and the blood-brain barrier. Giving (non-Phe) LNAA are therefore considered to result in lower Phe and higher LNAA levels in the brain [4,6]. Some centers prescribe LNAA in particular for individuals who struggle to control their blood Phe levels with dietary Phe restrictions alone [6].

Recent animal studies have shown LNAA to be as effective as the Phe-restricted diet to restore brain monoamines [251,252]. Burlina et al. reported increased blood Tyr levels in 12 adults with PKU and improved adherence in taking LNAA rather than the regular protein substitutes [253]. There is no data for children or during pregnancy. Further research is needed to determine the optimal dosage and amino acid ratio's.

Recommendation 44

There is inadequate evidence to recommend the routine use of LNAA for PKU. They should not be given to children or during pregnancy.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (80 %), mostly agree (10 %), partially agree (10 %), mostly disagree (0 %), completely disagree (0 %)

8.4. Nutritional requirements

In PKU, the nutritional intake of energy, macronutrients and micronutrients should meet the same dietary reference values as for the healthy population. Dietary assessment plays a central role in ensuring

that individuals with PKU meet their nutritional needs, for which several techniques or tools can be used to estimate this [254,255]. Catabolism and protein deficiency should be avoided as this can lead to poor blood Phe control.

Recommendation 45

In PKU, the nutritional intake of energy, macronutrients and micronutrients should meet the same average estimated amounts/dietary reference values as for the healthy population.

For all age groups, there should be focus on achieving a balanced intake of all nutrients, avoiding catabolism or deficiency but preventing excess of any nutrient that may lead to over nutrition or toxicity. Supplementary nutrients (vitamins, minerals and LC-PUFA's) added to Phe-free/low Phe protein substitutes should be in the amounts that will at least meet normal population "dietary reference values."

Assessment of dietary intake should be performed at each clinic visit with extra attention directed to patients who are non-adherent, do not have prescribed Phe-free/low Phe protein substitutes (with added micronutrients), or who are at higher risk of nutritional deficiency.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.5. Low protein foods, fruits vegetables

There is both short and long-term evidence from three studies in different PKU international centres that indicates that the consumption of fruits and vegetables containing Phe ≤ 75 mg/100 g without weight measurement or restriction is not associated with elevated blood Phe [6]. Potatoes are an exception as they contain a variable Phe content and their effect on blood Phe control of their "free" inclusion in the diet requires further testing. There is also evidence in children that fruit and vegetables containing Phe ≥ 76 mg/100 g increases blood Phe to above target range, and their intake should be calculated and measured as part of the daily Phe allocation [256]. A list of fruits and vegetables allowed without measurement in PKU is available in Table 3 of the PKU dietary handbook [7].

Special low protein foods e.g., bread and pasta are an essential part of a Phe restricted diet supplying an important source of energy and dietary variety. In patients with classical PKU, they may supply up to 50 % of energy intake [186,257,258], although their energy contribution is variable and commonly dependent on individual natural protein tolerance. Patients on pharmaceutical treatments, with a higher natural protein tolerance, have limited need for special low protein foods. Special low protein foods are high in carbohydrate, and there is evidence that in some countries they contain more energy and fat than regular foods [259], although this is not reported in all countries [260]. However, they commonly contain isolated starches such as maize and potato starch, may contain added sugar and may be higher in sodium than regular foods [260].

Although manufacturers must use approved food ingredients and food additives according to EU law, there is little legislation controlling their nutrient composition. On food labels, manufacturers must add nutrient information about their energy, fat, carbohydrate and sodium content, but vitamins and/or minerals need only be declared if they are present in "significant amounts".

Recommendation 46

Fruits and vegetables (except potatoes) containing Phe ≤ 75 mg/100 g of food can be safely given without measurement or estimation in a Phe restricted diet without loss of blood Phe control. Some unrestricted fruit and vegetables should be encouraged in the diet in early life to encourage long-term healthy feeding patterns.

Level of evidence: moderate (B)

Strength of recommendation: **strong**

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(continued)

Expert opinion: completely agree (85 %), mostly agree (15 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 47**

Special low protein foods should be considered as an important energy source in the diets of people with PKU. However, it is important that their energy, fat, sugar, and salt content should not exceed what is normally recommended for any food.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.6. Breastfeeding

Breast milk is universally recognized as the optimal source of nutrition for healthy infants up to the age of 2 years [261–265]. The mean protein composition of mature, term milk is estimated to be approximately 0.9 to 1.2 g/dL and has a low protein:energy ratio [262,266]. In PKU, evidence indicates when used combination with a Phe-free amino acid formula it is safe for infants [6]. Growth is normal, satisfactory blood Phe control is achieved and maternal stress is reduced [267]. Impact on neurodevelopmental outcome and immune status is difficult to assess associated with the rarity of PKU, the partial/limited intake of breast milk and variable ages at which breast milk is stopped.

In PKU, different methods of breast milk administration have been used, including a pre-measured volume of Phe-free amino acid formula before each breast feed or alternate breast and Phe-free amino acid formula feeds [268]. There is no evidence to show that any one method leads to better blood Phe control [254] or has advantage with infant growth. However, administering Phe-free amino acid formula before breast feeds is associated with a longer duration of breast feeding, although the reason for this is unclear and requires further study [269].

Recommendation 48

In infants with PKU, breast-feeding in combination with a Phe-free infant L-amino acid formula should be encouraged.

Level of evidence: moderate (B)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.7. Aspartame

Aspartame (E951), a non-nutritive sweetener, is a synthetic dipeptide known as N-L-alpha-aspartyl-L-Phe methyl ester. It is completely hydrolysed to free Phe (50 %), aspartic acid (40 %) and methanol (10 %) and should be avoided in a Phe restricted diet. It is added to soft drinks, chewing gums, desserts, jelly and tabletop sweeteners and some medications, particularly oral solutions. EU food regulations state that it is mandatory that aspartame addition is identified on food labels, but it is not mandatory for alcohol, and its presence may not be easily identifiable when food/drink labels cannot be read at the point of purchase or issue e.g., in restaurants or vending machines [270]. Also importantly, the amount of Phe in foods/drinks remains undisclosed and the quantity may vary even within the same brands purchased in different countries [271], while brands may change their quantity without notice. In PKU, accidental aspartame consumption is common [270].

Suitable non-Phe containing sweeteners include sucralose, saccharin, steviol glycosides and acesulfame potassium. Neotame (E961) contains aspartame but the peptide linkage between aspartic acid and Phe is resistant to hydrolysis, reducing Phe availability [272]. Although this sweetener may not be identifiable on food labels by name,

it is safe in PKU.

Recommendation 49*

- The artificial sweetener aspartame should be avoided in patients on a Phe restricted diet, especially in beverages and table top sweeteners (in which the aspartame content is high).
- Readily accessible information about the presence of aspartame in all foods, drinks and medications (including draft drinks and alcohol) should be mandatory.
- Manufacturers should voluntarily declare the amount of Phe provided in foods and drinks on labels.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.8. Dental care

Good dental health is important, particularly as dental caries causes pain and infection, with consequential elevation of blood Phe levels. There are an increasing number of studies reporting dental health in children with PKU but not in adulthood. Overall, there is more dental caries in the primary dentition and higher levels of acid erosion in children with PKU than the general population [273,274]. Decayed, missing and filled surfaces index is high compared to controls [274,275].

There is little data to suggest that the condition PKU itself affects teeth and oral health but there are three studies suggesting a higher prevalence of enamel developmental defects in PKU [276–278] which is also reported in another IMD, methylmalonic aciduria [279].

In PKU, studies suggest that dental caries and associated infection is related to a Phe restrictive diet and poor dental hygiene practices [273,274]. A frequent intake of carbohydrate and the potentially acidic protein substitutes increases the risk of dental caries and erosion [273,274]. In PKU, irregular tooth brushing and low use of interdental cleaning products leading to plaque accumulation is reported [273,274]. A high prevalence of untreated caries with irregular dental health checks is common [273,274,280].

Therefore, in PKU, it is essential that dental caries prevention should be encouraged from a young age. Prevention of dental caries and periodontal diseases is dependent on oral hygiene, dental care and maintenance of regular dental visits. A good dental health routine with regular dental health checks is essential. Rinsing the mouth out with water after protein substitute consumption (to prevent the solution sticking to the teeth), taking protein substitute quickly, limiting sweet foods and drinks to mealtimes, and discouraging the use of infant feeding bottles after 1 year of age are important.

Recommendation 50**

Caregivers of children with PKU should be encouraged to seek early dental advice and implement a robust and comprehensive prevention programme

Level of evidence: moderate (B)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.9. Disordered eating

Disordered eating and eating disorders are not the same. Eating disorders (anorexia nervosa, bulimia nervosa) are disturbances of eating or eating related behaviour that results in an altered consumption of food significant enough to impair the individual's physical health or psychosocial functioning. This is diagnosed according to specific and narrow criteria. Disordered eating is abnormal behaviours focused around eating or feeding that does not fit the pattern of a specific eating disorder. It is a change in normal eating pattern.

People with PKU on a Phe restricted diet have limited exposure to different dietary experiences from early infancy and entrenched abnormal dietary patterns may be established in early childhood.

Although disordered eating has not been systematically studied in PKU, abnormalities in eating behaviour, including restrictive eating and food neophobia have been identified in children and adults [281–285]. Negative attitudes surrounding food and body image are also reported [286–288].

Eating behaviours associated with disordered eating include irregular, chaotic eating patterns, anxiety/discomfort associated with specific foods, restrictive eating, refusal to deviate from a rigid food comfort zone, binge eating, emotional eating, feelings of guilt and shame associated with eating, feelings of loss of control around food, preoccupation with food, and a weight and body image that negatively impacts on quality of life [133]. It may be associated with excessive weight gain, food conflicts, poor psychological health (e.g., anxiety or depression), or associated with parental anxiety and parenting style [289]. It may lead to nutritional imbalance.

