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# Comprehensive Assessment of Nutrition, Metabolic Profiles, and Patient–Caregiver Knowledge in Phenylketonuria, with Dedicated Evaluation of Previously Understudied Foods for Phenylketonuria Diet

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# Abstract

Phenylketonuria (PKU) is the most common inherited disorder of amino acid metabolism. Lifelong treatment is based on a low-phenylalanine (Phe) diet supplemented with Phe-free amino acid formulas. Although early diagnosis and treatment substantially improve patient outcomes, long-term dietary therapy may affect nutrient intake, metabolic parameters, nutritional status, and treatment adherence. To date, no comprehensive study has been conducted in Latvia evaluating dietary habits, biochemical parameters, metabolic control, disease-related knowledge, and the nutritional composition of foods relevant to PKU dietary management.

The aim of this Doctoral Thesis was to assess anthropometric parameters, dietary habits, nutrient intake, and biochemical markers in Latvian paediatric patients with PKU, to analyse the nutritional composition of foods relevant to the PKU diet, and to evaluate disease-related knowledge among patients and their caregivers. The study was conducted between 2019 and 2025 at the Children's Clinical University Hospital. The first part of the study included 41 paediatric patients with PKU and an age- and sex-matched healthy control group. Nutritional intake was assessed using 31 valid 72-hour food diaries, while biochemical evaluation included measurements of minerals, lipid profile, fatty acid status, glucose, vitamin D, phenylalanine, and tyrosine concentrations. In the second part of the study, 34 foods commonly available in Latvia were analysed for their protein, amino acid, and phenylalanine content. The third part evaluated PKU-related knowledge among patients and caregivers and analysed metabolic control data collected between 2023 and 2025.

The study demonstrated that overweight or obesity was present in 32% of patients with PKU compared with 17% of participants in the control group. Female patients with PKU had a significantly higher body mass index than healthy female controls (19.0 vs. 16.7 kg/m²; $p = 0.027$). Dietary analysis showed that patients with PKU consumed less dietary fat than both the control group and the amounts recommended in Latvian dietary guidelines, whereas micronutrient intake exceeded recommended levels across all age groups. Erythrocyte fatty acid analysis revealed significant alterations in fatty acid status, including a higher omega-6/omega-3 ratio (6.1 ± 1.58 vs. 5.01 ± 1.19; $p = 0.043$), a higher arachidonic acid/eicosapentaenoic acid (Ara/EPA) ratio (43.94 ± 20.29 vs. 29.5 ± 10.18; $p < 0.001$), and a lower linoleic acid/dihomo-γ-linolenic acid (LN/DGLA) ratio (5.5 ± 1.74 vs. 6.47 ± 2.13; $p = 0.008$). In addition, HDL cholesterol concentrations were significantly lower in patients with PKU ($p < 0.001$), suggesting possible alterations in lipid metabolism and a potentially increased cardiometabolic risk.

The analysis of food composition allowed refinement of PKU dietary recommendations in Latvia. Of the 34 analysed food products, 18 contained less than 75 mg Phe / 100 g and were classified as suitable for unrestricted consumption, whereas 16 products were recommended for restricted use within the PKU diet. Assessment of metabolic control demonstrated an age-related deterioration in Phe control, with the highest Phe concentrations and Phe/Tyr ratios observed among adolescents. Knowledge assessment revealed that patients had lower levels of PKU-related knowledge than their caregivers, and better understanding of the disease and dietary treatment was associated with improved metabolic control. These findings highlight the need for enhanced educational programs and continuous multidisciplinary support for individuals with PKU and their families.

This Doctoral Thesis represents the first comprehensive assessment of nutritional status, nutrient intake, fatty acid profiles, biochemical parameters, metabolic control, disease-related knowledge, and food composition relevant to PKU dietary management in Latvia. The findings provide evidence-based data for improving clinical care, refining dietary recommendations, and developing targeted educational interventions aimed at enhancing treatment adherence and long-term health outcomes in patients with PKU.

**Keywords:** phenylketonuria, phenylalanine, diet, nutrient profiling, knowledge, adherence

# Anotācija

**Visaptverošs uztura, metabola profila un pacientu–aprūpētāju zināšanu novērtējums pacientiem ar fenilketonūriju: iepriekš maz pētītu pārtikas produktu analīze un to piemērotība slimības diētai**

Fenilketonūrija (FKU) ir biežākā iedzimtā aminoskābju vielmaiņas slimība, kuras ārstēšanas pamatā ir mūža zema fenilalanīna (Phe) satura diēta un Phe nesaturošu aminoskābju maisījumu lietošana. Lai gan agrīna diagnostika un ārstēšana būtiski uzlabo pacientu prognozi, ilgstoša diētas terapija var ietekmēt uzturvielu uzņemšanu, vielmaiņas rādītājus, uztura statusu un ārstēšanas līdzestību. Latvijā līdz šim nav veikts visaptverošs pētījums, kurā vienlaikus analizēti FKU pacientu uztura paradumi, bioķīmiskie rādītāji, metabolā kontrole, zināšanas par slimību un FKU diētā izmantojamo pārtikas produktu sastāvs.

Promocijas darba mērķis bija izvērtēt antropometriskos rādītājus, uztura paradumus, uzturvielu uzņemšanu un bioķīmiskos parametrus Latvijas bērniem ar FKU, analizēt FKU diētai nozīmīgu pārtikas produktu uzturvērtību, kā arī novērtēt pacientu un viņu aprūpētāju zināšanas par slimību un tās uztura terapiju. Pētījums tika veikts no 2019. līdz 2025. gadam Bērnu klīniskās universitātes slimnīcā.

Pirmajā pētījuma daļā tika iekļauts 41 bērns ar FKU un vecuma un dzimuma ziņā atbilstoša kontroles grupa. Uztura analīzei tika izmantota 31 derīga 72 stundu uztura dienasgrāmata, savukārt bioķīmisko rādītāju izvērtēšanai noteikti minerālvielu, lipīdu, taukskābju, glikozes, D vitamīna, fenilalanīna un tirozīna rādītāji. Otrajā pētījuma daļā laboratoriski analizēti 34 Latvijā pieejami pārtikas produkti, nosakot to olbaltumvielu, aminoskābju un fenilalanīna saturu. Trešajā daļā tika novērtētas FKU pacientu un viņu aprūpētāju zināšanas par slimību un diētas terapiju, kā arī analizēti metabolās kontroles dati 2023.–2025. gadā.

Pētījumā konstatēts, ka liekais svars vai aptaukošanās bija sastopama 32 % pacientu ar FKU, salīdzinot ar 17 % kontroles grupā, savukārt meitenēm ar FKU ķermeņa masas indekss bija statistiski nozīmīgi augstāks nekā veselām vienaudzēm (19,0 pret 16,7 kg/m²; p = 0,027). Uztura analīze parādīja, ka pacienti ar FKU uzņēma mazāk uztura tauku nekā kontroles grupa un Latvijas uztura rekomendācijās noteikts, bet mikroelementu uzņemšana visās vecuma grupās pārsniedza rekomendēto līmeni. Eritrocītu taukskābju profila analīzē tika konstatētas statistiski nozīmīgas izmaiņas, salīdzinot pētījuma un kontroles grupu, tostarp augstāka omega-6 un omega-3 attiecība (6,1 ± 1,58 pret 5,01 ± 1,19; p = 0,043), augstāka arahidonskābes un

eikozapentaēnskābes attiecība (43,94 ± 20,29 pret 29,5 ± 10,18; $p < 0,001$) un zemāka linolskābes un dihomo-γ-linolēnskābes attiecība (5,5 ± 1,74 pret 6,47 ± 2,13; $p = 0,008$). Tika konstatēta arī statistiski nozīmīgi zemāka augsta blīvuma lipoproteīnu holesterīna koncentrācija ($p < 0,001$), kas kopumā var liecināt par iespējamām izmaiņām lipīdu vielmaiņā un potenciāli paaugstinātu kardiometabolisko risku.

Pārtikas produktu sastāva analīze ļāva precizēt FKU diētas rekomendācijas Latvijā. No 34 analizētajiem produktiem 18 saturēja mazāk nekā 75 mg Phe / 100 g un tika atzīti par piemērotiem lietošanai bez ierobežojumiem, savukārt 16 produktus rekomendēts lietot ierobežotā daudzumā. Metabolās kontroles izvērtējums apliecināja, ka līdz ar vecumu pasliktinās Phe koncentrācijas kontrole, augstākās Phe koncentrācijas un Phe/Tyr attiecības novērojot pusaudžu vecuma grupā.

Zināšanu izvērtēšanā tika konstatēts, ka pacientu, kuri vecāki par 12 gadiem, zināšanu līmenis ir zemāks nekā aprūpētājiem, kuriem ir bērni vecumā līdz 12 gadiem, un labāka izpratne par slimību un diētas terapiju bija saistīta ar labākiem metabolās kontroles rādītājiem. Šie rezultāti uzsver nepieciešamību pilnveidot izglītojošās programmas un nodrošināt nepārtrauktu multidisciplināru atbalstu pacientiem ar FKU un viņu ģimenēm.

Promocijas darbs ir pirmais visaptverošais pētījums Latvijā, kurā izvērtēts FKU pacientu uzturs, uzturvielu uzņemšana, taukskābju profils, bioķīmiskie rādītāji, metabolā kontrole, zināšanas par slimību un FKU diētā nozīmīgu pārtikas produktu sastāvs. Iegūtie rezultāti sniedz zinātniski pamatotu informāciju FKU pacientu aprūpes pilnveidošanai, uztura rekomendāciju precizēšanai un mērķētu izglītojošu programmu izstrādei, veicinot labāku ārstēšanas līdzestību un ilgtermiņa veselības iznākumus Latvijā.

**Atslēgvārdi:** fenilketonūrija, fenilalanīns, uzturs, uzturvielu noteikšana, zināšanas, līdzestība

# Table of Contents


Abstract ..... 3

Anotācija ..... 5

Abbreviations used in the Thesis ..... 9

Introduction ..... 11
- Aim of the Thesis ..... 12
- Objectives of the Thesis ..... 12
- Hypotheses of the Thesis ..... 12
- Novelty of the Thesis ..... 13

1 Literature review ..... 14
- 1.1 Nutrition impact on human health ..... 14
- 1.2 Elimination diets ..... 16
- 1.3 Phenylketonuria ..... 17
  - 1.3.1 History of PKU discovery ..... 17
  - 1.3.2 Epidemiology ..... 17
  - 1.3.3 Phenylalanine metabolism ..... 18
  - 1.3.4 PKU genetics ..... 20
  - 1.3.5 PKU diagnosis ..... 20
  - 1.3.6 PKU classification ..... 21
  - 1.3.7 Clinical picture of untreated PKU patients ..... 22
  - 1.3.8 PKU management ..... 22
  - 1.3.9 Health risks for treated PKU patients due to periodically high Phe levels ..... 29
  - 1.3.10 Adverse effects on health and nutritional outcomes due to a low-protein diet ..... 29
  - 1.3.11 Nutritional status of PKU patients ..... 30
  - 1.3.12 Adherence and knowledge of a low-Phe diet ..... 31
  - 1.3.13 Nutritional assessment of PKU patients ..... 32

2 Methods and Materials ..... 34
- 2.1 Ethical statement ..... 34
- 2.2 Informed consent statement ..... 34
- 2.3 Study description ..... 34
- 2.4 Part I of the Study ..... 35
  - 2.4.1 Description of the PKU population ..... 35
  - 2.4.2 Anthropometric parameters ..... 36
  - 2.4.3 Food diary ..... 37
  - 2.4.4 Food diary analysis ..... 37
  - 2.4.5 Analysis of biochemical parameters ..... 38
  - 2.4.6 Methods of statistical analysis of the Part I of the Study ..... 39
- 2.5 Part II of the Study ..... 39
  - 2.5.1 Food selection for nutrition analysis ..... 39
  - 2.5.2 Methods of analysing nutritional profiling of foods for PKU ..... 40
- 2.6 Part III of the Study ..... 44
  - 2.6.1 Questionnaire evaluating patient and caregiver knowledge of PKU and dietary treatment ..... 44
  - 2.6.2 Questionnaire data analysis ..... 45
- 2.7 Methods of statistical analysis ..... 45

3 Results ..... 46
- 3.1 Patient characteristics ..... 46
- 3.2 Food diary analysis ..... 47
  - 3.2.1 Macronutrient intake in the PKU diet ..... 47