There is little data about the rate of disordered eating in patients on adjunct pharmaceutical treatments, although there is early evidence that patients with intransient abnormal eating behaviours may not change their dietary patterns when dietary protein intake is increased [285].

Generally, disordered eating is lower in severity and intensity and does not fit the pattern of a specific eating disorder such as anorexia nervosa and bulimia nervosa [288,290]. However, in studies with small patient numbers, there is some evidence suggesting that patients with PKU have a higher incidence of eating disturbance [283,286,288]. It is also established that individuals with disordered eating are at greater risk of developing eating disorders [291], and Bilder et al. reported from a retrospective USA insurance database comparing adult patients with PKU ($n = 3714$), diabetes mellitus ($n = 7060$) and the general population ($n = 22,726$) that the prevalence of eating disorders was 3.4 % in PKU, 1.9 % in diabetes mellitus and 0.9 % in the general population [292]. Eating disorders are associated with serious physical and psychosocial health outcomes, so any evidence of disordered eating in PKU patients should be carefully monitored [287] and early referral to eating disorder specialists should be considered if an eating disorder is suspected.

Increased awareness among health professionals about potential disordered eating in patients with PKU is required and there is a need to develop validated screening tools to use in combination with reliable methods for nutritional assessment to evaluate and monitor any disordered feeding. Patients with eating disorders require specialist care and it is important that the PKU team work closely alongside with an eating disorders team to ensure the latter have full understanding of the treatment needs of PKU.

Recommendation 51**

Early referral to an eating disorders team may be considered if there are any clinical indications.

Level of evidence: low (C)

Strength of recommendation: **conditional**

Expert opinion: completely agree (80 %), mostly agree (20 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.10. Overweight

The prevalence of overweight and obesity is frequently studied in PKU clinics. Some studies report higher occurrence of overweight and obesity compared to controls or the general population [191,293,294], while others report a similar prevalence compared to controls or the

general population [193,215,216,295].

There is a tendency for an increased prevalence in females [296] and in those with poor metabolic control [294,297]. In patients on sapropterin treatment there are contradictory results when comparing to patients on a Phe-restricted diet only [209,298].

Although in PKU, the prevalence of overweight is probably similar to the general population, the metabolic consequences of increased adiposity should not be neglected. Many studies have underlined the importance of identifying and monitoring any metabolic derangement caused by adiposity [174,191,299,300], and its potential impact on cardiovascular disease risk [151,293,299]. In PKU, a significantly higher fasting TG/HDL ratio has been reported [215].

While it is unknown if special low protein foods and protein substitutes have any causative factor [257], protein substitute composition has changed throughout the history of treatment [232]. Interestingly, overweight in PKU has been associated with a variation in the gene responsible for the Phe transport across the blood-brain barrier [301,302]. The tendency for a lower amount of weekly physical activity is associated with a lower energy expenditure that may contribute to obesity [303]. Overweight, and if possible, body composition together with cardiometabolic markers should be monitored in patients with PKU [192].

Recommendation 52**

In PKU, preventive life-style strategies should be implemented to avoid overweight and associated comorbidities.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.11. Exercise

Few reports have described physical activity practices in patients with PKU [287,303–305], but they should be encouraged to do the same amount of exercise as in healthy individuals without PKU [306]. Grunert et al. studied the impact of exercise on Phe metabolism, finding that the mean blood Phe remained stable after a 20 min bicycle-exercise whereas mean blood Tyr declined significantly by 16 % [105]. Another study showed no statistically significant differences for blood Phe, Tyr, Phe/Tyr ratio and BCAA levels after 30 min of controlled exercise [307]. Strenuous exercise may negatively impact on blood Phe levels caused by muscular catabolism, but Sumanszki et al. showed that strenuous exercise (peak treadmill test to exhaustion) increased blood Phe levels by only 5 % [308]. Even though patients with PKU showed similar basal metabolic rate, similar aerobic capacity and workload peak compared with controls, patients with poor metabolic Phe control showed lower VO₂ consumption [307]. Patients with PKU may also have lower creatine levels when compared to controls which may negatively affect their exercise performance [309]. Patients should be informed about the risks of using dietary supplements, containing high amounts of natural protein and/or aspartame for sports activities [310].

Nutritional adjustments may be necessary in patients with PKU performing regular exercise or sports, allowing them to maintain acceptable metabolic control while attaining maximal athletic performance [311]. Emphasis should be given about the distribution of protein substitute throughout the day with a post-exercise dose of Phe-free/low Phe protein substitute to promote protein synthesis. As recommended for the general population, a carbohydrate-rich diet should be maintained with specific attention to the pre- and post-exercise meals. Fat-rich foods should be avoided pre, during and post exercise meals. Maintaining adequate hydration is essential. Nutritional balance should

be promoted to achieve an optimal micronutrient status [311].

Recommendation 53**

Patients with PKU should be encouraged to participate in exercise and sporting activities, and maintain adequate nutrient and energy intake to ensure optimal performance.

Level of evidence: low (C)

Strength of recommendation: strong

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.12. Burden of diet

In PKU, treatment introduces a financial, time and psychosocial burden for adults and children with PKU and their family caregivers, affecting their ability to follow the treatment regimen. This may be more challenging with advancing age. There may be extra direct “out-of-pocket” costs of living with PKU such as expenditure on foods for special medical purposes (FSMP) e.g. special low protein foods and protein substitutes, medications, laboratory tests, blood Phe monitoring, and healthcare visits (clinic and travel costs) [114,312–315]. Loss of earnings is an indirect cost. Adults or family caregivers may take unpaid time off work to attend appointments or caregivers may stop work or reduce their working hours to care for their child with PKU [316,317].

Health care reimbursement systems or public health insurance systems vary from country to country and some patients may only have access to limited insurance coverage for protein substitutes but not special low protein foods. Some reimbursement or insurance systems may not extend into adulthood [114,318] or coverage may be for part of a country only. This has led to families deriving financial support from grandparents, loans, personal savings or sale of assets [319]. Some patients may stop dietary treatment as they cannot afford the cost of FSMP.

Even when FSMP are provided by the national health care systems, there may be delays in receipt of supplies, chronic under-dispensing of essential dietary products [315,320] or access may be limited because of complex and encumbered health administration systems [260]. Delayed processing of applications by insurance systems may lead to temporary cessation of dietary treatment. Some health care providers must conduct regular re-negotiation of their FSMP contracts with manufacturers, leading to frequent changes in the type of protein substitute formulations available for patients, increasing the dietary challenges and frustrations for patients [317].

Cross-sectional surveys indicate that the management of PKU is associated with a significant time burden [316,321], particularly for family caregivers caring for children with PKU. This includes extra time for shopping, food preparation and supervising the dietary treatment. Dietary management is complex and time-consuming, requiring good knowledge of the protein content of foods, recipes, cooking skills, continuous food measurement, as well as good organizational and parenting skills. Meal preparation time is lower in patients treated with sapropterin [322]. Family caregivers, when they are working may have difficulty finding reliable people to care for their children [323].

Social exclusion is also commonly described, particularly when eating outside the home [133,324], with some restaurants unwilling to prepare low protein meals.

In conclusion, a Phe restricted diet in PKU is associated with significant economic, time, practical and social burden in all countries, that renders dietary management very challenging to implement.

Recommendation 54**

Governments (health systems) should develop policies to ensure state-funded/health insurance reimbursement of special low protein foods and Phe-free/low Phe protein substitute availability for all patients with PKU of all ages on any dietary restrictions.

(continued on next column)

(continued)

Level of evidence: low (C)

Strength of recommendation: strong

Expert opinion: completely agree (80 %), mostly agree (15 %), partially agree (5 %), mostly disagree (0 %), completely disagree (0 %)

8.13. Support

A diagnosis of PKU is a life-changing event [289]. Caring for children with PKU brings additional stress and responsibilities for family caregivers and their families and they experience concern and fear about their child's short- and long-term health. Some studies report a higher incidence of maternal anxiety and depression in caregivers, compared with mothers of healthy controls. This negatively impacts on their quality of life and psychosocial wellbeing [325,326], although this is not reported by all [6,327,328]. The coping behaviours and resilience of caregivers is highly variable, influencing the need for individualized support. Programs such as the Healthy Living Triple P parenting intervention designed for families of children with chronic health conditions may help increase parental self-regulation and positive parenting practices [329].

Adults with PKU commonly express many frustrations particularly about the demands of treatment, they feel guilt, uncertainty about aging, and the social impacts of the disorder [133]. Adults have described the loss of social support from their family members [330]. It is important that IMD teams tailor support, respecting their autonomy, but also empowering them to actively seek and maintain social support from their family, partners, and through social networking with others with PKU.

Positive, non-judgmental, and continual interactions with health professionals are important in establishing effective patient-professional relationships and acceptance of PKU [330]. Patients and caregivers commonly describe the importance of coordinated actions between health professionals [330] and they report issues associated with the health care systems outside of PKU specific care, particularly when encountering providers unfamiliar with PKU [331]. The repeated need to explain PKU is exasperating for both patients and family caregivers. Patients highlight the lack of communication between local health care and specialist teams [133], emphasizing the need for improved coordination and collaboration between health care providers. They also have requested the need for patient-held electronic medical records) [323,332].

Recommendation 55*

- Health professional support from the IMD team should be offered throughout life in order to encourage normal, healthy outcomes. Support should be individualized according to different family conditions.
- Empower patients / parents by:
 - o helping them to actively seek and maintain social support from other patients and families with PKU
 - o giving them access to personal healthcare records to help them understand their treatment goals, health status and enable them to monitor their progress.
- Communication should be optimised between all health care providers, both within and outside specialty health care teams.
- Any barriers to accessing care should be addressed as these are likely to have an impact on quality of life and negatively affect the outcomes of individuals with PKU and their families.

Level of evidence: low (C)

Strength of recommendation: strong

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.14. Education

Patient education lies at the core of managing treatment for PKU effectively. Self-care that enables acceptable metabolic control and social outcomes should be promoted. Practically, caregivers should be encouraged to equip children with suitable knowledge about PKU and its treatment from an early age. They should actively involve siblings and other family members [333]. Periodic patient/caregiver re-education throughout life is essential.

All educational materials should be readily accessible, accurate and understandable. Caregivers reflect that the most effective educational method is “one to one counselling” delivered by health professionals [334], although PKU mobile apps have an increasing role [335]. Better treatment knowledge is associated with improved adherence [336], but findings are not consistent [337], and it may not change patient and caregiver attitudes towards a Phe restricted diet [338].

Recommendation 56**

- Effective education should be considered essential to help enable lifelong self-care that will assist in the attainment of satisfactory blood Phe control and social outcomes.
- Nutrition education should begin in early childhood. All educational tools and information should be appropriate for age and level of understanding.
- Periodic assessment of patient/caregiver knowledge with educational updates should be considered important.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

8.15. Illness

It is well established that Phe levels increase during fever and illness, associated with catabolism. Although patients with PKU are not at acute risk of metabolic decompensation, blood Phe levels are likely to remain high until symptoms have abated. In addition, all young children experience more illness episodes in their early years when, in PKU, they are particularly vulnerable to the effects of sustained high and fluctuating blood Phe levels.

Although during illness a formal emergency regimen is not normally necessary as acute metabolic decompensation does not occur, it is important to prevent an excessive rise in Phe levels, by administration of Phe-free/low Phe protein substitutes and high carbohydrate drinks. Adequate hydration should be maintained, and oral rehydration solutions (aspartame-free) are suitable. In patients receiving dietary therapy only, there is no evidence for decreasing natural protein intake during illness episodes. Antipyretics should be given for high temperatures following normal age/weight-based recommendations. For the speedy treatment of infections, it may be better to use aspartame containing medications, until a suitable aspartame free medication is sourced. However, in chronic disease and long-term medication use, it is better to find alternative aspartame free medications. Medications sweetened with aspartame may contain Phe from 1 to 25 mg per dose, but some preparation may contain more (e.g. Plenvu [Norgine], an aspartame containing bowel cleaning preparation powder contains 493 mg/dose of Phe).

Prevention of infectious disease is important and all children with PKU should be encouraged to complete their child health vaccination program.

Some case studies of children on sapropterin treatment report high blood Phe levels with infection [339–343]. A reduction in Phe intake may be necessary, particularly in young children, but no studies have

demonstrated the effectiveness of this.

Co-existent and chronic disease is not uncommon in patients with PKU [164], and may adversely affect blood Phe control mainly associated with infections, cachexia, sepsis, surgery, trauma, chemotherapy, steroid therapy, chronic vomiting, mouth/teeth infections, and inability to eat [164,344,345], as well as vaccinations. Malnutrition is particularly common in cancer. This may be associated with the presence of a tumour or the medical and surgical anticancer treatments. For adult patients, total protein requirements are increased (> 1 g/kg day and up to 1.5 g/kg). In contrast, in patients with gastrointestinal disorders, with uncontrolled malabsorption, blood Phe may be lowered [164]. In all patients with co-existent chronic disease, it is important to advise and support the non-PKU medical teams and help with treatment strategies for PKU [346]. Drugs such as methotrexate (acting as a mild immunosuppressant that exhibits anti-inflammatory activity) may increase blood Phe levels associated with a decrease in protein synthesis. Methotrexate is also reported to inhibit dihydropteridine reductase (DHPR) enzyme, potentially increasing blood Phe levels.

With surgery, oral intake should not be stopped for an extended period preoperatively as it causes a catabolic state. Anaesthetists may accept ORS/glucose orally as clear fluids prior to surgery. The amount of glucose can be determined using the generic metabolic emergency protocol tool via <https://www.emergencyprotocol.net> or BIMDG website. If a patient requires major or emergency surgery, intravenous glucose should be given during and after surgery [347].

Due to a possibility of post operative paraparesis in vitamin B12 deficient individuals with PKU, it is recommended that nitrous oxide, which inactivates B12-dependent methionine synthase, [347,348] should be avoided in patients at risk of vitamin B12 deficiency.

Generally anesthesia with propofol, especially if used for short periods, can be used without difficulty, although it was reported to cause hyperthermia in a child with PKU without clear reason [349].

Recommendation 57*

In children with PKU, during acute illness in order to prevent an excessive rise in blood Phe levels, some measures should be considered including encouraging the intake of Phe-free/low Phe protein substitute and high carbohydrate supplements. Hydration should be maintained. No other dietary changes are necessary. In children treated with sapropterin, a reduction in natural protein intake may be necessary.

Level of evidence: low (D)

Strength of recommendation: **conditional**

Expert opinion: completely agree (70 %), mostly agree (25 %), partially agree (5 %), mostly disagree (0 %), completely disagree (0 %)

8.15.1. Total parenteral nutrition

Parenteral nutrition in children with inborn errors of metabolism is rarely necessary, but if required, it presents a challenging therapeutic dilemma. Suitable total parenteral nutrition formulas are not available for PKU and there is very limited information about the management and outcome of patients with PKU requiring treatment with parenteral nutrition. Infants, particularly those who are premature, and young children requiring long-term parenteral nutrition, are likely to be at risk from permanent brain damage if blood Phe levels cannot be controlled [6]. In contrast to studies reported on in the first edition of the guideline on PKU, studies in very premature babies have shown that due to their rapid growth rate, associated with high Phe tolerance (up to 70 to 110 mg/kg/day) in the first weeks of life, blood Phe levels do not always increase markedly despite using parenteral nutrition solutions with a standard amino acid composition [350]. There are reports of a specially prepared Phe-free intravenous amino acid solution and a commercial preparation with a high content of branched chain amino acids designed

for use in hepatic failure being used in PKU [6,351]. Even if the need for this specific Phe-free parenteral formula is rare, suitable parenteral formula with global accessibility should be developed.

9. Special groups

9.1. Maternal PKU

High blood Phe during pregnancy has a teratogenic effect on the developing foetus causing growth retardation, microcephaly, intellectual disabilities and birth defects, including congenital heart defects (CHD) [6]. In line with multiple previous reports [6], a recent publication of Yildiz et al. described an association between higher blood Phe levels and an increased incidence of birth defects [26].

Pregnancy in women with PKU, should be considered as high risk, requiring follow up by an experienced metabolic team. Good communication with obstetric teams is important, as they may have limited experience with managing pregnancy in women with PKU and will require clear guidance on the potential impact of suboptimal metabolic control, monitoring of foetal growth and organogenesis, and the post-delivery follow up of children at risk of the maternal PKU syndrome.

As discussed in section 6.4, foetal blood Phe is higher than maternal blood Phe concentration due to active placental transport [87]. Although this is true and would favour lower target Phe levels in maternal blood, lower target Phe concentrations have not been evaluated or shown to be really necessary. Therefore, in line with the first European PKU guidelines, blood Phe should be maintained between 120 and 360 $\mu\text{mol/L}$ from preconception onwards (see section 6.4). Women with untreated blood Phe levels $<360 \mu\text{mol/L}$ do not require treatment.

Blood Phe should be checked at least weekly in the pre-conception period and at least twice weekly during pregnancy (see section 7.2.2).

For optimal preconception treatment, stable blood Phe control within the recommended therapeutic target range should be maintained before women with PKU conceive. Contraceptive strategies should only be discontinued after stable blood Phe within the target range have been achieved for at least 2 weeks. The time to reach stable blood Phe varies between women as it is influenced by personal circumstances, access to protein substitutes and special low protein foods, responsiveness to sapropterin, and family support, though women are generally highly motivated. As maternal PKU treatment needs considerable effort, referral to a fertility centre should be considered for well-controlled patients along with their partners who have unsuccessfully tried to conceive for 6 months.

Unplanned pregnancies should ideally be prevented by continuous education (starting preadolescence) and family planning. To prevent unplanned pregnancies, women at childbearing age should be counselled regarding family planning and contraceptive use. Ford et al. in a survey from the United Kingdom reported 18 % ($n = 23 / 128$) unplanned PKU pregnancies [352]. Women with an unplanned pregnancy should be seen within 24 h, without any delay, to initiate dietary treatment. Immediate commencement of dietary treatment and controlling blood Phe levels can be difficult.

Women require support from their partner/family and health care professionals [353]. Pregnancy is associated with fears and distress [352,353]. Ford et al. reported that 60 % of 273 women were concerned about causing harm to their baby, 48 % feared having an unplanned pregnancy and some even avoided physical intimacy [352]. When looking at coping strategies, women also emphasised the importance of support from another mother with PKU who has experienced pregnancy and access to psychological services [353,354].

Following delivery, women continue to need support from their health care professionals. Ford et al. reported that 48 % of 93 women had low mood or sadness, 41 % were depressed, 34 % felt unable to cope and 33 % said they could not manage their own PKU as well as take care

of their baby [352]. An offspring with a birth defect or severe health problems must be treated and monitored after birth. In the offspring of mothers with poor metabolic control during pregnancy, an echocardiogram and follow-up at the same frequency as preterm born infants should be considered to exclude birth defects and developmental problems.

Recommendation 58

Maternal PKU should be considered as a high-risk pregnancy, requiring counselling and the support of a multidisciplinary team to ensure good metabolic control and optimal outcome.

Level of evidence: very low (D)

Strength of recommendation: strong

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 59*

Detailed follow-up by ultrasound with screening for organ development at 18–22 weeks is strongly recommended (similar to routine pregnancy management in most countries). If metabolic control is sub-optimal, then additional growth and development scans are strongly recommended.

Level of evidence: very low (D)

Strength of recommendation: strong

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 60

Blood Phe should be monitored at least once weekly pre-conception and at least twice weekly during pregnancy.

Level of evidence: expert opinion

Strength of recommendation: strong

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 61

Referral to a fertility centre along with their partners should be considered in women who are unable to conceive after 6 months of unprotected sexual activity.

Level of evidence: very low (D)

Strength of recommendation: strong

Expert opinion: completely agree (90 %), mostly agree (10 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 62

Significant effort should be undertaken to avoid any unplanned pregnancies in women with PKU. Timely education and effective contraceptive methods are key elements.