3.2.2 Associations between biochemical parameters and nutrient intake ..... 48
3.3 Part II – Results of nutritional information in food ..... 55
3.4 Part III – PKU patients' and their caregivers' knowledge of PKU and dietary treatment ..... 58
3.4.1 Knowledge and self-reported dietary management among caregivers and patients with PKU ..... 58
3.4.2 Metabolic control of PKU patients ..... 62
4 Discussion ..... 65
4.1 Analysis of food diary results of Latvian PKU paediatric population ..... 65
4.2 Anthropometric parameters of PKU paediatric population ..... 66
4.3 Nutrient intake and biochemical parameters status ..... 68
4.4 Nutritional profiling of foods appropriate for PKU ..... 76
4.5 Knowledge and awareness of the PKU diet among patients and their caregivers ..... 81
4.6 Limitations of this study ..... 83
4.7 Key Strengths and Practical Contributions of the Study ..... 84
Conclusions ..... 86
Proposals ..... 87
Publications and reports on topics of Doctoral Thesis ..... 88
References ..... 89
Acknowledgments ..... 103
Annexes ..... 104
Annex 1 ..... 105
Annex 2 ..... 106
Annex 3 ..... 109
Annex 4 ..... 115
Annex 5 ..... 116
Annex 6 ..... 117
Annex 7 ..... 118
Annex 8 ..... 120

# Abbreviations used in the Thesis

| | |
|---|---|
| AA | Amino acid |
| ALA | Alpha-linolenic acid |
| Ara | Arachidonic acid |
| BBB | Blood-brain barrier |
| BH2 | Dihydrobiopterin |
| BH4 | Tetrahydrobiopterin |
| BMI | Body mass index |
| Ca | Calcium |
| CCUH | Children's clinical university hospital |
| CHO | Carbohydrate |
| CNS | Central nervous system |
| cPKU | Classical phenylketonuria |
| DHA | Docosahexaenoic acid |
| EPA | Eicosapentaenoic acid |
| E.S.PKU | European Society for Phenylketonuria and Allied Disorders Treated as Phenylketonuria |
| FA | Fatty acids |
| FD | Food diary |
| Fe | Iron |
| HDL | High-density cholesterols |
| HPA | Hyperphenylalaninaemia |
| IMD | Inherited metabolic disorder |
| IQ | Intelligence quotient |
| Kcal | Kilocalories |
| LC-PUFA | Long chain polyunsaturated fatty acids |
| LDL | Low-density cholesterol |
| LNAA | Large neutral amino acids |
| MHA | Mild hyperphenylalaninaemia |
| MHRL | Ministry of Health of the Republic of Latvia |
| mPKU | Mild phenylketonuria |
| MUFA | Monounsaturated fatty acids |
| NBS | Newborn screening |
| PAH | Phenylalanine hydroxylase |
| PKU | Phenylketonuria |
| Phe | Phenylalanine |

| | |
|---|---|
| PS | Protein substitute |
| RDI | Recommended daily intake |
| SFA | Saturated fatty acids |
| SLPF | Special low protein food |
| Tyr | Tyrosine |
| WHO | World Health Organization |
| Zn | Zinc |

# Introduction

Phenylketonuria (PKU) is the most common inborn error of amino acid metabolism. PKU is caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH), which converts phenylalanine (Phe) into tyrosine (Tyr) (Blau et al., 2010). As a result, phenylalanine accumulates in the blood. If left untreated, high Phe concentrations are neurotoxic and cause severe mental retardation, seizures, and motor deficits.

To date, the main PKU treatment method is a strict low-phenylalanine diet with supplementation of a Phe-free L-amino acid formula (MacDonald et al., 2020). Phe is an essential amino acid and is present in all protein-containing foods, such as meat, milk products, eggs, and fish. In classic PKU (cPKU), the mean Phe intake per day is approximately 4 to 6 grams of natural protein, or 200–300 mg of natural phenylalanine from food (van Wegberg et al., 2025). This low-protein diet is very strict and can cause many nutrient deficiencies; for example, phenylalanine-rich products are a source of animal fat, especially saturated fatty acids and long-chain polyunsaturated fatty acids (LC-PUFA) (Okano et al., 2016).

Effective PKU management hinges on the knowledge of, and compliance with, the treatment by both patients and their caregivers. Studies have shown that inadequate knowledge among caregivers correlates with poor dietary adherence, leading to suboptimal metabolic control and compromised biochemical parameters (Kaloteraki et al., 2025). Eating habits, influenced by the patient's and caregiver's understanding of dietary requirements, significantly impact nutritional intake and metabolic outcomes. For instance, it has been found that reintroducing a low-volume, nutrient-enriched protein substitute improved eating behaviour and nutrient intake in non-compliant PKU patients (Yagudina et al., 2024).

Another important aspect of PKU dietary management is the availability of reliable food composition data. Patients with PKU require precise information on the natural protein and Phe content in foods in order to determine which products must be calculated as protein exchanges and which may be consumed in controlled amounts or eaten freely. However, for many fruits, vegetables, starchy roots, and other potentially suitable low-protein foods, detailed and locally relevant nutritional composition data may be limited or unavailable (Araújo et al., 2017). This creates uncertainty in dietary planning and may unnecessarily restrict food variety, reduce dietary quality, and increase the treatment burden. Therefore, the nutrimental analysis of foods that could potentially be included in the PKU diet is important for improving practical dietary recommendations and supporting more flexible, nutritionally adequate meal planning.

Despite the clinical importance of these factors, data on nutrient intake, biochemical status, metabolic control, dietary knowledge, adherence, and food composition in the Latvian PKU population remain limited. The absence of country-specific evidence restricts

the development of tailored educational materials, practical dietary tools, and national recommendations. Investigating these aspects in the Latvian context is therefore essential.

International studies demonstrate that PKU knowledge and dietary adherence are important determinants of treatment success: Witalis et al. (2017) reported that only 45% of patients knew their daily Phe intake recommendations and only 27% knew the Phe content of at least three out of four investigated food products, while a recent systematic review confirmed that adherence to a low-Phe diet is influenced by patient age, education, family factors, and professional support. Investigating these aspects in Latvia is therefore necessary to identify nutritional risks, knowledge gaps, and adherence challenges, as well as to develop evidence-based recommendations for improving PKU dietary management and long-term metabolic outcomes (Witalis et al., 2017).

**Aim of the Thesis**

To evaluate anthropometric characteristics, biochemical parameters, metabolic control, and disease-related knowledge in Latvian paediatric patients with phenylketonuria, and to determine the nutritional composition of food products relevant to PKU dietary management.

**Objectives of the Thesis**

1 To evaluate the anthropometric parameters and dietary intake of PKU patients in comparison with a control group;
2 To analyse biochemical parameters in PKU patients, including fatty acid profile concentrations, and to compare these indicators with those observed in a control group;
3 To determine the nutritional composition of food products that could potentially be consumed by PKU patients, with a particular emphasis on phenylalanine content;
4 To assess the knowledge and understanding of PKU patients and their caregivers regarding the disease and its dietary management;
5 To evaluate metabolic control in PKU patients during the period from 2023 to 2025 based on Phe monitoring data.

**Hypotheses of the Thesis**

1 Paediatric patients with PKU differ from healthy controls in dietary intake, anthropometric parameters, and selected biochemical markers.
2 Detailed nutritional characterisation of less commonly consumed foods may identify additional products suitable for inclusion in the phenylketonuria diet, thereby expanding dietary variety and flexibility.

3 Higher knowledge and better adherence to dietary treatment are associated with better metabolic control in patients with PKU.

**Novelty of the Thesis**

This Doctoral Thesis provides the first comprehensive assessment of nutritional status, biochemical profiles, metabolic control, and disease-related knowledge among patients with phenylketonuria in Latvia. Unlike previous studies that have usually focused on isolated aspects of PKU management, this work integrates dietary intake, anthropometric parameters, biochemical markers (including fatty acid status), food composition analysis, and patient–caregiver knowledge within a single study framework.

A novel aspect of the Thesis is the evaluation of fatty acid profiles and lipid metabolism indicators in Latvian paediatric patients with PKU compared with healthy controls. This allows a deeper understanding of how a lifelong low-phenylalanine diet may influence not only Phe control but also broader metabolic and cardiovascular risk-related parameters.

Another important contribution is the laboratory analysis of locally available foods that may be relevant for the PKU diet. By determining the protein and phenylalanine content of selected foods, the study provides practical evidence for classifying products as suitable for unrestricted consumption or as requiring calculation as phenylalanine/protein exchanges. This is particularly important because locally relevant food composition data for PKU dietary management are limited.

The Thesis also provides the first structured assessment in Latvia of PKU-related knowledge and self-reported dietary management among both patients and caregivers. By comparing knowledge levels with metabolic control, the study identifies clinically relevant educational gaps and highlights the need for targeted education, especially during the transition from caregiver-led management to independent self-management.

# 1 Literature review

## 1.1 Nutrition impact on human health

Adequate nutrition is a fundamental determinant of children's health and development. Due to its profound impact on growth, cognitive function, and long-term health outcomes, child nutrition represents a critical focus of global research efforts (Puri et al., 2023).

To support optimal physical and cognitive development, it is essential that a child's diet be nutritionally adequate and well-balanced. The human body requires energy to sustain vital functions and perform daily activities, which is derived from food consumption. Energy intake is typically measured in kilocalories (kcal). The daily energy requirements of children vary according to several factors, including age, sex, body weight, height, and level of physical activity (Butte, 2006).

A healthy diet is characterised by the consumption of macronutrients in balanced proportions that adequately meet the body's energetic and physiological demands without leading to excessive intake, while also ensuring the sufficient provision of micronutrients and hydration to support normal physiological functions (Neufeld et al., 2023). Macronutrients – including carbohydrates, proteins, and fats – serve as the primary sources of energy necessary for sustaining cellular processes and daily physiological activities. In contrast, micronutrients, comprising vitamins and minerals, are required in smaller quantities but are indispensable for normal growth, metabolic regulation, development, and overall physiological function (Cena & Calder, 2020).

To ensure adequate energy intake and meet micronutrient and macronutrient requirements (Table 1.1) (VM, 2017), it is essential that the daily diet includes a diverse selection of items from the major food groups. These include cereal products and starchy vegetables (e. g., potatoes); fruits and vegetables; milk and dairy products; meat and fish; as well as alternative protein sources such as eggs, legumes, nuts, seeds, and dietary fats and oils (Cena & Calder, 2020; Neufeld et al., 2023; Rajasekaran, 2017).

Table 1.1

**Recommended energy intakes for the paediatric population of Latvia** (VM, 2017)

| Age | Gender | Average Body Weight (kg) | Average Height (cm) | Energy (kcal/day) | Proteins (% Energy) | Fats (% Energy) | CHO (% Energy) |
|---|---|---|---|---|---|---|---|
| 0–6 mo. | boys | 6 | 60 | 590 | – | – | – |
| | girls | 6 | 60 | 520 | – | – | – |
| 7–12 mo. | boys | 9 | 71 | 730 | 7–15 | 30–40 | 45–60 |
| | girls | 9 | 71 | 720 | 7–15 | 30–40 | 45–60 |
| 1–3 yrs. | boys | 13 | 90 | 1040–1290 | 10–15 | 30–40 | 45–60 |
| | girls | 13 | 90 | 960–1220 | 10–15 | 30–40 | 45–60 |
| 4–6 yrs. | boys | 20 | 112 | 1280–1680 | 10–15 | 30–35 | 45–60 |
| | girls | 20 | 112 | 1200–1570 | 10–15 | 30–35 | 45–60 |
| 7–10 yrs. | boys | 28 | 132 | 1520–2280 | 10–20 | 30–35 | 45–60 |
| | girls | 28 | 132 | 1400–2120 | 10–20 | 30–35 | 45–60 |
| 11–14 yrs. | boys | 45 | 157 | 2040–2740 | 10–20 | 30–35 | 45–60 |
| | girls | 46 | 157 | 1920–2430 | 10–20 | 30–35 | 45–60 |
| 15–18 yrs. | boys | 66 | 176 | 2630–3230 | 10–20 | 30–35 | 45–60 |
| | girls | 55 | 163 | 2240–2560 | 10–20 | 30–35 | 45–60 |

CHO – carbohydrates, yrs. – years, mo. – months

Carbohydrates represent the principal source of dietary energy and are most abundantly found in grains, fruits, legumes, and vegetables (Hess et al., 2017). From a health perspective, the consumption of whole grains is favoured over refined grains, as the milling process removes the germ and bran, significantly reducing fibre and micronutrient content (Benisi-Kohansal et al., 2016). Meta-analyses of prospective cohort studies have consistently demonstrated that a higher intake of whole grains is associated with a reduced risk of coronary heart disease, stroke, cardiovascular disease, and various forms of cancer, as well as a lower risk of all-cause, cardiovascular, cancer-related, respiratory, diabetic, and infectious disease mortality (Aune et al., 2016; Benisi-Kohansal et al., 2016).

Fresh fruits and vegetables not only provide energy but are also rich in dietary fibre, which enhances satiety and contributes positively to gastrointestinal health, lipid profiles, and glycaemic regulation (McRorie & McKeown, 2017). Furthermore, fruits and vegetables are important sources of phytochemicals – such as polyphenols, phytosterols, and carotenoids – which are bioactive compounds believed to underlie many of the protective health effects attributed to fruit and vegetable consumption (Probst et al., 2017).