Level of evidence: very low (D)

Strength of recommendation: strong

Expert opinion: completely agree (100 %), mostly agree (0 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 63

Following delivery a woman with PKU should receive standard obstetric care and be supported to continue standard treatment for adults with PKU.

Level of evidence: very low (D)

Strength of recommendation: strong

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

9.1.1. Diet in maternal PKU

Pregnancy is a state of rapid tissue accretion and is accompanied by changes in energy and nutrient requirements [355]. Increased tissue accumulation from the foetus, placenta, uterine, and breast tissues paired with expansion of blood and extracellular fluid volume requires increased nutrient supply [356,357]. Good nutrition of women before and during pregnancy will contribute to optimal foetal growth, improved perinatal survival, and the potential for better long-term health in both the mother and offspring.

9.1.1.1. Natural protein tolerance. Low blood Phe levels in maternal PKU are associated with intrauterine growth restriction (IUGR). The longer the duration of blood Phe below 120 $\mu\text{mol/L}$, the higher the risk of IUGR. The placenta requires adequate levels of amino acids for growth and development to supply sufficient nutrients to the foetus. In non PKU pregnancies, low maternal protein intake is linked to intra-uterine growth restriction (IUGR) as well as reduced postnatal growth and feed efficiency [358].

It is well established that from 16 to 20 weeks' gestation until the end of gestation, Phe tolerance is likely to increase as foetal growth accelerates. In PKU pregnancy, while very low blood Phe levels are not considered to be beneficial for good foetal outcome, the definition of a precise safe lower blood Phe is unclear. As the foetus grows, extra Phe is considered to be necessary to prevent blood Phe levels below <120 $\mu\text{mol/L}$ and the dietary Phe prescription should be increased by at least 50 mg/day (1 g/day protein) to 100 mg/day (2 g/day protein) if the blood Phe level is < 120 $\mu\text{mol/L}$. The exact required amount will depend on various factors such as foetal growth, energy intake and the blood Phe level. Some women may need practical help in choosing suitable foods of higher protein content.

Recommendation 64

Low blood Phe levels <120 $\mu\text{mol/L}$ should be considered with care during pregnancy.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 65

Phe intake should be increased by 50–100 mg/day immediately when blood Phe is <120 $\mu\text{mol/L}$ anytime during pregnancy.

Level of evidence: very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (80 %), mostly agree (20 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

9.1.1.2. Weight gain and energy requirements. Energy in pregnancy is needed: 1) for adequate maternal weight gain to ensure the growth of the foetus, placenta and associated maternal tissues; 2) to support the increased metabolic demands of pregnancy; 3) to maintain adequate maternal weight, body composition and physical activity throughout the gestational period and 4) to provide sufficient energy stores to assist in lactation after delivery [359]. Published guidelines for maternal energy requirements do vary between “expert” groups and are outlined in the European PKU Guidelines 2017 [6].

In maternal PKU, low energy intake may lead to poor blood Phe control associated with catabolism and weight loss. Weight loss is common, particularly in the first trimester of pregnancy. In PKU pregnancies, low pre-pregnancy weight and poor weight gain are associated with microcephaly. In both PKU and non-PKU pregnancies, inadequate gestational weight gain is associated with increased risk of low birth-weight infants and small for gestational age infants. Therefore, close monitoring of energy intake and body weight changes is essential to prevent the occurrence of catabolic states, leading to higher blood Phe levels and impairing metabolic control.

In general, excessive gestational weight gain is a risk factor for gestational diabetes mellitus, pregnancy-induced hypertension, pre-eclampsia, stillbirth, macrosomia and caesarean delivery [360]. Maternal pre-pregnancy overweight/obesity is positively associated with reduced fertility, increased risk of miscarriage, gestational diabetes, large for gestational age, preterm delivery, macrosomia, birth trauma, caesarean delivery, still birth, postpartum complications (e.g., thrombosis and infection) and subsequent offspring overweight/obesity

[361,362].

Recommendation 66

Weight loss should be avoided in maternal PKU to avoid Phe toxicity particularly in the first trimester. At the same time, women should maintain a healthy pre-conception weight with excess maternal weight gain discouraged during pregnancy. Weight should be carefully managed.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (90 %), mostly agree (10 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

9.1.1.3. Total protein requirements. Total protein requirements are not accurately defined in maternal PKU but pregnancy, a state of rapid tissue accretion, is accompanied by an increase in protein requirements. Most protein deposition occurs in the third trimester. The recommended additional protein intake recommended [356] in non-PKU pregnancy is 1 g/day during the first trimester, with an extra 10 g/day during the second trimester and 31 g/day during the third trimester, with an efficiency for protein utilization estimated to be 42 % [356]. Several systematic reviews in non PKU pregnancy have reported that increased maternal protein intake and a balanced energy/protein supplementation were associated with increased birth weight and a significant reduction in the risk for small for gestational age infants [363–365].

Earlier PKU studies reported that insufficient total protein intake in maternal PKU is associated with sub-optimal blood Phe control, lower birth weight and length of the newborns and an independent risk factor for the development of congenital heart defects (CHD) with a higher rate of CHD in the infants born to women who consumed <50 % of the recommended protein intake in the first trimester [6]. Furthermore, low levels of plasma amino acids from low intakes of Phe-free protein substitutes are associated with a higher risk of congenital heart disease in infants [6].

The existing estimations for protein requirements of (non-PKU) pregnant women are based on factorial calculations derived from nitrogen balance studies [366]. More recently, using the 24-h IAAO technique, it showed that the mean estimated protein requirement during early (16 week) and late (36 week) stages of pregnancy was 1.2 g/kg/day and 1.52 g/kg/day, respectively, which exceeds the current FAO/WHO/UNU requirements [367]. This requires further investigation.

Recommendation 67*

To compensate for an increase in protein synthesis, additional protein is strongly recommended for healthy pregnant women. Total protein requirements in pregnancy are uncertain, but it is known that extra protein is deposited particularly in the last trimester of pregnancy

In trimester 1 and 2, an additional 20 % of Phe-free/low Phe protein equivalent from protein substitute extra to the FAO/WHO/UNU 2007 requirements should be adequate. In the third trimester, a further additional 30 g/day protein is advised. In most pregnancies, because of improved protein tolerance this should mainly be provided by natural protein.

In lactation, an extra 20 g/day protein equivalent is required in the first 6 months of breast feeding.

Level of evidence: very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (70 %), mostly agree (30 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

9.1.1.4. Tyrosine supplementation. Tyr is a semi-essential amino acid. The FAO/WHO/UNU 2007 suggests the aromatic amino acids (Phe/Tyr combined) requirement for healthy adults is only 30 mg/g protein [356]. Therefore, women taking 75 g/day total protein would require 2250 mg Tyr daily. All Phe-free/low Phe protein substitutes contain Tyr. The amounts of Tyr in 60 g/day protein equivalent from protein

substitutes vary between 5000 and 7000 mg/daily. Thereby, Tyr requirements are met by Phe-free/low Phe protein substitutes and extra Tyr supplementation may not increase lower blood Tyr levels observed.

It should be noted that Tyr levels may be low in healthy (non PKU) women who are pregnant. McBride reported blood Tyr levels from DBS (390 cards) using tandem mass spectrometry from 170 pregnancies from non PKU, healthy women (5/1–39/6 weeks' gestation) with singleton pregnancies. Tyr levels declined from the first trimester ($25.7 \pm 9.7 \mu\text{mol/L}$) to the second trimester ($21.7 \pm 6.7 \mu\text{mol/L}$) and then remained relatively the same in the third trimester ($21.0 \pm 6.6 \mu\text{mol/L}$) [368].

The effects of both low Tyr or Tyr supplementation on foetal growth are not known. If there is concern about Tyr deficiency, an early study in healthy young men showed that evaluation of pre to post prandial Tyr change could be considered to evaluate Tyr intake [369].

Recommendation 68*

Tyr intake should be at least 6 g/day. This is usually supplied by Phe-free/low Phe protein substitute, providing the entire daily prescribed amount is consumed (and spread through the day).

If there is persistent low blood Tyr, check that all the protein substitute is being consumed.

Level of evidence: very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (75 %), mostly agree (20 %), partially agree (0 %), mostly disagree (5 %), completely disagree (0 %)

9.1.1.5. Emergency diet during unplanned pregnancy. Individuals with PKU should be encouraged to contact the metabolic clinic immediately to report a positive pregnancy result and strict dietary treatment should begin without delay. They should be assessed by a physician and dietitian within 24 h of notification. It is essential to lower the blood Phe level quickly to within the therapeutic target range.

All high protein foods should be stopped, Phe-free/Low Phe protein substitute commenced, with supplementation of special low protein foods. Practical details are described in the PKU dietary handbook to accompany the 2017 PKU guidelines [7]. PKU clinics should supply women with all essential dietary products and any medication until their own supply can be established through their healthcare or insurance systems.

Baseline blood Phe levels, anthropometry, nutritional biochemistry should be established. Re-training about the dietary Phe restriction and home blood Phe sampling is essential.

Recommendation 69*

Women with unplanned pregnancy should be contacted by their metabolic centre the day of notification as rapid blood Phe reduction is essential. Dietary treatment should start immediately (within 24 h). It should meet protein requirements from Phe-free /low Phe protein substitute and low Phe foods to ensure adequate energy intake.

As a starting point, the initial Phe allocation should be the same as the Phe tolerance when aged between 1 and 5 years.

Blood Phe should be measured pre-treatment commencement.

Level of evidence: very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (75 %), mostly agree (25 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

9.1.1.6. Nausea and vomiting/hyperemesis. Symptoms of nausea and vomiting in maternal PKU should be identified promptly and treated aggressively, particularly if associated with poor appetite, inability to take the full protein substitute prescription, weight loss, dehydration and electrolyte imbalance, and high blood Phe levels.