Dietary proteins serve both as an energy source and as providers of amino acids, including essential amino acids that cannot be synthesised endogenously by the human body. Protein is obtained from both animal (e. g., meat, dairy products, fish, and eggs) and plant (e. g., legumes, soy products, grains, nuts, and seeds) sources, with animal-derived proteins

generally regarded as superior due to their comprehensive amino acid profiles, high digestibility, and greater bioavailability (Lonnie et al., 2018).

Fats are a fundamental nutrient and can be classified as simple or complex lipids and lipid derivatives, which include fatty acids. Fatty acids are distinguished by the number of double bonds in the chain, with saturated fatty acids (SFA) containing no double bonds, monounsaturated fatty acids (MUFA) containing one double bond, and polyunsaturated fatty acids (PUFA) containing at least two double bonds (Banaszak et al., 2024).

Omega-3 long-chain polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are dietary lipids associated with a wide range of health-promoting effects (Su et al., 2008). These fatty acids are incorporated into various tissues, including cellular membranes, where they influence membrane fluidity and participate in anti-inflammatory pathways. EPA and DHA are especially important during critical life stages, such as foetal development and ageing, due to their roles in neural, visual, and cardiovascular functions (Swanson et al., 2012). DHA is a structural component of cellular membranes and is highly concentrated in the cerebral cortex and retina. EPA and DHA serve as precursors to a class of bioactive lipid mediators – such as resolvins and protectins – that exhibit anti-inflammatory and pro-resolving properties, potentially contributing to the prevention and management of several chronic diseases (Banaszak et al., 2024; Dunstan et al., 2007).

## 1.2 Elimination diets

Elimination diets are widely implemented in the diagnostic and therapeutic management of food allergies, intolerances, and inherited metabolic disorders (IMD). These dietary interventions demand a high level of precision and adherence; however, numerous barriers may impede optimal compliance. Factors such as limited health literacy, socioeconomic constraints, monotonous dietary options, and anxiety related to dietary restrictions can significantly compromise adherence to the prescribed regimen (Malone & Daley, 2024).

In clinical contexts where an elimination diet is required due to underlying health conditions, there is a high risk of multiple nutrient deficiencies. Dietary management is a fundamental component of therapeutic strategies for inherited metabolic disorders, often involving the restriction of specific macronutrients such as fats, carbohydrates, or proteins, depending on the enzymatic defect involved. One of the most prevalent inborn errors of metabolism is PKU, which necessitates a lifelong low-phenylalanine (i. e., protein-restricted) diet to prevent neurotoxicity and cognitive impairment. Insufficient intake of essential

micronutrients due to dietary restrictions – particularly protein, calcium, vitamin D, iron, and zinc – may adversely affect normal growth and developmental processes (Durban et al., 2021).

## 1.3 Phenylketonuria

Phenylketonuria (E70.0, ORPHA:716, OMIM# 261600) is a rare autosomal recessive inborn error of phenylalanine (Phe) metabolism caused by variants in the gene encoding phenylalanine hydroxylase (PAH). PAH normally converts Phe into tyrosine (Tyr) requiring the cofactor tetrahydrobiopterin (BH4), molecular oxygen, and iron (van Wegberg et al., 2025). Elevated levels of Phe damage nerve cells and cause significant motor and intellectual disabilities (Blau et al., 2010).

### 1.3.1 History of PKU discovery

PKU was first described by Asbjørn Følling in 1934, a paediatric resident from Norway, who named the condition *oligophrenia phenylpyruvica*. He examined the urine of two siblings with mental retardation for "ketone bodies" using ferric chloride; instead of the expected purple colour, a green hue appeared during testing (Woolf & Adams, 2020). Dr. Følling identified the substance as phenylpyruvic acid – which had not been previously described – and determined that it originates from the essential amino acid phenylalanine (Woolf & Adams, 2020).

In 1949, two doctors from the United Kingdom, Dr. L. Woolf and Dr. D. Vulliamy, suggested that mental retardation develops due to increased concentrations of phenylalanine or one of its metabolites in the brain. They hypothesised that this impairment could be alleviated by reducing the concentration of Phe in the blood and described three options for its management; however only one – the dietary restriction of natural Phe – proved practicable (Woolf & Vulliamy). PKU newborn screening, introduced by Robert Guthrie in the 1960s, uses a bacterial inhibition test to detect elevated Phe levels in the blood shortly after birth (la Marca et al., 2023; Talebi & Eshraghi, 2024).

### 1.3.2 Epidemiology

The prevalence of PKU differs significantly across ethnic groups and geographic regions worldwide. It is most common among individuals of white or East Asian descent, with an estimated prevalence of approximately 1 in 10 000–15 000 live births. In Europe, prevalence rates vary widely; the incidence of PKU ranges from 1:850 in the Karachay-Cherkess Republic (Russia) (Gundorova et al., 2018) to only 1:112 000 live births in Finland (Guldberg et al., 1995). PKU occurs less often in Japan, with an incidence of 1:125 000 (Hillert et al., 2020; Okano et al., 2011).

The prevalence of PKU in Latvia is approximately 1:6190 newborns (unpublished data), whereas it is 1:6700 in Estonia (Lillevali et al., 2018) and 1:9300 in Lithuania (Kasnauskiene et al., 2003). In comparison, the worldwide prevalence is 1:23 930 (Hillert et al., 2020; Kreile et al., 2020).

The severity of the PAH deficiency determines the PKU phenotype, which ranges from mild hyperphenylalaninaemia HPA (MHPA) (120–360 μmol/L) to classical PKU (cPKU) (> 1200 μmol/L) based on untreated blood Phe levels (Balci et al., 2025). The global phenotypical distribution of PKU is 62% cPKU, 22% mPKU, and 16% mild hyperphenylalaninaemia (MHPA). A genotype-phenotype pattern has been identified across Europe, with cPKU predominating in the east, mPKU in the southwest, and mild hyperphenylalaninaemia in the south (Hillert et al., 2020).

### 1.3.3 Phenylalanine metabolism

Phe is an essential amino acid, which means it cannot be synthesised by the human body. After protein ingestion and digestion, phenylalanine is absorbed from the gastrointestinal tract into the liver via the portal vein; Phe comprises 3–7% of all dietary protein. The cofactor – tetrahydrobiopterin (BH4) is essential to perform Phe hydroxylation (Figure 1.1). BH4 is synthesised from guanosine triphosphate mainly in the liver and proximal renal tubules in the kidneys, and is recycled after Phe hydroxylation through enzymatic reduction. During the conversion of Phe to Tyr, BH4 is oxidised to quinonoid dihydrobiopterin (Laurie, 2022). In individuals with recessively inherited pathogenic variants in the PAH gene, the activity of the PAH is either completely absent or significantly reduced. Since approximately 90% of daily dietary Phe intake is metabolised through this pathway, PAH deficiency leads to the accumulation of Phe in the body (Figure 1.1) (van Spronsen et al., 2021).

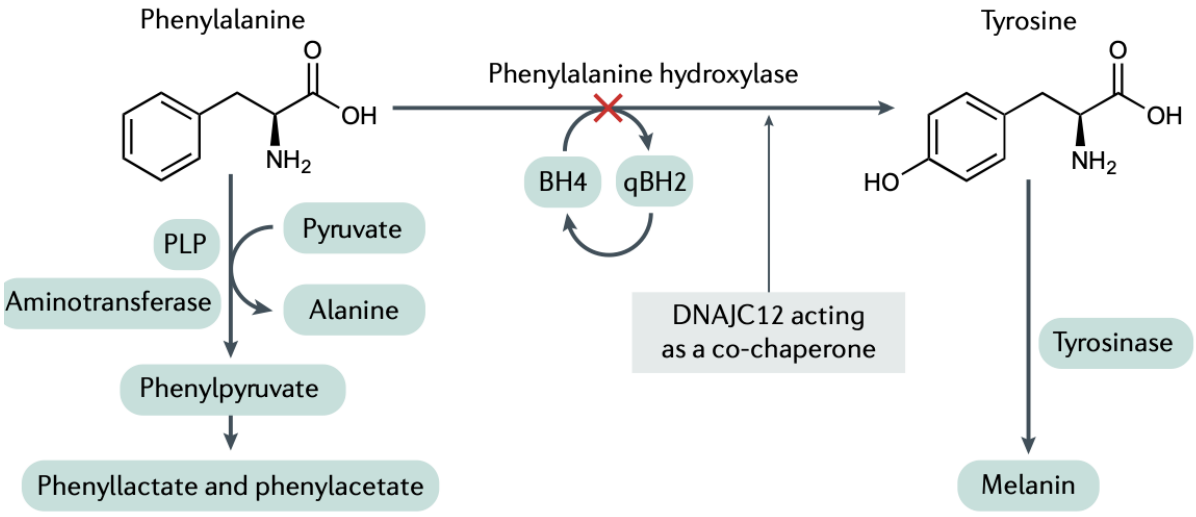



Figure 1.1 **Phenylalanine metabolism in PKU** (van Spronsen et al., 2021)

BH4 – tetrahydrobiopterin; qBH2 – quinonoid dihydrobiopterin;
DNAJC12 – DnaJ Heat Shock Protein Family (Hsp40) Member C12;
PLP – pyridoxal 5'-phosphatase

Additionally, Tyr levels are lower than those in individuals with normal PAH function (Remmington & Smith, 2021).

Net protein balance is defined as the difference between skeletal muscle protein synthesis and breakdown (Breen & Phillips, 2011); however, there is still uncertainty regarding the precise amount of Phe required for net protein turnover. Studies suggest that 10–20% of typical dietary Phe intake is used for net protein turnover, and the other 80–90% of Phe is converted to Tyr (Blau et al., 2010; Elhawary et al., 2022).

Phe undergoes transamination to form phenylpyruvic acid as an alternative to hydroxylation by PAH. Phenylpyruvic acid is subsequently excreted in the urine as phenylacetic acid, phenylacetylglutamine, and phenyllactic acid. However, this metabolic pathway is significantly less efficient than hydroxylation (Laurie, 2022).

Large neutral amino acids (LNAAs), including Phe and Tyr, cross the blood–brain barrier (BBB) by facilitated diffusion through the L-type amino acid transporter 1 (LAT1, also known as Solute Carrier Family 7 Member 5, SLC7A5). Elevated blood Phe levels inhibit the transport of other LNAAs into the brain and reduce their concentrations. Low concentrations of LNAA in the brain impair synthesis of cerebral protein and cause a deficiency of monoamine neurotransmitters (Dobrowolski et al., 2022). Phe also inhibits the activity of tyrosine hydroxylase and tryptophan hydroxylase in the brain; these enzymes are crucial for the synthesis of neurotransmitters such as dopamine and serotonin. This mechanism could be linked to the high prevalence of anxiety, depression, and other mental disorders in individuals with PKU (Van Vliet et al., 2018; Whitehall et al., 2024).

### 1.3.4 PKU genetics

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 are known to be associated with PAH deficiency (van Wegberg et al., 2025):

- The majority of the variants (60%) are missense, usually resulting in protein misfolding and/or the impairment of catalytic functions. These involve a single nucleotide change leading to the substitution of one amino acid for another in the PAH protein. A notable example is p.Arg408Trp (c.1222C>T), which is prevalent in various populations, particularly in Eastern Europe.
- Splice-site mutations constitute about 22.6% of PAH mutations. These affect the normal splicing of pre-mRNA, potentially leading to aberrant or nonfunctional proteins. An example is c.1066-11G>A, a common pathogenic variant that activates a cryptic splice site, resulting in the insertion of additional amino acids and a loss of enzyme activity.
- Nonsense mutations, which introduce premature stop codons, account for approximately 2.9% of PAH mutations (Elhawary et al., 2022).

These PAH variants are catalogued in the locus-specific databases PAHvdb and BioPKU (Hillert et al., 2020).

### 1.3.5 PKU diagnosis

Most PKU cases are diagnosed through newborn screening (NBS) rather than after the onset of clinical presentation. However, there are still countries where NBS is not established or is only partially established. Before NBS is performed, the newborn must receive breastmilk or infant formula. NBS involves collecting a blood sample on Guthrie filter paper from a healthy newborn through a heel prick, ideally between 24 and 72 hours after birth, although screening times may vary in different countries. Tandem mass spectrometry (TMS) is the preferred method for analysing dried blood spots, although some laboratories also utilise alternative techniques, such as enzymatic methods or high-pressure liquid chromatography (Zakaria et al., 2016). Tests should be repeated if the Phe level is above 120 µmol/L. If the Phe level remains in the range of 120–360 µmol/L after the second test, dietary treatment should not be initiated, but Phe levels must be routinely monitored. However, dietary treatment must be initiated immediately if the Phe level exceeds 360 µmol/L, ideally before symptoms appear or within the first 10 days of life, and no later than two months of age (M.Auzenbaha et al., 2021; van Spronsen et al., 2021).

Molecular analysis with the identification of pathogenic variants in the PAH (homozygous or compound heterozygous state) can be used to confirm the diagnosis molecularly (M.Auzenbaha et al., 2021; van Wegberg et al., 2025).

### 1.3.6 PKU classification

There is no consensus regarding phenotype classification. Historically, PKU was mainly categorised into three types based on pre-treatment blood Phe levels: classical PKU, mild PKU, and mild hyperphenylalaninaemia:

- Classical PKU (cPKU ORPHA:79254): This is the most severe form, characterised by significantly elevated Phe levels (> 1200 µmol/L before treatment is initiated) due to minimal or no activity of PAH (< 1%). Without strict dietary management, it can result in severe intellectual disability. In cPKU, the tolerated Phe intake is less than 20 mg/kg/day, but not exceeding 250–350 mg of Phe per day.
- Mild PKU (mPKU ORPHA:79253): Less severe than classical PKU, this form still involves moderately elevated Phe levels (900–1200 µmol/L) and requires dietary management to prevent neurological complications; PAH enzyme activity is approximately 1–5%. In the case of mPKU, 25–50 mg/kg/day of Phe can be tolerated, up to a maximum of 400–600 mg per day.
- Mild hyperphenylalaninaemia (MHP ORPHA:79651): Phe levels are slightly elevated (360–600 µmol/L). Dietary intervention may not always be necessary, but regular monitoring is still required. PAH enzyme activity is above 5%, and more than 50 mg/kg/day of Phe can be tolerated in MHP (Camp et al., 2014; Talebi & Eshraghi, 2024).

These classifications are crucial for adjusting treatment and dietary management plans to meet the specific needs of PKU patients with varying levels of severity. However, recently, this classification has not been used because patients can be diagnosed before reaching their highest levels of Phe (Dalei & Adlakha, 2022; de Groot et al., 2010; Elhawary et al., 2022).

According to the latest European Society for Phenylketonuria and Allied Disorders Treated as Phenylketonuria (E.S.PKU) guidelines, 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 (van Wegberg et al., 2025).

### 1.3.7 Clinical picture of untreated PKU patients

If PKU is left untreated, it causes severe intellectual disability – with an intelligence quotient (IQ) or developmental quotient (DQ) < 20–40, epilepsy, and psychotic behaviours such as severe hyperactivity, destructiveness, self-injuring behaviours, and agoraphobia. Anxiety, depression, speech difficulties, and neurological impairments (movement difficulties, tremors) also occur (Blau et al., 2010; de Groot et al., 2010; Feldmann et al., 2019; Whitehall et al., 2024; Woolf & Adams, 2020). Data indicate that lower IQ levels can persist even in early-treated PKU patients, despite newborn screening (NBS) and the early initiation of a phenylalanine-restricted diet. Long-term studies suggest that cognitive function in adults with PKU remains stable over time, although it consistently falls below the levels observed in healthy individuals. Moreover, early discontinuation or loosening of dietary restrictions during adolescence has been associated with adverse cognitive outcomes in later adulthood (Feldmann et al., 2019).

### 1.3.8 PKU management

The treatment targets differ slightly between Europe and other regions globally. According to the E.S.PKU guidelines, the goal of treatment is to maintain blood Phe concentrations within the target range of 120–360 µmol/L in patients up to 12 years of age. In treated PKU patients aged > 12 years, the target Phe levels should be 120–600 µmol/L, and in pregnancy, target Phe levels should be 120–360 µmol/L (van Wegberg et al., 2025). However, in the United States of America, the goal is to maintain Phe concentrations below 360 µmol/L across all age groups (Camp et al., 2014; Greene & Longo, 2014; Smith et al., 2025).

Another parameter used to monitor the metabolic status of PKU patients is the Phe/Tyr ratio. It has been described before that Phe/Tyr ratio < 6 is associated with poorer inhibition control and with a normal executive function outcome (Sharman et al., 2009; Sharman et al., 2012; van Wegberg et al., 2025).

Nowadays, two primary modalities of PKU treatment are available: dietary and pharmaceutical therapies (van Wegberg et al., 2025).

#### Dietary management

Since the early 1950s, when the first patient with PKU received dietary treatment, a low-Phe diet has remained the cornerstone of PKU management (Woolf and Adams, 2020). Dietary treatment in PKU aims to provide sufficient Phe intake to promote growth while preventing the excessive accumulation of Phe in the blood and brain by strictly limiting natural Phe and protein intake. The cornerstone of a low-Phe diet is the combination of a Phe-free amino acid formula and a low-protein diet; here, supplementation with a Phe-free formula –

variously referred to as synthetic protein, an amino acid mixture, a supplement, or a protein substitute – is essential (MacDonald et al., 2020).

All protein substitutes are Phe-free or contain very low levels of phenylalanine. The intake of additional vitamins and minerals can be achieved through these amino acid mixtures or dietary supplements, thereby ensuring normal growth and development by preventing potential nutrient deficiencies (Aguiar et al., 2015; Daly, Evans, et al., 2021; MacDonald et al., 2020).

In most individuals with PKU, natural protein or dietary Phe intake is limited to 25% or less of typical intake levels in order to keep blood phenylalanine levels within the target ranges set by the European PKU Guidelines. To maintain Phe levels within these target ranges, all foods rich in protein should be excluded from the diet (van Wegberg et al., 2025).

Foods that are restricted in a low-Phe or low-protein diet are meat, poultry, fish, seafood, eggs, dairy products, legumes, nuts, seeds, soya, grains, and grain-based products (bread, flour, pasta). All of those products, including artificial sweeteners – aspartame (E951), acesulfame potassium – aspartame (E950), gelatine, plant algae, and spirulina – must also be excluded from the diet (MacDonald et al., 2020).

The sources of natural Phe are foods that can be consumed in limited amounts and are called “exchange” foods. Exchange foods are classified based on different upper cut-off values. Specifically, any food with a protein or Phe content above the designated cut-off point is considered an exchange food. This includes plant-based alternative drinks with a protein content exceeding 0.5 g per 100 g, as well as fruits and vegetables that contain more than 75 mg of Phe per 100 g (Araújo et al., 2017; MacDonald et al., 2020). Those foods can be consumed in calculated and measured amounts, and they serve as natural protein sources, typically calculated as 1 g of protein or 50 mg exchanges of Phe. A specific daily quantity of Phe or natural protein is determined individually and adjusted based on the levels of Phe in the blood (MacDonald et al., 2020).

According to the PKU handbook, the following groups of products can be consumed without restriction:

- Fruits and vegetables containing ≤ 75 mg/100 g (except potatoes);
- Foods containing protein ≤ 0.5 g/100 g;
- Low protein special foods containing Phe ≤ 25 mg/100 g (low-protein bread, pasta, milk replacements);
- Sugar, jams, fats, and oils;
- Spices;
- Salt (MacDonald et al., 2020).

Patients with PKU should be assigned a daily Phe allowance based on their personal tolerance to ensure a stable phenylalanine/protein intake. Various methods exist for calculating Phe/protein intake, which can be measured either in grams of protein per day or milligrams of Phe. Across Europe, healthcare professionals use one of five different calculation systems, all of which effectively support acceptable blood Phe control.

These five approaches are:

- Phe exchange system (e. g. 50, 25, 20 mg of Phe);
- 1 g protein exchanges;
- 1 g protein exchanges (but using the Phe analyses of fruit and vegetables and calculating the weight that provides 50 mg Phe);
- Paper lists/apps listing the Phe content of foods;
- Paper lists/apps listing the protein content of foods (MacDonald et al., 2020).

Every method has its own advantages and limitations, but there is little evidence to show that any particular method is better than another (Pinto et al., 2023).

The amount of amino acid (AA) calculated in grams per kilogram provided by Phe-free formula influences the regulation of blood Phe levels and helps to prevent severe nutrient deficiencies. Because a low-Phe diet often lacks essential nutrients vital for childhood development, micronutrient imbalances are a major concern for PKU patients, who are at an increased risk of developing deficiencies in zinc, selenium, iron, vitamin $B_{12}$, and folate (Anton-Păduraru et al., 2025; Robert et al., 2013). It has been previously described that intake of protein substitutes decreases Phe concentration in the blood; studies have also shown that Phe tolerance increases when total protein intake from protein substitutes is increased (Acosta, 1996; Daly, Evans, et al., 2021; MacDonald et al., 2006).

Protein substitutes play a crucial role at the blood–brain barrier (BBB). LNAA, including Phe, compete for LAT1, a transporter that facilitates the entry of amino acids into the brain. Phe has a particularly high affinity for LAT1, making protein substitutes the only source of competitive LNAA needed to limit excess Phe entry into the brain (Pietz et al., 1999).

Despite their significant pharmacological effects on Phe metabolism and long-term physical and neurological outcomes, the impact of AA formulas is often overlooked in scientific discussions. Similarly, the gut regulates AA absorption across the epithelial membrane. Phe is transported through a carrier-mediated, sodium-dependent process requiring energy. As with the blood–brain barrier, LNAAs in the gut are transported via LAT1, which phenylalanine binds to with high affinity (Daly, Evans, et al., 2021).

To summarise the role of protein substitutes in PKU, they:

- supply LNAAs that compete at the BBB for access to the LNAA transporter (LAT1), thereby reducing the risk of excessive Phe entering the brain and causing neurocognitive harm;
- support normal growth and cellular functioning;
- supply Tyr, which is an essential amino acid in case of PKU;
- regulate blood Phe levels;
- prevent protein and other essential nutrient deficiencies (Daly, Evans, et al., 2021).

According to the E.S.PKU guidelines the total protein intake should supply 40% more than the FAO/WHO/UNU safe levels of protein intake (van Wegberg et al., 2017). According to data published by A. Aguiar et al. in 2015, the median range dosage of protein equivalent (natural protein + protein substitute) expressed in g/kg/day prescribed in Europe differs across the regions (see Table 1.2 (Aguiar et al., 2015)).

Table 1.2

**Median dosage of total equivalent in g/kg/day prescribed by region**
Adapted from A. Aguiar et al. (Aguiar et al., 2015)

| Age Group | Western Europe (N = 25) | Northern Europe (N = 24) | | Southern Europe (N = 10) | Eastern Europe (N = 4) |
|---|---|---|---|---|---|
| | Belgium Netherlands France Germany Switzerland | United Kingdom Ireland (n = 17) | Norway Sweden (n = 7) | Italy Portugal Spain Turkey** | Hungary Poland Russia |
| Infants < 1 year | 2–2.5[4] | 2.5–3[7] | 2.5[2] | 2.5[2] | 2.5 |
| 1–3 years | 1.5–2.4[1] | 2.5–3[7] | 2–2.5[1] | 2–2.5[1] | 2–2.5 |
| 4–10 years | 1–1.5[4] | 2–2.5[7] | 1.5–2 | 1.5–2 | 1.5–2 |
| > 10 years | 1–1.5[1] | 1–1.5[5] | 1–1.5[1] | 1–1.5[1] | 1–1.5 |
| Maternal patients | 1–1.5[4] | 1–1.5[9] | 1.5–2[4] | 1.5–2[4] | 1–1.5 |

Suffix numbers are the number of centres who did not provide data in each age category.
** Turkey belongs to Southeastern Europe but is categorised in this overview in Southern Europe.

To maximise the utilisation of amino acids and minimise the variation in Phe concentrations, protein substitutes should be taken frequently, a minimum of three times per day (Aguiar et al., 2015).

As previously demonstrated by MacDonald A et al. the greater the amount of protein substitute consumed between waking and 4 p.m., the greater the decrease in Phe concentrations. Likewise, when protein substitute was administered every four hours over a 24-hour period, there was a marked stabilisation in Phe concentrations, which reduced Phe variability (MacDonald et al., 1998).

The variety of protein substitutes has significantly increased since the early 2000s. These substitutes are now available in various forms, including AA powders, capsules, tablets, bars, and liquids. Most of these formulas contain added carbohydrates, fats, vitamins, and minerals. This period also saw the rise of pre-packaged and pre-measured products, enhancing convenience, precision, compliance, and simplicity for healthcare providers when prescribing protein substitutes (Aguiar et al., 2015; Daly, Evans, et al., 2021).

**Types of Phe-free formulas:**

- **Spoonable low-volume protein substitutes** – developed in 2000 for young children with PKU, these are based on Phe-free AAs mixed with starch and a small amount of water to form a gel or paste that resembles weaning food (MacDonald et al., 2004). This low-volume, fat-free, lower-calorie, and more concentrated AA substitute aids the transition to a second-stage product beginning at 6 months of age, when the initiation of complementary feeding is advised. Normal infant feeding patterns, teething, and illnesses can cause product rejection in late infancy, requiring parents to be patient and consistent in their approach (Daly, Evans, et al., 2021; Evans et al., 2018; Evans et al., 2016).
- **Ready-to-drink liquid protein substitutes:** First manufactured in 2005, flavoured Phe-free AA pouches were launched as 'ready to drink' options. These low-volume, lower-calorie protein substitutes were practical and compact, promoting greater independence for children and adolescents. Furthermore, Patients felt less self-conscious when consuming a liquid compared to a powder preparation (MacDonald et al., 2006). The pharmacological effectiveness of these lower-volume substitutes did not negatively impact nutritional biochemistry or Phe levels, which either remained stable or improved (MacDonald et al., 2004). However, one potential issue was gastrointestinal discomfort (constipation or diarrhoea), linked to the hyperosmolar concentration of these lower-volume substitutes, indicating that all concentrated AA products should be consumed with additional water (Ford et al., 2018).
- **Protein substitute tablets:** Around the year 2000, AA tablets and modular systems were introduced. Modular systems combine amino acid tablets, capsules, liquids, powders, or bars to meet daily protein substitute needs, offering flexibility in selection; however, the daily requirement was approximately 70 tablets. Furthermore, these tablets were not nutritionally complete, necessitating additional vitamin and mineral supplementation. Tablets provide an alternative for adolescents and adults who find traditional protein substitutes challenging to take (MacDonald et al., 2003).