It is important to establish the onset, duration, frequency, and severity of symptoms, including the impact on oral intake. The Pregnancy-Unique Quantification of Emesis (PUQE) score, a validated

questionnaire, will help assess the severity of symptoms [370–372].

Safe anti-emetic therapy and acid reducing medications should be considered with persistent vomiting and symptoms of dyspepsia and indigestion. Advice from the family physician or obstetrician should be sought early. Useful strategies for persistent nausea, vomiting, dyspepsia and indigestion are given in the PKU dietary handbook [7] and <https://www.nhs.uk/pregnancy/related-conditions/common-symptoms/vomiting-and-morning-sickness>.

Recommendation 70

In maternal PKU, nausea and vomiting should be promptly and effectively treated as this may decrease the intake of Phe-free/Low Phe protein substitute and energy intake leading to poor blood Phe control, poor maternal weight gain and increased risk to the infant.

Level of evidence: low (C) / very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (90 %), mostly agree (10 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

9.1.1.7. Folic acid supplementation. In PKU and non-PKU, folic acid pre- and peri-conceptionally is essential in the prevention of neural tube defects (NTDs). Therefore, 400 $\mu\text{g/day}$ should be taken by women with PKU at normal risk of a NTD before conception and throughout the first 12 weeks of pregnancy (van Wegberg 2017). Although protein substitutes contain (variable amounts) of folic acid, adherence may be sub-optimal, and additional acid supplementation should be given.

In addition, in overweight/obesity, the UK Royal College of Obstetricians and Gynaecologists recommend folic acid supplementation of 5 mg/day if BMI is 30 or above (<https://www.rcog.org.uk/for-the-public/browse-all-patient-information-leaflets/being-overweight-in-pregnancy-and-after-birth-patient-information-leaflet/>). NHS England also recommends 5 mg/day folic acid to high-risk pregnancies e.g., diabetes (<https://www.nhs.uk/pregnancy/keeping-well/vitamins-supplements-and-nutrition/>).

Iodine is another important nutrient in foetal development associated with its involvement in thyroid function and foetal brain development [373]. Iodine requirements increase in early pregnancy to 200 $\mu\text{g/day}$ [374]. In PKU, protein substitutes supplemented with iodine are the main source, providing around 80 μg for each 20 g protein equivalent. If patients are following a vegan diet and taking only a low daily amount of protein substitute, they are vulnerable to iodine deficiency and intake should be carefully monitored.

Recommendation 71

At least 400 μg of folic acid daily should be given to all women with PKU considering pregnancy and for the first 12 weeks of pregnancy (planned and unplanned) irrespective of the folic acid content of supplemented Phe-free/low Phe protein substitute.

Level of evidence: moderate (B)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

9.1.1.8. DHA and EPA. The foetus and neonate should receive LC-PUFA in amounts sufficient to support optimal neural, visual, and cognitive development. DHA also reduces the risk of early premature birth. In the last trimester of pregnancy, it is estimated there is a foetal accretion of 67 mg of omega-3 fatty acids per day, mainly DHA, and around 5 % is delivered to the brain (3.1 mg/d) [375]. In a Cochrane review, that included 70 trials with 19,927 women, preterm birth (< 37 weeks) and early preterm birth (< 34 weeks) were reduced in women receiving omega-3 LCPUFA compared with no omega-3. They suggested that the optimum dose was a daily supplement containing between 500 and 1000 mg/day of long-chain omega-3 fats starting at 12 weeks of pregnancy [376]. Supplementation of up 1000 mg/day has been suggested

by others [377].

Generally, all pregnant and lactating women should aim to achieve an average daily intake of at least 200 mg/day DHA [6]. Many (but not all) Phe-free/low Phe protein substitutes contain DHA, and usually would supply 120–150 mg of DHA for each 20 g protein equivalent.

Recommendation 72

A daily intake of ≥ 200 mg/day of DHA (n-3) should be provided to all women with PKU who are considering pregnancy and during pregnancy.

Level of evidence: high (A)

Strength of recommendation: **strong**

Expert opinion: completely agree (80 %), mostly agree (20 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

9.1.1.9. Nutrient monitoring. Inadequate maternal micronutrient status has significant potential to adversely affect many developmental processes in the foetus with both immediate and longer-term consequences. Poor maternal nutrition has been linked with early embryogenesis and foetal growth abnormalities, cardiovascular disease risk, and metabolic and renal dysfunction [378–380]. In particular micronutrients (including iron, zinc, folic acid, and other vitamins) contribute to epigenetic modifications during organogenesis in early pregnancy [381,382]. Therefore, it is important to assess biochemical nutritional status of key nutrients pre-conception and at the start of pregnancy, with further monitoring recommended only if there are concerns about dietary adherence or if biochemical/clinical deficiency has been noted earlier in the pregnancy.

The usefulness of laboratory tests for assessment of nutritional status in pregnancy is limited due to changes in blood volume, blood levels of nutrients or in nutrient-dependent enzymes or reactions, which most likely reflect normal physiologic changes that occur in pregnancy rather than a state of nutrient deficiency [357].

Recommendation 73*

Key nutrients that are suggested to be measured pre-conception and at the start of pregnancy are: folic acid, vitamin B12 (homocysteine or methylmalonic acid), MCV and ferritin, full blood count, and quantitative plasma amino acids. Additional monitoring is only advocated during trimester 2 and 3 if adherence with dietary treatment is sub-optimal or deficiency has been detected.

Level of evidence: low (C) / very low (D)

Strength of recommendation: **conditional**

Expert opinion: completely agree (60 %), mostly agree (35 %), partially agree (0 %), mostly disagree (5 %), completely disagree (0 %)

9.1.1.10. Breast-feeding and lactation. Non-PKU infants of maternal PKU women can metabolize the Phe contained in their mother's breast milk without difficulty. Even if an individual with PKU has an infant with PKU she is still able to breast feed providing the breast milk is given in combination with a Phe-free infant formula. However, there is little published information describing the outcome of breast feeding in mothers with PKU, both when they have an affected and unaffected child with PKU. In particular, it may be prudent for women with PKU, who have an infant with PKU to aim to maintain their blood Phe < 600 $\mu\text{mol/L}$, although this may not always be possible in the first days after giving birth because of the involution of the uterus and hormonal changes. When the mother and infant both have PKU, the decision to breast feed should be done in consultation with the mother, her partner and the health professional team.

In women with PKU, the Phe content of maternal breast milk is highest immediately after birth (up to 238 mg/100 mL) but decreases to between 90 and 130 mg/100 mL [6]. A study reported the Phe content of breast milk was 86 mg/100 mL at day 6 post-partum and 74 mg/100 mL at day 13 post-partum [6]. However, overall, there is limited data about the Phe content of breast milk from women with PKU with different

blood Phe control.

Recommendation 74

In maternal PKU, breast-feeding infants either unaffected or affected by PKU is advocated.

Level of evidence: very low (D)

Strength of recommendation: **conditional**

Expert opinion: completely agree (85 %), mostly agree (15 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

9.1.1.11. After delivery. Mothers with PKU should be encouraged to breast feed their infants. Mothers need guidance about their own nutritional needs during breastfeeding whilst following a Phe restrictive diet. Lactation does increase energy (by 500 kcal/day) and protein (20 g/day) requirements in the first 6 months post-partum [359]. The tolerance during lactation is unreported, but may be higher than in patients not breastfeeding but published data is needed [7].

Many women stop dietary Phe restriction shortly after childbirth. They may be unable to cope with the pressures of strict dietary management and caring for their baby. Women may have difficulty following strict dietary management after adjusting to higher protein intakes in the last trimester of pregnancy. Many describe anxiety, depression, tiredness and inability to focus well after the birth of the baby and some describe how they struggle with day-to-day childcare [352]. Women require practical and emotional support from health professionals, so they are not left isolated to cope with parenting.

Women should also be encouraged to return to a healthy weight post pregnancy. They also need to be aware that the postpartum period is a high-risk time for unintended pregnancy and they should be encouraged to restart contraception after birth.

Recommendation 75*

Continuing, attentive care to women in the postpartum period is strongly advised. During lactation, it is strongly recommended to provide practical and nutritional support, whether they have chosen to continue dietary treatment or not.

Level of evidence: very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

9.1.1.12. Education programmes for maternal PKU. The first European PKU guideline reported about the importance of education programmes for maternal PKU [6]. This includes preconceptional dietary training which increases the success rate of achieving Phe target before conception compared to women without training [383], supporting the impact of this recommendation. Women need training about every aspect of treatment with specific details outlined in the PKU dietary handbook [7].

Recommendation 76*

Educational programmes with practical skills and emotional support should be provided to women both before and during pregnancy. Education of supporting partners and/or family members/friends should be encouraged so they can support the woman with maternal PKU.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

9.2. Late diagnosed / untreated PKU

NBS for PKU has a very high negative predictive value, meaning patients with PKU are rarely missed by NBS. Therefore, late diagnosed or untreated PKU patients are typically found in countries in which there is no NBS available, no national coverage or only recently implemented

NBS [34–38]. In countries with a long history of NBS, immigration and to a lesser extent technical or follow-up failures are reasons for a missed diagnosis [24].

It is important that untreated patients are identified, as every patient deserves to be treated. Treatment should be discussed as it may improve outcome or quality of life. Hatimi et al. evaluated QoL in 82 late diagnosed patients with PKU (diagnosed between 1 and 180 months) and reported lower physical, psychological, social and environmental health, which was strongly influenced by their level of education [384].

In children up to the age of 8 years, (some) reversibility of IQ loss may occur with a Phe restricted diet. Treatment in previously untreated PKU patients may also improve some behaviours (*aggressive behaviour, stereotypic behaviour, self-injury, hyperactivity, restlessness, irritability, sleep disorders, anxiety, mood, social interaction, verbal communication*), neurological dysfunction (*attention span, alertness, short-term memory, motor skills, seizures, spasticity, tremors*), physical appearance (*eczema, skin rash, body odour, hair colour*), or reduce medication needs (*sedatives, anti-psychotic, anticonvulsants*) [6].