- **Casein glycomacropeptide with amino acids (CGMP-AA)**: First introduced in the USA as a protein substitute for PKU in 2008, CGMP is derived from whey through anion exchange chromatography, although the final product contains trace amounts of aromatic amino acids, including Phe. CGMP-AA differs from amino acid substitutes; approximately 40% of the product consists of amino acids, while the rest is made up of a bioactive peptide. These peptides originate from glycomacropeptides and are recognised for their better taste and palatability (Daly et al., 2022; Ney et al., 2008).
- **Slow-release protein substitute:** Developed in 2014, there are currently limited published data supporting their effectiveness. A short-term observational study utilising prolonged amino acids in individuals with PKU demonstrated good tolerance, fewer gastrointestinal issues, and no variations in blood Phe levels (MacDonald et al., 2020) (Daly, Evans, et al., 2021).

**Summary of available supplements for dietary therapy:**

- Phe-free formula therapy serves as the central treatment for PKU patients, supporting intellectual development and helping individuals achieve normal IQ levels. It ensures normal growth and development throughout life; however, it requires strict dietary restrictions, which may lead to psychological and neurological challenges due to the avoidance of protein-rich foods. Another disadvantage is that these medical and formulated products are often expensive (Dalei & Adlakha, 2022; Giovannini et al., 2012; Hafid & Christodoulou, 2015).
- Glycomacropeptide formula is enriched with branched-chain AA, inherently possesses low Phe concentration and promotes better palatability and satiety in the diet. High-purity GMP products can effectively supplement integral AAs, though they also increase the cost of treatment (Dalei & Adlakha, 2022; Giovannini et al., 2012; Hafid & Christodoulou, 2015; Rose et al., 2019).
- LNAAs compete with Phe concentrations across the BBB, helping to reduce its levels in the brain. They have been shown to improve executive functions. Despite these advantages, LNAAs can only be used as a supplement to diet therapy. Their administration requires vigilant and strategic management within specialised metabolic centres. Additionally, they are generally more suitable for adults who face difficulties in maintaining strict dietary control (Dalei & Adlakha, 2022; Matalon et al., 2006).

**Pharmaceutical therapy**

Currently, the only pharmacological treatments available for PKU include supplementation with sapropterin dihydrochloride (a synthetic form of BH4, marketed as Kuvan® by BioMarin Pharmaceutical Inc., Novato, CA, USA) and enzyme replacement therapy with pegvaliase. However, sapropterin dihydrochloride is effective only in patients with

significant residual PAH activity. As a result, it may not be suitable for individuals with classical PKU who have no residual enzymatic activity and rely on strict dietary management (Scala et al., 2024; van Wegberg et al., 2017; Vockley et al., 2014).

In contrast, pegvaliase is a PEGylated version of the recombinant enzyme phenylalanine ammonia lyase, derived from *Anabaena variabilis*. This innovative enzyme substitution therapy is administered subcutaneously and breaks down Phe into ammonia and trans-cinnamic acid (Thomas et al., 2018), thereby functioning independently of residual enzymatic activity. Pegvaliase is commercially available under the brand name Palynziq®. Pegvaliase therapy is appropriate for PKU patients older than 16 years (Camp et al., 2014; Scala et al., 2024).

The most common side effects associated with Palynziq® (which may affect more than 7 in 10 people) include reactions at the injection site, joint pain and allergic reactions. The most significant allergic reactions include acute systemic allergic reactions, angioedema (swelling under the skin in areas such as the face, throat, arms. and legs), and serum sickness (allergic reaction caused by animal proteins or serum) ("Palynziq | European Medicines Agency (EMA),").

Also, several other medications are under development. First, sapropterin (a synthetic BH4 analogue) is a lipophobic molecule that is unstable *in vivo* and quickly oxidised in the gut and plasma to BH2, which is either cleared by the kidneys or enzymatically converted to intracellular BH4. As a result, sapropterin administration leads to a significant increase in plasma BH2, but only a moderate rise in intracellular BH4. In contrast, sepiapterin is a more stable molecule that is rapidly absorbed systemically and effectively transported into cells via active membrane transporters, such as the human Equilibrative Nucleoside transporter-2 (hENT2). Once inside the cells, sepiapterin is quickly converted to BH4 through the pterin salvage pathway (Bratkovic et al., 2022; Smith et al., 2019). Unlike sapropterin, sepiapterin efficiently crosses the blood-brain barrier and is transported into brain cells. Administration of sepiapterin results in a more substantial increase in intracellular BH4 levels in target tissues such as the liver, kidneys, and brain compared to sapropterin (Bratkovic et al., 2022; Harding, 2024; Hasegawa et al., 2005; Ohashi et al., 2017; Smith et al., 2025).

Primary and base editing methods are also being studied to correct pathogenic variants in the PAH gene that cause PKU. In mouse studies, these methods have successfully normalised Phe levels without affecting liver function. The company is working on an oral therapy, SYNB1934, which has shown significant reductions in Phe levels during Phase 2 clinical trials. This therapy uses a specially engineered bacterial strain to break down Phe in the digestive tract. Preliminary results indicate an average Phe reduction of up to 34% in patients, with most participants experiencing clinically significant improvements ("Synlogic Announces Positive

Top-Line Phase 2 Data for Phenylketonuria (PKU); SYNB1934 Advances to Phase 3 – Synlogic,").

Meanwhile, Sanofi is conducting clinical trials for SAR444836, a one-off intravenous gene therapy designed to enable patients to maintain normal Phe levels without dietary restrictions ("Gene Therapy Study for Adults with Phenylketonuria (PKU) | Clinical Research Studies Listing,").

Despite the ongoing development of various medications, the primary treatment for PKU remains the restriction of natural Phe intake (Keskin et al., 2022).

### 1.3.9 Health risks for treated PKU patients due to periodically high Phe levels

Main complications are prevented, and clinical outcomes are generally good if treatment is started immediately after newborn screening. Although mild cognitive impairments may still occur, IQ scores tend to be slightly lower than those of healthy controls. A four weeks' delay in starting treatment causes a decline in the IQ score of approximately four points, and neuropsychological impairments may still be present. Those complications can appear due to high Phe levels resulting from unstable metabolic control and/or concurrent illnesses (de Groot et al., 2010; Van Vliet et al., 2018; van Wegberg et al., 2017).

Cognitive impairment is linked with elevated blood Phe levels and/or reduced blood Tyr levels. However, Tyr levels do not directly correlate with cognitive outcomes in PKU, and Tyr supplementation alone does not prevent severe intellectual disability. On the other hand, the relationship between cognitive outcomes and blood Phe levels is well-established. Elevated blood Phe levels increase brain Phe concentrations, which are widely recognised as neurotoxic (de Groot et al., 2010; Van Spronsen et al., 2001).

### 1.3.10 Adverse effects on health and nutritional outcomes due to a low-protein diet

Body composition is a key component of health, and it typically refers to the quantification of body fat and muscle mass – changes that cannot be adequately assessed by body weight or body mass index (BMI) (Javed et al., 2015). In classical PKU, maintaining a low-Phe diet requires significant changes to typical eating habits. Most individuals with PKU must avoid high-biological-value proteins. Instead, they rely on low-Phe or Phe-free protein substitutes as their primary source of essential nitrogen, which is crucial for metabolic stability, proper growth, and lean body mass development (Dokoupil et al., 2012). Since skeletal muscle accounts for 40–45% of body weight and serves as the main storage for peptides and free amino acids, dependence on synthetic nitrogen sources may negatively impact body composition (Wu, 2016). Lean mass relies on amino acid availability, and compared to natural protein, protein substitutes provide amino acids less efficiently in terms of absorption and utilisation

(Daly, Högler, et al., 2021; MacDonald et al., 2006). The care of individuals with PKU necessitates a multidisciplinary approach and ongoing support to ensure positive outcomes. Even with average Phe levels maintained within the treatment range, body composition can still be affected (MacLeod et al., 2009).

Inconsistencies have been noted in reports regarding differences in fat mass percentage and body composition between individuals with PKU and control groups (Doulgeraki et al., 2014; MacLeod et al., 2009). It was previously observed by White et al. (White et al., 1982) that BMI in children with PKU is increased; similar observations have been reported in other studies, indicating increased prevalence rates of overweight and obesity in comparison to the general population in female adult and paediatric populations (Gokmen Ozel et al., 2014). In 2021, Rodrigues et al conducted a meta-analysis and found that BMIs of healthy controls and PKU patients are similar, but in the case of patients with the classical PKU form, a significantly higher BMI was observed (Robertson et al., 2022; Rodrigues et al., 2021).

Early research identified calcium and vitamin D deficiencies as common issues among adult individuals with PKU (Rovelli et al., 2023). However, modern Phe-free L-amino acid supplements now contain calcium and vitamin D levels that exceed recommended requirements (Demirdas et al., 2015; Mirás et al., 2013; van Wegberg et al., 2017). Despite these advancements, osteopenia continues to affect patients who maintain strict low-Phe diets and achieve good metabolic control. Elevated calciuria in PKU patients suggests that calcium deficiency is not the underlying cause. This points to other contributing factors in the development of bone disease in PKU (Demirdas et al., 2017; Mirás et al., 2013).

### 1.3.11 Nutritional status of PKU patients

Several factors can influence the nutritional status of individuals with PKU, including the bioavailability of nutrients, variations in the composition of available protein substitutes, differences in management strategies among metabolic centres, and the accessibility of multidisciplinary care (Venegas et al., 2024).

As previously mentioned, elimination diets can lead to a significant risk of developing deficiencies in micronutrients such as selenium, zinc, iron, calcium, polyunsaturated fatty acids, and vitamin D (Kose and Arslan, 2019). The main components of a low-protein diet for PKU are fruit and vegetables that are naturally low in protein and Phe, special low-protein foods (SLPF) and Phe-free protein substitutes. Although SLPF are low in protein, they contain high amounts of carbohydrates and fat (Pena et al., 2015).

Intriguingly, a deficiency in docosahexaenoic acid (DHA) has also been associated with osteopenia in this population (Mirás et al., 2013). PKU patients have a higher risk of inadequate

long-chain polyunsaturated fatty acids (LC-PUFA) intake than healthy populations. LC-PUFA are essential components of structural lipids in all tissues. DHA and arachidonic acid are the most important LC-PUFA of the omega-3 and omega-6 series, respectively. LC-PUFA are essential for normal central nervous system (CNS) development, and a fatty acid deficiency may lead to disturbances in CNS function (Campoy et al., 2012). Long-chain PUFA intake may be especially deficient after the age of 2 years because, during the first years of life, infants with PKU receive a special Phe-free L-amino acid formula that is enriched with LC-PUFAs; due to high protein content, nutrient-dense foods rich in LC-PUFAs (such as fatty fish, eggs, or offal) are excluded from the diet of children with PKU (Giovannini et al., 2012; Yilmaz et al., 2023).

### 1.3.12 Adherence and knowledge of a low-Phe diet

A diagnosis of PKU in an infant fundamentally changes the lives of parents and caregivers, and it has a significant psychological impact. The knowledge that their actions can affect the neurocognitive outcomes of their children is a heavy responsibility (Ford et al., 2018).

Studies have shown a decline in dietary adherence with increasing patient age, particularly starting from adolescence. An inability to adhere to the diet causes poor metabolic control, elevates Phe concentrations, and worsens neurocognitive outcomes and executive functioning (Beghini et al., 2024; García et al., 2017; Pinto et al., 2023). The main reasons why patients with PKU exhibit low dietary adherence are restricted food options, the unpleasant taste of specialised low-protein foods and protein substitutes, burdensome routines, challenges with dietary socialisation, the limited availability of special low-protein foods, the unintentional consumption of aspartame, and insufficient knowledge about dietary treatment among PKU patients and their caregivers. Healthcare professionals should aim to ensure dietary management is not excessively restrictive, thereby reducing the need for frequent dietary calculations or protein tracking for patients and their caregivers to improve adherence to the diet in adolescents and adults (Bernstein et al., 2017; Hansen et al., 2020; Pinto et al., 2023; Waisbren et al., 2007).

Following dietary guidelines (MacDonald et al., 2020; van Wegberg et al., 2025) requires appropriate knowledge and commitment to these recommendations, initially by parents and later by adolescent and adult patients. The mindset of caregivers and patients often influences whether and how the recommendations of therapists are implemented. It is established that various socioeconomic and psychosocial elements can enhance patient adherence to dietary guidelines, just as other factors may lead to the discontinuation of PKU therapy (Cazzorla et al., 2018; Ford et al., 2018). However, there is a scarcity of studies focusing on the understanding of PKU therapy among patients and their parents, and its effect on dietary

compliance. Still, the importance of family dynamics and their role in treatment adherence has often been underscored. This highlights the necessity of assessing the knowledge level of PKU patients and their families (Bilginsoy et al., 2005).

Dietary adherence in PKU is a complex behaviour influenced by various factors, including individual patient traits, family dynamics, social support, and healthcare system structures (Bilginsoy et al., 2005). Gaining insight into these factors is essential for designing targeted interventions that boost adherence and enhance health outcomes for individuals with PKU. Previous research has identified several barriers to adherence, such as the restrictive nature of the diet, a lack of social support, and limited access to specialised care. However, a comprehensive understanding of the factors affecting adherence across different age groups and contexts remains insufficient (Ford et al., 2018; Gokmen-Ozel et al., 2011).

Poor commitment to treatment is a common challenge in managing chronic diseases. While counselling and education can help improve adherence, it is important to recognise that knowledge alone is not a strong predictor of compliance (Bilginsoy et al., 2005; van Wegberg et al., 2025). Intervention programs, such as summer camps, may lead to temporary enhancements in knowledge, but these increases typically do not result in better adherence or metabolic control in the long run (Ford et al., 2018). A study published by Witalis et al. (2017) indicated that there is a need to develop interactive educational programs about the Phe content in food products, because only 33–40% of patients were aware of the Phe content in common products such as apples, tomatoes, or potatoes (Witalis et al., 2017). Research shows that educational interventions lead to long-term dietary knowledge, whereas improvements in metabolic control are often only temporary (Singh et al., 2000; Witalis et al., 2017). Additionally, failing to adhere to recommendations for protein substitutes increases the risk of nutritional deficiencies (Rohde et al., 2013).

### 1.3.13 Nutritional assessment of PKU patients

Nutritional assessment is included in the minimum requirements for the management and follow-up of patients with PKU defined in the E.S.PKU guidelines. Indeed, a clinical nutritional assessment should be conducted at every outpatient clinic visit, including a 24-hour dietary recall or a 72-hour food diary (FD) (van Wegberg et al., 2025). One of the main parts of a nutritional assessment is dietary evaluation, which includes measuring the intake of energy, natural protein, Phe, and other nutrients. Usually, a 24-hour food recall or a 72-hour FD is used for dietary evaluation by dietitians. However, collecting and analysing food diaries can be very challenging. (Pinto et al., 2023) Below are listed the most common challenges:

- It can be complex for individuals, who do not cook regularly and are not familiar with weighing foods;
- It requires high cooperation from caregivers, and its application can be limited by the characteristics of the target population;
- Respondents may improve their dietary habits unintentionally through self-reflection, or they may alter their diet intentionally to avoid the burden of detailed reporting, or even choose not to report actual intake (Kristjansdottir et al., 2006; Ortega et al., 2015).

Even if an FD is correctly filled, for specialists, it may be difficult for specialists to calculate Phe levels, as the absence of standardisation in food composition databases presents a considerable hurdle. Variability in Phe content among different foods and brands (in the case of commercially available foods) can lead to inconsistencies, making it difficult to determine precise Phe intake levels (Araújo et al., 2017). Food Composition Tables and searchable databases provide information on nutrients, but data regarding the amino acid content of foods are frequently either unavailable, scarce, or outdated (Araújo et al., 2017; Demirkol et al., 2011).

# 2 Methods and Materials

## 2.1 Ethical statement

All participants or their legal guardians gave written informed consent. The study was conducted according to the Declaration of Helsinki, and the protocol was approved by the Central Medical Ethics Committee of Latvia (Nr.1/19-02-11) (Annex No 1).

## 2.2 Informed consent statement

All participants and their caregivers who took part in this study gave written informed consent, allowing their clinical data to be used for research. The informed consent template and its detailed description are included in the Annex No 2.

## 2.3 Study description

The study took place from 2019 until 2025 at the Rare Disease Coordination Center, Children's Clinical University Hospital (CCUH), and it consisted of three parts:

- **Part I –** Assessment of dietary intake and biochemical parameters in patients with PKU: At the first phase, all patients aged 1–18 years at the time of the study (from 2019 to 2020) diagnosed with PKU were invited to participate. The families of the affected children were asked to:
  - Provide blood samples for the evaluation of biochemical parameters (detailed description in the Section 2.4.5), including plasma Phe concentrations.
  - Record detailed food diaries over three consecutive days, documenting all foods and beverages consumed.
  - The collected dietary records and biochemical data were subsequently analysed and compared to those obtained from a age- and sex-matched control group without known disorders.
- **Part II –** Evaluation of nutritional composition of preselected foods with unknown or doubtful nutritional composition:
  - During the analysis of data from food diaries, it was identified that existing food composition databases often lacked accurate nutritional information for locally available Latvian food products commonly consumed by the PKU patient population. To address this limitation, a curated list of relevant food items was developed and compiled in consultation with patients and their caregivers. Selected food products were subjected to laboratory analysis to determine their macronutrient and micronutrient content, including full amino acid profiles. These data were subsequently incorporated into the dietary assessments to improve

the precision of nutritional analysis and to enhance dietary planning for patients with PKU.

- **Part III** – Evaluation of patient and caregiver knowledge regarding PKU and low-protein diet management:
  - In the third part, patients with PKU and their caregivers were surveyed using a structured questionnaire. The purpose of this phase was to assess their knowledge and understanding of the disease, its metabolic implications, and the specific requirements of a low-protein diet essential for effective PKU management. The findings from this survey aimed to identify knowledge gaps and inform the development of targeted educational strategies to improve disease management outcomes.

## 2.4 Part I of the Study

### 2.4.1 Description of the PKU population

In total, 122 patients with PKU were identified or diagnosed in Latvia between 1987 and 2021. Of these, 39 were unable to receive the necessary follow-up care for various reasons, such as death (n = 1), emigration (n = 2), or being lost to follow-up due to a lack of treatment adherence (n = 36). The remaining 83 patients regularly (at least once a year) visited the outpatient clinic to see an IMD doctor and a dietitian.

At the time of the research, 46 of the diagnosed patients receiving follow-up care were under 18 years of age, while the remaining 37 were adults over 18.

These patients with PKU adhered to their regular treatment plan, which involved following a protein-restricted diet and using the prescribed amount of a Phe-free amino acid formula that also contained essential vitamins and trace elements. Of the 46 paediatric patients, 36 (78%) had cPKU, five (11%) had the mild PKU form, and five (11%) had MHPA. Additionally, five patients were receiving sapropterin dihydrochloride treatment, a pharmaceutical form of tetrahydrobiopterin (BH4) that aids in PKU management.

The inclusion criteria for **Part I** of the study were as follows:

- Patients aged between 1 and 18 years;
- Patients diagnosed with the cPKU and mild PKU.

The following criteria were used to exclude individuals from **Part I** of the study:

- Patients aged below 1 year or above 18 years;
- Patients or their parents who refused to undergo the blood tests necessary for the study;
- Patients with MHPA;

- PKU patients receiving BH4 therapy.
- Individuals receiving nutritional supplements (with the exception of vitamin D and fish oil) or adhering to other specific dietary restrictions.

Patients under the age of one year were excluded from the study due to several important methodological and physiological considerations. Firstly, the volume of blood required for analysis exceeded the amount that could be safely collected from this age group. Secondly, at this developmental stage, infants had not yet commenced complementary feeding, which resulted in a non-differentiated dietary profile and significant variability in nutrient intake. Thirdly, their nutrition was primarily based on specialised amino acid formulas, which were often fortified with polyunsaturated fatty acids, potentially influencing the study outcomes.

Adult patients were also excluded from the study, as dietary restrictions during childhood are known to have the most profound and lasting effects on health outcomes. Additionally, adults typically exhibit lower treatment adherence and are more likely to present with comorbid conditions, which may confound the interpretation of results and obscure causal relationships.

The control group was randomly selected, without known comorbidities, and sex-matched with PKU patients. All healthy control participants did not receive supplements except vitamin D and fish oil and had no dietary restrictions. They also had to sign the agreement paper to participate in the study, fill a food diary, and take the blood tests.

### 2.4.2 Anthropometric parameters

Anthropometric parameters – specifically height and weight – were measured in patients during follow-up visits every three months and analysed using World Health Organization (WHO) growth percentile charts. Body mass index (BMI) was used to analyse body composition according to the following equation (Casadei & Kiel, 2025):

$$BMI = \frac{weight\ (kg)}{height\ (m)2}$$

BMI was analysed using World Health Organization BMI percentile (pc) charts which are age-and gender-appropriate:

- Severe underweight: BMI < 3 pc;
- Underweight: BMI < 15 pc;
- Normal weight: BMI ≥ 15pc and < 85 pc
- Overweight: BMI ≥ 85 pc and < 95 pc;
- Obesity: BMI ≥ 95 pc. (Organización Mundial de la, 2007).

### 2.4.3 Food diary

During their outpatient visits, all paediatric PKU patients who received dietary treatment (n = 41) and the caregivers of the control group participants were instructed to record all foods and beverages consumed by the child over three consecutive days, including one weekend day. This was done to complete a detailed 72-hour FD (a record of all food and beverages consumed within this period) and bring it to their next appointment or send it to the dietitian via e-mail. For younger children, the food diary was completed by parents or legal guardians. For older children and adolescents, the food diary was completed by the participant with parental assistance when necessary.

Information on food brand names, as well as cooking and preparation methods, was included, and portion sizes were to be reported as accurately as possible using common unit sizes or, preferably, by weighing the food. Participants were contacted to clarify unclear or missing entries when necessary.

Eight (20%) caregivers and patients refused to complete or did not provide a completed FD, and two (5%) FDs were invalid due to missing information on the precise amounts of food or because they contained beverages incompatible with the dietitian's instructions.

### 2.4.4 Food diary analysis

The 72-hour FD data were analysed using a nutrient content database created from the particulars of local and foreign food nutrients and Nutritics Nutritional Software (*Nutritics*, 2021) and Microsoft Office 365 Excel software (*Microsoft Excel*, 2025). The analysed parameters included the daily intake of:

1 Total energy (measured in kilocalories, kcal);
2 Total protein;
3 Natural Phe (for PKU patients only);
4 Amino acid supplements (for PKU patients only);
5 Total fat;
6 Carbohydrates (CHO);
7 Micronutrients – zinc, selenium, and iron.

If an FD was not completed according to the instructions or information was missing (such as missing data regarding the amount of a product or an unknown food brand), it was excluded from the analyses (n = 2). All data were compared with Ministry of Health of the Republic of Latvia (MHRL) recommendations (VM, 2017). Participants who provided an FD were divided into groups by age: 1–3 years of age (n = 5), 4–6 years of age (n = 11), 7–12 years of age (n = 8), and 12–18 years of age (n = 7) (MacDonald et al., 2020; van Wegberg et al., 2025). The distribution of energy from macronutrients in children with PKU and

age-matched controls was compared against the national dietary recommendations issued by the MHRL.

### 2.4.5 Analysis of biochemical parameters

After completing the FD, all respondents were asked to undergo overnight fasting blood tests to analyse their levels of micronutrients (zinc, selenium, and ferritin), lipids (total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and triglycerides (TG)), erythrocyte fatty acids (FAs), glucose, and vitamin D. Vitamin D measurements were assessed year-round, without restriction to a specific season. Blood tests were performed in the E. Gulbis Laboratory (LVS EN ISO 15189:2013) and by the CCUH Laboratory (LVS EN ISO 15189).

Venous blood sample was obtained. Methods are described in Table 2.1.

Table 2.1

**Biochemical Parameter Assessment Methods**

| Biochemical parameters | Unit | Analysing methods | Instrument | Laboratory |
|---|---|---|---|---|
| Zinc | µg/dL | Photometric methods | Atellica CH, SIEMENS | E. Gulbis Laboratory |
| Selenium | µg/dL | Nuclear mass spectrometry | PerkinElmer Nexion, 2000 ICP MS | E. Gulbis Laboratory |
| Ferritin | µg/dL | Immune fermentative chemiluminescence methods / Electrochemiluminescence | ATELLICA IM Analyser, SIEMENS Healthcare | E. Gulbis Laboratory |
| Lipids (Total cholesterols, LDL, HDL) | Mmol/L | Enzymatic colour reaction / enzymatic reaction | Atellica CH, SIEMENS | E. Gulbis Laboratory |
| Fatty acids status | % | Gas chromatography technology | GC-FID | E. Gulbis Laboratory |
| Glucose, | Mmol/L | Enzymatic hexokinase method | Atellica CH, Siemens | E. Gulbis Laboratory |
| Vitamin D | ng/ml | Electrochemiluminescence method | Atellica IM, Siemens | E. Gulbis Laboratory |
| Amino acids (Phe, Tyr) | mg% | Ultra Performance Liquid Chromatography (UPLC) | An Ultra Performance Liquid Chromatograph equipped with a UV detector (UPLC-UV system) | CCUH Laboratory |

LDH – low-density lipoprotein cholesterol, HDL – high-density lipoprotein cholesterol, Phe – phenylalanine, Tyr – tyrosine, CCUH laboratory – Children's Clinical University Hospital laboratory, % of FA – All peaks were subjected to integration, and subsequently, the proportional content of each FA component was determined as the percentage of its peak area relative to the total peak areas of all FA components

The method used for the determination of amino acids (Phe, Tyr) was Ultra Performance Liquid Chromatography (UPLC), where the separation of a mixture of amino acids occurs under

chromatographic conditions with subsequent semi-quantitative determination using a UV detector that reacts to compounds that absorb ultraviolet or visible light.