Even untreated adults with PKU, with severe intellectual disabilities, may benefit from the introduction of treatment [6]. Brown et al. reported reduction in nursing time, hospitalizations, outpatient clinic visits and medications, which led to reduced mean annual costs [6]. Unfortunately, not all untreated adults will improve, and so far, it has not been possible to predict which patients will respond to treatment.

Untreated adults with PKU usually have severe intellectual disability and challenging behaviour problems that have high support needs and consequently they live in residential or supported accommodation. Interestingly, there are reports of untreated patients with PKU that have escaped intellectual disability, although several of them have neurological symptoms like tremor and headache as well as behavioural issues needing psychiatric care or treatment [17,18].

Initiating treatment in previously untreated symptomatic adult patients, should be carefully considered with the caregivers/family members and should be supervised by a metabolic team. Care providers and/or family members require education about the practicalities of dietary treatment. Clear information should be given about the potential benefits of treatment and the timespan. In the first European PKU guidelines, it was stated that a treatment trial in untreated PKU patients should last at least 6 months [6]. However, in some patients this might be too short, as it might take longer to see any effect and it also might take some months to reach metabolic stability.

Recommendation 77*

Intellectual disability and/or neurological deficits with or without behavioural and psychiatric problems in unscreened children and adults should initiate diagnostic tests for PKU.

Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 78a*

In late-diagnosed children and adolescents with PKU, treatment should always be initiated, aiming to keep blood Phe levels within the recommended therapeutic target range.

Recommendation 78 b*

In untreated adults with PKU, introduction of treatment can be beneficial in several domains, but an individual decision in the best interests of the patient should be taken.

A treatment trial of at least 6 months should be considered to evaluate the effect.

Level of evidence: very low (D)

Strength of recommendation: **strong**

Expert opinion: completely agree (90 %), mostly agree (10 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 79**

Late diagnosed and untreated PKU patients should have access to all treatment options. However, careful consideration on an individual basis is necessary.

(continued on next column)

(continued)

Level of evidence: expert opinion

Strength of recommendation: **conditional**

Expert opinion: completely agree (80 %), mostly agree (15 %), partially agree (5 %), mostly disagree (0 %), completely disagree (0 %)

10. Pharmacological treatments

10.1. Sapropterin

Tetrahydrobiopterin (BH4) is the naturally occurring cofactor of PAH, Tyr and tryptophan hydroxylase and for the production of nitric oxide. Pharmacological treatment with sapropterin dihydrochloride, a synthetic form of BH4, has been approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) as a treatment option for patients with PKU of all ages [6]. A subset of PAH-deficient patients can benefit from sapropterin treatment beginning as early as the neonatal period. However, the drug is still not reimbursable in all countries.

Sapropterin acts as a pharmacological chaperone which increases the residual PAH activity in a subset of PKU patients. It has been well established by clinical trials that sapropterin supplementation can safely reduce blood Phe levels and/or increase natural protein tolerance in sapropterin-responsive patients [6,385–392]. Patients with high residual PAH activity have a greater probability of sapropterin response, but a minority of patients with lower residual enzyme activity also may benefit from sapropterin treatment [6].

Studies examining QoL with sapropterin treated PKU have reported conflicting results. A meta-analysis by Evers et al. found no differences before and after relaxing Phe restriction with sapropterin treatment [187]. This is possibly explained by the use of generic QoL questionnaires. Using a PKU-specific QoL questionnaire, Douglas et al. however, reported an increase in the QoL score and the subscales “impact” and “satisfaction” in 17 patients after 12 months of sapropterin treatment [121]. In addition, cross-sectionally, patients treated with sapropterin scored better on several domains when compared to patients treated with diet using the PKU-specific QoL questionnaire [6,124].

Associated with sapropterin usage and associated lower blood Phe levels some but not all studies have reported improved executive functioning, behaviour and neuro-imaging findings [6,138]. A few studies have reported improvement in behaviour ($n = 8$), cerebral glucose metabolism ($n = 5$) and neural activation ($n = 4$) even when blood Phe is not lowered [6,393].

Discontinuation of sapropterin treatment should be considered if:

- 1) the patient experiences side effects that cannot be eliminated or adequately managed, or.
- 2) blood Phe levels consistently exceed the upper target range and if there is no improvement in control when a maximum drug dosage of 20 mg/kg is prescribed and.
- 3) if nutritional status has deteriorated since commencement of drug treatment, for example development of morbid obesity.

10.1.1. Identification of potential sapropterin-responders and long-term responders

The identification of potential sapropterin responsive patients can be done by genotype and/or a sapropterin loading test. The genotype may predict or exclude sapropterin -responsiveness to some degree, but information is not available on all genotypes. Alleles known to be responsive are listed in the BIOPKU database (www.biopku.org), including the allelic phenotype values (APVs). Wettstein et al. reported that residual PAH activity and the APV predicted sapropterin responsiveness in 71 % of patients [394]. Sapropterin responsiveness was less

frequent in patients with APV < 2 (patients with two null variants have a APV of 0) and more common in those with APV > 5 [395]. Sapropterin treatment is not recommended in patients with two null variants, as there is an absence of residual PAH protein so they should not be responsive.

The first European PKU guidelines provided some guidance on sapropterin response testing [6]. First, a 48 h test was recommended. If a sapropterin loading test is performed in the neonatal period, it should not take longer than 24 h to avoid delays in treatment of nonresponsive PKU patients. However, a negative 24 h neonatal test does not rule out long-term sapropterin response [396]. Likewise, the 48-h test can miss sapropterin responsive patients, although the frequency is dependent on the definition of a long-term responder [397].

In patients with a negative result but a suggestive phenotype and/or genotype, repetition of the test under different conditions should be considered. These include higher blood Phe levels [6], a longer observation period, a different sapropterin dose or sapropterin divided into two doses [398]. Testing for sapropterin responsiveness is done by the analysis of blood Phe levels after the administration of a single dose of sapropterin of 20 mg/kg. There is however data that some patients respond better to 10 mg/kg [399]. Several protocols for sapropterin loading tests are published [6,400]. Although arbitrary, a 30 % reduction in blood Phe levels from baseline is widely accepted to define a potential sapropterin response. A lower cut-off increases the number of false positives [397].

Long term response should be proven in a treatment trial by increasing the natural protein intake first, followed by Phe-free/low Phe protein substitute reduction and lowering of sapropterin dosing [400]. Adequate outcomes have been described with doses up to 20 mg/kg/day. This treatment trial can take several weeks to months and blood Phe levels need to be maintained within the therapeutic target range.

Critical evaluation to determine the clinical benefit of sapropterin therapy should be done. In the first PKU guidelines a long-term sapropterin response was defined as an increase of 100 % or more in natural protein and/or improved biochemical control (>75 % of Phe levels in target range). However, in practice several definitions are used and an increase of 100 % would mean that patients with a high natural protein tolerance pre-sapropterin use are less likely to readily fit this definition [401,402]. It should be acknowledged that there are differences in pre-sapropterin protein tolerance, and an increase of 100 % may not be fully reached, but meaningful changes in natural protein tolerance with reductions in Phe-free/low Phe protein substitute are still achieved.

Recommendation 80*

For every PAH-deficient patient, except those with 2 known null-variants, sapropterin responsiveness should be considered, to help increase their Phe tolerance and/or achieve better metabolic control.

Level of evidence: moderate (B)

Strength of recommendation: strong

Expert opinion: completely agree (95 %), mostly agree (5 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 81*

A sapropterin response test should be performed to detect patients who may benefit from pharmacological treatment with sapropterin.

Testing for sapropterin response should be performed by analysing change in blood Phe levels after the administration of a single dose of sapropterin (20 mg/kg/day). In the neonatal period a sapropterin loading test can be done before starting dietary Phe restriction, but should not be longer than 24 h to avoid delay in treatment. In infants and older patients the test should last 48 h (second sapropterin dose of 20 mg/kg after 24 h). If there is no response, longer test durations can be considered (max 7 days). In general, a Phe decrease of ≥ 30 % is considered a potential sapropterin response and the patient may proceed to a treatment trial.

Level of evidence: low (C)

Strength of recommendation: strong

(continued on next column)

(continued)

Expert opinion: completely agree (75 %), mostly agree (20 %), partially agree (5 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 82*

Long-term response should be proven in a treatment trial by adjusting the sapropterin dose, natural protein intake and Phe-free/low Phe protein substitute intake.

Long-term benefit is defined as a ≥ 100 % increase in natural protein intake and maintaining blood Phe in recommended therapeutic range or improved biochemical control (≥ 75 % of Phe levels in the recommended therapeutic target range).

Level of evidence: expert opinion

Strength of recommendation: conditional

Expert opinion: completely agree (70 %), mostly agree (30 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

10.1.2. Diet and follow-up

Two systematic reviews have examined the nutritional outcome with sapropterin treatment [187,189]. Ilgaz et al. reported 9 studies that investigated changes in weight and height z-scores with long term sapropterin treatment showing that in general weight and height for age z-scores remained within the normal range. In a meta-analysis, Evers et al. reported 2 single patient cohorts that had an increase in either BMI or weight and concluded that sapropterin treatment may lead to a small increase in weight and BMI. Ilgaz et al. described 8 studies that reported changes in micronutrient intakes and/or markers of nutritional status with long-term sapropterin treatment, indicating that vitamin and mineral supplementation may be necessary.

It is estimated that around half of patients can stop protein substitute prescription [189], but it should be reduced in parallel with any increase in natural protein intake. It is important to advise patients/caregivers that the natural protein is sourced from high biological quality protein sources as protein substitutes are usually a major source of nutrients (if they include vitamins and minerals). Unhealthy food choices will leave patients vulnerable to nutritional deficiency.

Recommendation 83**

Patients treated with sapropterin -with or without dietary Phe restriction- should have the same clinical, biochemical and nutritional follow-up as all other patients with PKU.