A fluorometric assay measured patients' Phe concentrations in dried blood spots (DBS) collected onto filter paper. The assay was based on a modification of the McCaman and Robins quantitative fluorometric method. Briefly, Phe eluted from the DBS formed a fluorescent complex with ninhydrin, which was enhanced by the dipeptide L-leucyl-L-alanine. During the reaction, the pH was controlled using a succinate buffer. A copper reagent was added to stabilise the ninhydrin-Phe complex and increase the signal before measuring the fluorescence. Fluorescence was measured using a Neonatal Phenylalanine kit (Labsystems Oy, Finland) and a microplate fluorometer (Fluoroskan Ascent; Labsystems Oy).

### 2.4.6 Methods of statistical analysis of the Part I of the Study

Food diaries from 33 families were screened; 31 (94%) met the quality criteria and were analysed. Continuous outcomes (energy/macronutrient intakes, energy shares, anthropometry, biochemical markers, including erythrocyte FA concentrations) were summarised as median ± SD. PKU vs control comparisons were performed using independent-samples t-tests and Mann–Whitney U tests.

Weight, height, and BMI were reported as median ± standard deviation (SD), while the PKU patient proportion and gender distribution were expressed as percentages (%).

Anthropometric parameters, BMI (kg/m²), and age- and sex-specific BMI percentiles were derived using standard paediatric growth references; overweight and obesity were classified by BMI percentile cut-points (overweight ≥ 85$^{th}$ to < 95th, obesity ≥ 95$^{th}$ percentile). Between-group differences in the prevalence of overweight and obesity (PKU vs control) were evaluated using Fisher's exact test.

## 2.5 Part II of the Study

### 2.5.1 Food selection for nutrition analysis

Patients with PKU and their families were invited to compile a list of food products they would like to have analysed for chemical composition. This informal survey was conducted among members of a WhatsApp group consisting of approximately 40 parents and 10 individuals living with PKU. This platform served as a practical communication channel for engaging directly with the Latvian PKU community and identifying foods of particular interest for further nutritional evaluation. Ten caregivers (25%) and five patients (50%) submitted responses, listing foods (n = 55) that present challenges for adhering to the dietary management of PKU. Subsequently, a team of four dietitians, three paediatricians, and one internist reviewed

the list and, following discussions with the caregivers, reached a consensus on which foods (n = 34) should be analysed for their Phe content. Preference was given to:

- Locally grown, traditional Latvian products – sorrel, rhubarb, nettle, leeks, spring onions, wild garlic, mushrooms (*Boletus edulis, Russula emetica, Chanterelle*), coriander, celery stalk and root;
- Foods with a lack of available nutritional composition data: microgreens of radish, sunflower, broccoli, garden cress, and sugar peas;
- Controversial foods with limited available nutritional data: broths of turkey, chicken, trout, lamb, venison, and pork.

### 2.5.2 Methods of analysing nutritional profiling of foods for PKU

Foods that were bought in the supermarket or picked, requiring no specific preparation, are listed in Table 2.2. All foods that were collected (wild, tinned or frozen) during harvest time, were vacuum-packed and stored at a temperature of −18 °C until analysis due to seasonality constraints. One kilogram of each product was used for the analysis.

Table 2.2

**Methods of food collection**

| No | Food Item | Method of Collection | Storage and Analysing Methods |
|---|---|---|---|
| 1 | *Aquafaba* | Manufactured “Rimi” | From cane |
| 2 | *Arugula* | Manufactured “I love Eco” ICA | Fresh |
| 3 | *Broccoli microgreens* | Manufactured “Daces zaļumi” | Fresh |
| 4 | Celery stalk | Manufactured (Rimi, origin Spain) | Fresh |
| 5 | Coriander | Picked in harvest season | Fresh |
| 6 | Garden cress microgreens | Manufactured “Daces zaļumi” | Fresh |
| 7 | Leeks | Picked in harvest season | Fresh |
| 8 | Nettle | Picked in harvest season | Frozen |
| 9 | Peas microgreens | Manufactured “Daces zaļumi” | Fresh |
| 10 | Radish microgreens | Picked in harvest season | Fresh |
| 11 | Raisins (black) | Manufactured “Zelta rieksts” | Dried |
| 12 | Raisins (gold) | Manufactured “Zelta rieksts” | Dried |
| 13 | Raisins (jumbo) | Manufactured “Zelta rieksts” | Dried |
| 14 | Rhubarb | Picked in harvest season | Frozen |
| 15 | Sorrel, fresh | Picked in harvest season | Fresh, frozen |
| 16 | Spring onions | Picked in harvest season | Fresh |
| 17 | Sugar snap peas | Manufactured (Rimi, origin Egypt) | Fresh |
| 18 | Sunflower microgreens | Manufactured “Daces zaļumi” | Fresh |
| 19 | Wild garlic | Picked in harvest season | Frozen |

For the purposes of the analysis, the food samples were prepared using standard boiling and cooking procedures on *Electrolux*® induction cooktops. Cooking was conducted at power level 7, corresponding to an estimated temperature range of 200–250 °C. Methods of preparation described in Table 2.3.

Table 2.3

**Methods of food collection and sample preparation techniques**

| No | Food Item | Method of Collection | Method of Preparation |
|---|---|---|---|
| 1 | Cep *(Boletus edulis)* | Picked in harvest season | Chopped and cooked in the pan for 18 minutes, frozen, tempered |
| 2 | Turkey boneless meat | Manufactured (Rimi, origin Latvia) | 1 kg of turkey boneless meat was used to cook turkey broth – boiled in 3 litres of water, cooking time 40 minutes |
| 3 | Chicken boneless meat | Manufactured (Kekavas food, origin Latvia) | 1 kg of chicken boneless meat was used to cook chicken broth –, boiled in 3 litres of water, cooking time 40 minutes. |
| 4 | Lamb rib bones | Manufactured (Farm-based meat producer) | 1 kg of lamb rib bones was used to cook lamb broth, boiled in 6 litres of water, cooking time – 1 hour 20 minutes |
| 5 | Broth (venison) | Manufactured (farm-based meat producer) | 1 kg of deer shin bones was used to cook venison broth – boiled in 6l of water, cooking time 1 hour 20 minutes. |
| 6 | Broth (trout) | Manufactured (Rimi, origin France) | 1 kg of trout fillet was used to cook trout broth, boiled in 3l of water, cooking time 40 minutes |
| 7 | Broth (pork) | Manufactured (Rimi, origin Poland) | 1 kg of pork shoulder meat was used to cook pork broth, boiled in 3 litres of water, cooking time 40 minutes |
| 8 | Celery root | Manufactured (Rimi, origin Netherlands) | 1kg of celery root, cut and boiled for 30 minutes |
| 9 | Champions mushroom (brown) | Manufactured (Rimi, origin Lithuania) | Chopped and cooked in the pan for 10 minutes |
| 10 | Champions mushroom (white) | Manufactured (Rimi, origin Lithuania) | Chopped and cooked in the pan for 10 minutes |
| 11 | Chanterelle | Picked in harvest season | Collected during harvest time, washed, cooked for 30 minutes and frozen, tempered, cooked |
| 12 | Portobello mushroom | Manufactured (Rimi, origin Lithuania) | Chopped and cooked in the pan for 20 minutes |
| 13 | Russula, boiled | Picked in harvest season | Boiled in 3l of water for 20 minutes, frozen |
| 14 | Shiitake mushroom | Manufactured (Rimi, origin Lithuania) | Chopped and cooked in the pan for 20 minutes |
| 15 | Sorrel, boiled | Picked in harvest season | Boiled in 3l of water for 5 minutes, frozen |

The initial analysis of the 34 selected foods was performed by *J.S. Hamilton Poland Sp. z o.o.* (LVS EN ISO/IEC 17025:2017). AA were analysed by PB-53/HPLC – determining the profile of AA (in total – with peptide bonds and free AA) using pre-column reverse-phase high-performance liquid chromatography ranging from 0.005% to 10%.

Phe was determined in the unoxidized sample, which was hydrolysed with a hydrochloric acid solution (C = 6 N). The AAs were converted to the corresponding phenyl thiocyanate derivatives and determined by reverse phase high-performance liquid chromatography with ultraviolet or diode array detector detection (wavelength 254 nm). The content of each AA in the test sample was determined by the internal standard method. The result was expressed as a mass fraction, in a percentage to two decimal places. Samples were analysed according to the following norms and regulations: *PB-116 ed. III of 11 August 2020* for crude protein. The protein content of all 34 chosen foods was calculated from the total nitrogen content using the conversion factor N × 6.25, with total nitrogen determined via the Kjeldahl method.

Total sugars were analysed based on European Union regulations, utilising method PB-429 ed. I of 28.09.2021. Analytes from the samples were extracted with water, deproteinised, and clarified using Carrez solutions. During clarification, the following reaction occured:

$$2\ ZnSO_4 + K_4Fe(CN)_6 \rightarrow Zn_2Fe(CN)_6\downarrow + 2\ K_2SO_4$$

Colloidal zinc hexacyanoferrate (II), formed during the reaction in the form of a precipitate, co-precipitates high-molecular compounds such as proteins, pectins and tannins. The final determination was performed using the ion chromatography technique with pulse amperometric detection.

Fatty acids were analysed in accordance with EU regulations (PN-EN ISO 12966-1:2015-01; PN-EN ISO 12966-2:2017-05, excluding sections 5.3 and 5.5; PN-EN ISO 12966-4:2015-07. Fatty acids were determined in the fat fraction extracted by the PAK laboratory; subsequently, the fat samples were esterified and quantified using gas chromatography coupled with a flame ionisation detector.

All nutrients were determined according to Regulation (EU) No 1169/2011 of the European Parliament and of the Council (see Table 2.4).

Table 2.4

**Applied methods of laboratory analyses**

| Chemical components | Source of method / regulation |
|---|---|
| Energy (kcal/100 g) | Regulation (EU) No 1169/2011 of the European Parliament and of the Council |
| Carbohydrates (g/100 g) | Regulation (EU) No 1169/2011 of the European Parliament and of the Council |
| Fibre content (g/100 g) | AOAC 991.43:1994 |
| Protein content (g/100 g) | PB-116 ed. III of 11.08.2020 |
| Fat content (g/100 g)) | PN-A-82100:1985 (withdrawn) |
| Fatty acids (mg/100 g) | PN-EN ISO 12966-1:2015-01; PN-EN ISO 12966-2:2017-05 except p.5.3 and 5.5; PN-EN ISO 12966-4:2015-07; |

Six broths were analysed second time in the "Eurofins Labtarna Lietuva" laboratory (LST EN ISO/IEC 17025:2018). Nutrients such as protein, amino acids, fat, carbohydrates, and total energy were analysed (analysing methods described in Table 2.5), second test results compared with data from the first laboratory J.S. Hamilton Poland Sp. z o.o.

Table 2.5

**Analytical methods applied for nutrient profiling by *Eurofins Labtarna Lietuva***

| External Name | Method Source | Analytical Technique | Method Description |
|---|---|---|---|
| Protein | Internal Method | Kjeldahl (titrimetry) | Digestion with $H_2SO_4$ and catalyst; alkalization, distillation into boric acid; titration with HCl to determine nitrogen and protein content. |
| Amino acids (acid hydrolysis) | ISO 13903:2005 | IC-UV | Hydrolysis in HCl, pH adjustment, amino acids separated and detected via post-column derivatization with ninhydrin (440/570 nm). 1-point calibration. QA includes pet food standard. |
| Tryptophan | EU 152/2009 | LC-FLD | Alkaline hydrolysis, quantification by HPLC. Method reference: EU 152/2009 (G) = EN ISO 13904:2016. |
| Total digestible carbohydrates calculated from dietary starch and sugar profile | Internal Method | Calculation | Based on summation of AOAC 2014.10 and Sugar profile (HPAEC-PAD acc ISO 22148). In line with Cereal Chemistry 97 (2019) 114. Complements total dietary fibre methods like AOAC 2017.16. |
| Total fat | Internal Method | Gravimetry | Fat hydrolysed with Hydrotherm in HCl, filtered, dried. Extracted with Soxtherm using light petroleum, solvent evaporated, mass of extract measured. |

Statistical analysis was not applied to the nutritional composition data of food products because comparable datasets or standardised reference values were not available. Consequently, the findings are reported using descriptive analysis only.