Level of evidence: expert opinion

Strength of recommendation: strong

Expert opinion: completely agree (90 %), mostly agree (10 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

Recommendation 84**

- Monitoring dietary patterns, micronutrient intake, and biochemical nutritional status should be performed annually, particularly in patients who have stopped or reduced intake of protein substitute.
- Appropriate supplementation should be given if dietary intake is low of any nutrient, but special attention should be given to nutrients that may be at risk of deficiency including calcium, vitamin D, iron, and vitamin B12.
- Titration and reduction of protein equivalent from protein substitutes should be performed in parallel with any increase in natural protein intake.

Level of evidence: moderate (B)

Strength of recommendation: strong

Expert opinion: completely agree (75 %), mostly agree (25 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

10.1.3. Sapropterin treatment and pregnancy

Only a few case reports and small cohort studies on sapropterin in pregnant women with PKU are available. For this reason, it was recommended in the first European PKU guidelines, that sapropterin should only be considered when a women failed to achieve target blood Phe levels with a Phe restricted diet [6].

A limited number of new case reports have been published, all

reporting a healthy outcome [403–405]. However, the total number of case reports is still low. Even though it cannot be guaranteed that sapropterin is safe for the foetus, an increasing number of clinics are prescribing sapropterin throughout pregnancy. The risks of high blood Phe, especially during the first trimester of pregnancy are considered to clearly outweigh the risks of sapropterin administration during pregnancy. If a woman is pregnant but fails to achieve target blood Phe levels and response to sapropterin therapy is unknown, sapropterin administration should be considered.

Larger studies are necessary providing definitive evidence and evaluate if patients may remain on the same dose as before pregnancy.

Recommendation 85*

Pre-conception:

- Testing for sapropterin responsiveness may be considered, especially in patients with a suggestive phenotype/genotype and/or patients that fail to achieve target levels with a Phe restricted diet.

During pregnancy:

- Patients treated with sapropterin may continue to use this treatment option during pregnancy.
- Patients known to be unresponsive to sapropterin (genotype / negative sapropterin response test) should not be given sapropterin during pregnancy.
- If it is not known if a patient is sapropterin responsive, but the patient fails to achieve target Phe levels with a low-Phe diet, sapropterin administration should be considered.

Level of evidence: very low (D)

Strength of recommendation: **conditional**

Expert opinion: completely agree (70 %), mostly agree (30 %), partially agree (0 %), mostly disagree (4.6 %), completely disagree (0 %)

10.2. Pegvaliase

In 2018, the US Food and Drug Administration (FDA) approved the use of pegylated phenylalanine ammonia lyase (PEG-PAL, Pegvaliase), an enzyme substitution therapy for patients with PKU aged ≥ 18 years who have uncontrolled blood Phe levels ($> 600 \mu\text{mol/L}$) on their current treatment. In 2019, the European Medicines Agency (EMA) gave approval for individuals ≥ 16 years. Although experience in Europe is increasing, this treatment is not reimbursed in many countries.

Pegvaliase is a recombinant *Anabaena variabilis* phenylalanine ammonia lyase (rAvPAL) enzyme, produced in *Escherichia coli*. The enzyme is conjugated to polyethylene glycol (PEG) which increases its stability and decreases its immunogenicity. Pegvaliase converts Phe into ammonia and trans-cinnamic acid, which are both excreted in the urine. It is administered via daily subcutaneous injections and lowers blood Phe levels regardless of PAH residual activity. However, efficacy is different in individual patients and is unable to be predicted. Immunogenicity plays an important role in pegvaliase pharmacokinetics, primarily via drug clearance during early treatment [406].

Clinical trials with more than 350 patients exposed to the drug show that this is the first treatment that is able to normalise blood Phe levels in the majority of patients with PKU [80,137,407,408]. Within 24 months, 68.4 % of the 261 participants achieved blood Phe levels $\leq 600 \mu\text{mol/L}$, 60.7 % $\leq 360 \mu\text{mol/L}$, and 51.2 % $\leq 120 \mu\text{mol/L}$ [80]. In addition, some clinics have reported their own experience in which many patients were able to achieve blood Phe $< 360 \mu\text{mol/L}$ [409,410]. When patients finally respond to pegvaliase, which may take many months, blood Phe levels can decrease very quickly and may result in hypophenylalaninemia (blood Phe $< 30 \mu\text{mol/L}$). When this occurs, the natural protein intake should be rapidly increased, and this a challenge for some patients. If normal protein intake is already established, drug dose

should be reduced.

During the first 6 months of treatment, patients are at increased risk of hypersensitivity reactions, including anaphylaxis, and so require close monitoring. Adverse reactions are related to the non-IgE mediated formation of anti-PAL and anti-PEG antibodies and to immune complex formation. Most frequent reported adverse events are arthralgia (70.5 %), injection-site reactions (62.1 %), erythema (47.9 %), and headache (47.1 %) [80]. Typically, after 6 months, the adverse events decrease in frequency [80], as the immune response matures with time [406]. Almost all (96 %) adverse events are resolved without dose interruption or reduction [80]. However, severe reactions may lead to pegvaliase treatment cessation. Treatment cessation may also be associated with treatment burden, or delay in treatment response but there is limited data.

10.2.1. Treatment trial

Pegvaliase treatment is carried out using a stepwise titration schedule, referred to as the induction, titration and maintenance (I/T/M) period, to diminish and eventually overcome the effects of immunogenicity [406] and reduce hypersensitivity reactions [411]. The dosing starts at 2.5 mg/week and is gradually titrated to a maximum dose of 60 mg/day. Hollander et al. observed in a post-marketing cohort that patients tolerated a reduction in pegvaliase dose over time while maintaining efficacy [412]. Based on individual tolerability and responsiveness it may take several months to years to reach clinically significant efficacy.

Patients and their families should be well educated prior to starting treatment. It is important to correctly set expectations and to give extensive information about potential adverse events and mitigation measures. A protocol for the treatment trial has been published by Longo et al. including the prevention of adverse events and the management of anaphylaxis, arthralgia and injection site reactions [413]. Close clinical and biochemical follow-up during induction, titration and maintenance is strongly recommended [409,410].

Caution should be taken in patients who are unable to communicate issues associated with adverse events, who are unable to inject or who do not have access to emergency services.

Safety measures

- Follow the stepwise introduction and titration recommendations starting with 2.5 mg per week. Do not increase too fast.
- Use pre-medication antihistamines (H1 and H2 receptor antagonists) and antipyretics
- Involve a trained observer (i.e. partner or parent) that accompanies the patient for 1 h after each injection during the introduction and initial titration.
- Instruct patients how to use and to always carry an epinephrine autoinjector (epipen).
- Manage adverse events like arthralgia for example with a short course of steroids.
- Caution as to interference with other pegylated medications (previous and ongoing, i.e. some cytostatics).
- Consider permanently discontinuing pegvaliase in patients with severe acute systemic hypersensitivity reactions.
- Consider rechallenge at a lower dose and/or dose frequency in patients with less severe systemic hypersensitivity reactions.

Follow-up

- Blood Phe should be monitored at least once weekly until efficacy has been reached.

- Phe intake should be closely monitored and adapted according to metabolic control (see 10.2.2).
- Injection sites should be rotated, as administering the drug into scar tissue may reduce its effectiveness.

Recommendation 86**

- Patients with PKU >16 years of age who are not able to achieve metabolic control with pharmacological treatment with sapropterin dihydrochloride may be offered treatment with pegvaliase.
- Due to the safety profile close follow-up with specific safety measures are required and must be clearly explained to patients.

Level of evidence: high (A)

Strength of recommendation: **strong**

Expert opinion: completely agree (95 %), mostly agree (0 %), partially agree (5 %), mostly disagree (0 %), completely disagree (0 %)

10.2.2. Pegvaliase and dietary management

Although it is an expectation that adult patients on pegvaliase treatment should exceed protein intake of ≥ 0.8 g/kg/day, meeting expected normal, healthy requirements [285], not all patients reach this target [171,410]. If all the natural protein is supplied by vegan sources, due to its lower digestibility, 10–20 % extra protein (up to 1 g of protein/kg/day) is required to achieve a healthy nutritional status [414]. While it is aimed to remove the Phe-free/low Phe protein substitute, any reduction should be in parallel with the natural protein increase, and good quality nutrition from natural foods should be established before it is stopped. Some patients may have limited knowledge about what represents a healthy diet because of their dietary restrictions and so require extensive nutritional education.

There are limited reports about the actual nutritional quality of relaxed protein intakes with pegvaliase treatment and systematic data is necessary to understand the long-term nutritional effect of dietary protein liberalization. There is suggestion that micronutrient quality decreases and McWhorter et al. reported 6 patients who had been on pegvaliase for 3 years who consumed less than the dietary reference values for Vitamin D, A, K, E, folate, calcium, magnesium, potassium and essential fatty acids [171]. Another study reported low 25-hydroxy vitamin D (< 30 ng/mL) and/or low ferritin status [410]. Supplementation with vitamins, minerals and micronutrients is recommended if dietary intake or biochemical deficiency is detected [413]. Increasing overweight/obesity may occur with pegvaliase treatment [285], so monitoring and prevention of weight gain with increasing BMI is important.

Disordered or atypical eating behaviour with neophobia is also documented with pegvaliase treatment [285]. Patients on a Phe-restricted diet may be hesitant about eating new foods due to their taste, texture and smell. They may have no practical experience of cooking with higher protein foods.

Alopecia is associated with hypophenylalaninemia with pegvaliase [201]. Although the underlying mechanism is not understood, alopecia improves when increasing natural protein intake. Hypophenylalaninemia can be managed through dietary adjustments or drug dose modulation. Low fasting blood Tyr is also reported with pegvaliase, so should be monitored, but there is no information about the efficacy of Tyr supplementation. Low Tyr can also occur when protein substitutes are discontinued. Studies are needed to assess the potential nutritional impact of low blood Phe and/or Tyr.

Recommendation 87**

- The goal of therapy with Pegvaliase is to achieve a safe intake of protein (at least) of 0.8 g/kg/day from a mix of high and low biological protein, without the use of protein substitute or to decrease blood Phe levels in patients that are not on a protein restricted diet.
- Persistent hypophenylalaninemia should be avoided.

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Level of evidence: low (C)

Strength of recommendation: **strong**

Expert opinion: completely agree (85 %), mostly agree (15 %), partially agree (0 %), mostly disagree (0 %), completely disagree (0 %)

10.2.3. Pegvaliase and pregnancy

Due to a lack of data, the use of pegvaliase is currently not recommended during pregnancy or women in the pre-conception period. There is 1 case report in which pegvaliase was continued during an unplanned pregnancy and the baby had no congenital abnormalities [415].

When planning a pregnancy, a 4-week period should wash out pegvaliase, based on pharmacokinetic data [413]. Rohr et al. described the practicalities of discontinuing and resuming pegvaliase in a woman with PKU [416]. In her second pregnancy, she resumed pegvaliase during lactation as no pegvaliase activity was detected in any breast milk samples [416,417]. Similar to sapropterin, case reports on pegvaliase will increase knowledge and perspectives on treatment during pregnancy. This will potentially lead to revised guidance, for example, for women with PKU with poor dietary adherence or if they eat a normal protein intake prior to pregnancy.

10.3. Emerging therapies

New pharmacological therapies are being developed such as gene correction, gene therapy, mRNA therapy, enzyme substitution and cofactor therapy. Currently various forms of gene therapy are being studied in clinical trials [4]. Phase II results have been reported for the cofactor sepiapterin [92]. Sepiapterin is a natural precursor of intracellular BH4 and is a more stable molecule that is more efficiently transported into cells than sapropterin [92]. It is administered orally. Another option that seems promising is to decrease the re-absorption of Phe at renal tubular level [418], while other chaperones may be found [419].

11. Future requirements and research

Many of the issues mentioned in the section on future requirements and research in the first European PKU guidelines [6] are still valid, including identification and training of sufficient numbers of physicians with a broad interest in co-ordinating care for adult patients with inherited metabolic disorders, optimizing care in adult and elderly PKU, re-engaging adults in long term follow up (either without treatment or following unmonitored dietary treatments that many be nutritionally unbalanced), and home monitoring of blood Phe in PKU. It remains challenging to build up large databases in PKU when clinic numbers of patients are small unless there is good multi-team collaboration across centres and countries.

In order to improve the accuracy of the results of blood Phe measurements taken at home, it may help to use strategies for blood sampling other than DBS, preventing the influence of hematocrit and volume [420]. Also, in order to evaluate the value of Phe/Tyr ratio and Phe fluctuations in patient monitoring, routine evaluations of these biomarkers should be considered.

To date, data on PKU related potential co-morbidities such as heart, kidney and eye complications is limited and unclear. Future research may provide new data about the relationship between eye symptoms and neurological status of the brain. Future research should also focus on the origin and impact of dysbiosis of gut microbiota. i.e. to evaluate a potential link between dysbiosis and dysfunction of organs like brain, heart, bone and kidney.

Years after the introduction of sapropterin and pegvaliase, it is seen that some of the outcome of PKU may be related to the burden of dietary

treatment. Although much information remains to be established, it is clear that other therapeutic options are needed.

Further data is necessary to establish the long-term effect of strict metabolic control on cerebral functioning during the different age periods throughout life. In addition, more information about interindividual vulnerability together with better outcome prediction based on genotype (taking optimal treatment adherence for granted) is essential.

In order to collect more information about neurocognitive functioning, the widespread introduction of electronic versions of neurocognitive testing should be encouraged. This is expected to decrease the workload of neuropsychologists and enable better comparisons between results from various research groups. Also, in PKU, in order to fully assess the quality of life, and ensure that all relevant issues are assessed, a comprehensive and validated list of questions or prompts should be developed.

An important question that remains is the optimal target blood Phe levels for throughout life and in special conditions such as pregnancy. It is also important to fully establish the effect of childhood blood Phe levels on later outcome. The USA and the European PKU guidelines recommend different target blood Phe levels for adolescents and adult patients with PKU [421]. This can be explained by 1) lack of data with regard to blood Phe levels between 360 and 600 $\mu\text{mol/L}$, 2) a different perspective whereas the USA use lifetime Phe levels and the European PKU guidelines use blood Phe levels in different stages of development 3) differences in the dietary treatment efficacy because of dietary reimbursement issues in the USA and therefore a greater need for pharmacological treatment. However, this does not prove that the blood Phe targets from either the European or USA PKU guidelines are the optimal choice. Historically, it was considered that a blood Phe of 480 or 600 $\mu\text{mol/L}$ was safe for children, which is now considered unacceptable. The journey about the optimum blood Phe targets is ongoing and research groups should be encouraged to examine different targets to the ones suggested in the current guidelines. New studies should try to incorporate the recommendations given about neurocognitive assessment within this guideline.

12. Patient perspective by the ESPKU

The experience of living with PKU is shaped by scientific and medical knowledge and the diverse realities patients face in their daily lives. This perspective has been a driving force in the development and refinement of European Treatment Guidelines for PKU, as patients, caregivers, and healthcare providers work together to ensure the best possible care.

In 2013, the European Society for Phenylketonuria (ESPKU) took a significant step by defining minimum standards for PKU care and initiating the development of European Guidelines [9]. For many people living with PKU, it was difficult to understand why the same condition was treated differently across European countries. The European PKU Guidelines, published in 2017, marked a key milestone in addressing such disparities [6]. However, they also left many questions unanswered, particularly in terms of the full range of healthcare needs for PKU patients.

One of the most challenging issues is the lack of consensus concerning the upper blood Phe concentration thresholds for adult patients as a key distinction between “real healthcare needs” and “over- or undertreatment” in adult patients. Balancing medical needs with treatment feasibility in patients’ daily lives remains a complex issue not just for healthcare providers and researchers, but most importantly for patients themselves. Rather than working towards a minimal consensus, positions among health professionals have seemingly diverged further. For instance, the American ACMG guidelines are notably stricter than this European update [421], while some other healthcare providers challenge the impact of high Phe intake on cognition [422] and some even choose to deny any unmet needs of adults living with PKU [423]. Not surprisingly, the latter have caused considerable disbelief and frustration within the patient community [424].

Overall, this first update of the European PKU Guidelines is another important milestone in the ongoing process of describing the diverse (health)care needs of the PKU community, but we are not there yet. It remains essential that future research shifts its focus towards other patient-relevant questions beyond Phe levels and continues to generate the evidence necessary for future guideline updates. A range of concerns voiced by the aging PKU community remain largely unaddressed. These include i.e. mental health challenges, the impact of menopause on metabolism, multimorbidity and other increasingly relevant issues affecting long term health and quality of life. The patient perspective is clear: these concerns deserve greater attention and research.

In the near future, dynamic research and development activities on innovative therapeutic options are likely to offer new opportunities for optimizing PKU care. However, these innovations may not be available to all patients simultaneously. Access to therapies may vary significantly across different European healthcare systems and further complicate the European PKU care landscape. As a result, it is likely that the diversity of treatment strategies across Europe will again continue to grow, posing additional challenges for future updates of the European PKU Guidelines. Nonetheless, this evolution should be seen as a reminder of the need to continually adapt and refine (health)care and to better meet the ever-evolving needs of the PKU patient community rather than an obstacle in such a rapidly changing research and healthcare environment.

13. Conclusions

This is the first revision of the European PKU guidelines with twenty new topics added. These guidelines are intended to standardize care and help with a suggested course of action. In this first revision, there were some changes to the professionals contributing to the guidelines and included adult physicians, dieticians, pediatricians, and psychologists. Statements were reported with the degree of consensus, needing at least 75 % agreement for consensus. Data and experience have increased, but at the same time knowledge gaps still exist and many statements are at least partly based on consensus rather than evidence. Even though the scientific level of evidence is often low or very low, many recommendations are important and relevant.

Future research should focus on the pathophysiology, and the (long-term) impact of Phe in PKU on various domains. Finally, new treatments are emerging which will hopefully increase therapeutic options in the near future.

Although some statements were revised, they changed very little. However, importantly, they give more context about the relevance of the statement. For example more caution is given about upper blood Phe targets levels for adults with PKU as the strength of supporting evidence is low. Some of the new statements give a more complete picture of PKU diagnosis, care, and treatment. Some statements on diagnosis (DNA diagnostics, DNAJC12) and treatment (Pegvaliase) may change future PKU care.

CRediT authorship contribution statement

A.M.J. van Wegberg: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **A. MacDonald:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **K. Ahring:** Writing – review & editing, Methodology, Conceptualization. **A. Bélanger-Quintana:** Writing – review & editing, Methodology, Conceptualization. **S. Beblo:** Writing – review & editing, Methodology, Conceptualization. **N. Blau:** Writing – review & editing, Methodology, Conceptualization. **A.M. Bosch:** Writing – review & editing, Methodology, Conceptualization. **A. Burlina:** Writing – review & editing, Methodology, Conceptualization. **J. Campistol:** Writing – review & editing, Methodology, Conceptualization. **T. Coşkun:** Writing – review & editing, Methodology, Conceptualization. **F. Feillet:** Writing – review & editing, Methodology, Conceptualization. **M. Gżewska:**

Writing – review & editing, Methodology, Conceptualization. **S.C. Huijbregts**: Writing – review & editing, Methodology, Conceptualization. **V. Leuzzi**: Writing – review & editing, Methodology, Conceptualization. **F. Maillot**: Writing – review & editing, Methodology, Conceptualization. **A.C. Muntau**: Writing – review & editing, Methodology. **J.C. Rocha**: Writing – review & editing, Methodology, Conceptualization. **C. Romani**: Writing – review & editing, Methodology, Conceptualization. **F. Trefz**: Writing – review & editing, Methodology, Conceptualization. **F.J. van Spronsen**: Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization.

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The other authors (A.Bo., A.Bu., K.A., T.C., V.L. F.T.) declare no competing of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ymgme.2025.109125>.

Data availability

No data was used for the research described in the article.

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