## 2.6 Part III of the Study

### 2.6.1 Questionnaire evaluating patient and caregiver knowledge of PKU and dietary treatment

To assess the level of knowledge regarding PKU and adherence to a low-protein diet among patients with PKU and their caregivers, a cross-sectional questionnaire study was conducted. A structured questionnaire was developed and adapted for individuals diagnosed with PKU and for their primary caregivers. The survey instrument included sections on socioeconomic characteristics, as well as a shared core section addressing disease-specific knowledge and dietary practices related to PKU. The disease-related questions (n = 15) were identical for both groups. Participation in the questionnaire study was limited to patients with preserved cognitive function to ensure the reliability and validity of self-reported responses. Patients with late-diagnosed PKU and associated intellectual disabilities were therefore excluded from the study. The questionnaire included an explanation of the study's aims and encouraged respondents to answer as sincerely as possible.

In total, 61 PKU families were invited to participate in the study. Data collection was performed either in person during routine outpatient visits or through telephone interviews in 2025. Participants aged 12 years and older, including adults (n = 20) completed the questionnaire independently. For patients younger than 12 years, the primary caregiver (n = 30) completed the questionnaire on behalf of the patient. One patient declined participation, and ten patients could not be reached via telephone.

Knowledge regarding PKU itself and dietary treatment assessed using structured questionnaires and analysed separately for patients and caregivers. In addition, knowledge levels were compared between the two groups and evaluated in relation to metabolic control status, aiming to determine whether better disease- and diet-related knowledge was associated with more favourable biochemical outcomes.

Metabolic control was assessed using available blood phenylalanine (Phe) measurements from the period between 2023 and 2025. For the longitudinal comparison, patients were stratified into the following age groups: 1–3 years, 4–6 years, 7–12 years, and 12–18 years. Annual metabolic control was evaluated using the median Phe concentration for each patient. Metabolic control was considered good or acceptable when the annual median Phe concentration was below the recommended age-specific threshold: < 360 µmol/L for

children below 12 years of age, and < 600 µmol/L for patients above 12 years of age, according to age-specific treatment targets.

For the detailed 2025 analysis, metabolic control was additionally classified based on the proportion of blood Phe measurements within the recommended target range. Patients were categorised as having good metabolic control if at least 75% of all available Phe measurements in 2025 were below the maximum recommended age-specific threshold (< 360 µmol/L or < 600 µmol/L, depending on age). Patients who did not meet this criterion were classified as having poor metabolic control.

To ensure clarity and relevance, the questionnaire was piloted with five non-PKU individuals, including healthcare professionals (inherited metabolic disorder physicians, dietitians, a psychologist, and a nurse) and members of the general population. Based on their feedback, modifications were made to resolve ambiguities. Pilot participants were excluded from the main study.

The full questionnaire is available in Annex 3.

### 2.6.2 Questionnaire data analysis

Descriptive statistics were used to summarise questionnaire responses. The total number of correct answers was calculated by awarding one point per correct response, yielding a maximum possible score of 15. Correct response rates were calculated separately for caregivers and patients and compared between groups. Respondents were further stratified according to metabolic control status, and overall knowledge scores were compared between the good/average and poor metabolic control groups. Categorical variables were presented as n (%), while biochemical parameters, including Phe, Tyr, and Phe/Tyr ratio, were presented as medians.

## 2.7 Methods of statistical analysis

The statistical analysis was conducted using the Statistical Package for the Social Sciences IBM SPSS Statistics R 4.1.2 ( *IBM SPSS Statistics for Windows*) and Microsoft 365 Office Excel (*Microsoft Excel*, 2025). A p-value $< 0.05$ was considered statistically significant.

# 3 Results

## 3.1 Patient characteristics

Thirty-three families of PKU patients provided food diaries for the analysis, but 31 of the 33 FDs (94%) were suitable for it.

FD availability varied across age groups. In the 1–3 years group, 5 PKU families provided FD (62.5%). In the 4–6 years group, 11 families (92%) provided FD, whereas in the 7–12 years group, 8 FD (80%) were available. In the oldest group (13–18 years), FD were collected from 7 patients (58%). Figure 3.1 presents the distribution of analysed food diaries by age group and sex.

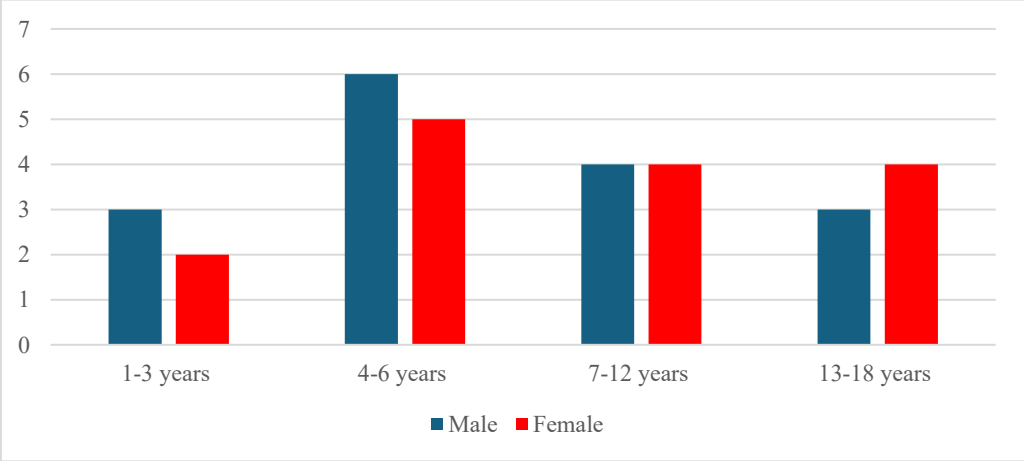



Figure 3.1 **PKU patients classification by age and sex**

The median age of male patients in the PKU group was 6.5 years, compared with 7.5 years in the control group (CG). Median body weight was 19.1 kg in the PKU group and 27.5 kg in the CG, while median height was 1.1 m and 1.3 m, respectively. The median BMI of male PKU patients was 16.9 kg/m² with a median percentile of the 50th, whereas in the CG, the median BMI was 17.1 kg/m², and median percentile was the 50th. No statistically significant differences were observed between male PKU patients and the CG.

The median age of female participants in both the PKU and control groups (CG) was 7 years. The median body weight was 26 kg in the PKU group and 20 kg in the CG, while median height was 1.2 m and 1.1 m, respectively. Female PKU participants had a statistically significantly higher median BMI (19.0 kg/m²) compared with female CG respondents (16.7 kg/m², $p = 0.027$), although the median BMI percentile did not differ significantly between the groups.

In total, 32% (n = 10) of PKU patients were overweight or obese. This included four males who were overweight and one who was obese. Among the female patients, four girls were obese and one was overweight. In the CG, 17% (n = 5) of participants were overweight

or obese – comprising two boys, while none of the girls were obese; one boy and two girls were overweight.

## 3.2 Food diary analysis

### 3.2.1 Macronutrient intake in the PKU diet

In the youngest age group (1–3 years; n = 5) energy and protein intakes (1236 ± 403 kcal/day and 42 ± 12 g/day, respectively) were slightly higher than in controls (973 ± 206 kcal/day and 44 ± 8 g/day), though the differences were not statistically significant ($p > 0.05$). CHO intake in the PKU group (210 ± 40 g/day) was higher than in the controls (122 ± 49 g/day), but this difference was not statistically significant ($p = 0.738$). Fat intake was statistically significantly lower in the PKU group (27 ± 16 g/day) compared to controls (40 ± 4 g/day; $p = 0.004$). In this group, children with PKU demonstrated a significantly lower proportion of energy derived from fat compared to the control group ($p = 0.012$). Conversely, the CHO energy share was significantly higher in the PKU group (68%), exceeding the control group (50%) ($p = 0.004$). Protein contribution to energy did not differ significantly between groups ($p = 0.125$).

In the age group of 4–6 years (n = 11) PKU patients exhibited lower median intakes of energy (1142 ± 371 kcal/day), protein (41 ± 14 g/day), and fat (29 ± 14 g/day) compared with controls (1461 ± 276 kcal/day, 55 ± 11 g/day, and 49 ± 7 g/day, respectively). CHO intake was similar between the groups. No statistically significant differences were observed in this age group across all nutrient categories ($p > 0.05$). PKU patients in this age group of 4–6 years had a significantly lower median fat energy share (23%) compared with controls (30%) ($p = 0.026$). Protein contribution was slightly lower in the PKU group (14%) compared with controls (15%) and was statistically significant ($p = 0.019$). No significant differences were observed in the CHO energy share ($p = 0.213$).

In the 7–12 years age group (n = 8) median energy and macronutrient intakes in both groups exceeded or were within the MHRL reference ranges. Although the PKU group had slightly higher CHO intake (260 ± 83 g/day vs. 232 ± 79 g/day in controls), the difference was not statistically significant ($p = 0.238$). No significant differences were observed in protein or fat intake. In the PKU group, fat contributed 25% of total energy, while protein and carbohydrate contributions were 12% and 53% respectively.

Adolescents in 13–18 years age group (n = 7) with PKU had lower energy (1682 ± 394 kcal/day) and fat intakes (48 ± 17 g/day) compared to controls (2051 ± 565 kcal/day and 76 ± 39 g/day, respectively), though these differences did not reach statistical significance ($p > 0.05$).

Among adolescents, PKU patients consumed significantly less energy from fat (26%) than recommended by the MHRL, though the difference between groups was not statistically significant. Interestingly, the protein contribution was higher in the PKU group (15%) than in controls (12%) and more closely aligned with national recommendations, with the difference reaching statistical significance ($p = 0.002$). CHO intake showed no significant differences between the groups ($p = 0.363$), though both groups remained below the recommended intake levels (See Annex 4 and Annex 5).

### 3.2.2 Associations between biochemical parameters and nutrient intake

This analysis compared the biochemical markers of zinc, selenium, and iron with their respective nutrient intakes in children with PKU and matched controls across different age groups. The results were evaluated in relation to national reference ranges according MHRL recommendations and are presented in Table 3.1.

- Zinc

Across all age groups, blood Zn levels in both PKU and control groups remained within the reference range (70–120 μg/dL). However, the nutrient intake of zinc in the PKU group consistently exceeded the recommended dietary intake, particularly in the 4–6-year group ($18 \pm 7$ mg/day vs reference 6–8 mg/day). Despite these higher intakes, there were no statistically significant differences in blood Zn levels between PKU and control groups ($p > 0.05$ in all age groups).

- Selenium

Selenium intake in PKU patients tended to be higher than reference values (e. g., $68 \pm 25$ μg/day in 1–3-year-olds vs reference 20–24 μg/day), yet no statistically significant differences in blood selenium levels or intakes were found between PKU and controls across any age group.

- Iron

Iron status showed the most notable trend. Median blood ferritin levels in the PKU group were higher than in controls across all age groups, although no statistical significance was observed in the youngest group (1–3 years: $27 \pm 13$ μg/dL in PKU vs $20 \pm 6$ μg/dL in controls; $p = 0.986$). Iron intake among PKU participants was markedly above recommended values (e. g., $17 \pm 5$ mg/day in 1–3-year-olds vs reference 7–9 mg/day), and it was consistently higher than in controls (who had sub-recommended intakes). However, no statistically significant differences were found for iron intake values between the groups ($p > 0.05$).

Table 3.1

**Biochemical comparison with nutrient intake in study groups – phenylketonuria and control group**

| Age group | Group | Zinc | | Selenium | | Ferritin / Iron | |
|---|---|---|---|---|---|---|---|
| | | Blood level µg/dl (±SD) | Nutrient intake* (mg / day) (±SD) | Blood level µg/dl (±SD) | Nutrient intake* (µg / day) (±SD) | Blood level µg/dl (±SD) | Nutrient intake* (mg / day) (±SD) |
| 1–3 years (n = 5) | PKU | 71 ± 8 | 13 ± 5 | 81 ± 13 | 68 ± 25 | 27 ± 13 | 17 ± 5 |
| | Control group | 104 ± 13 | 7 ± 2 | 68 ± 7 | 57 ± 45 | 20 ± 6 | 6 ± 1 |
| | p-value | 0.318 | 1.000 | 1.000 | 1.000 | 0.986 | 0.500 |
| | Reference | 70-120 | 5 - 7 | 50-150 | 20 - 24 | 20-200 | 7 - 9 |
| 4–6 years (n = 11) | PKU | 76 ± 19 | 18 ± 7 | 75 ± 13 | 59 ± 17 | 34 ± 48 | 19 ± 7 |
| | Control group | 107 ± 16 | 8 ± 1 | 60 ± 5 | 54 ± 10 | 22 ± 13 | 6 ± 2 |
| | p-value | 0.278 | 0.214 | 1.000 | 1.000 | 0.882 | 0.313 |
| | Reference | 70-120 | 6 - 8 | 50-150 | 24 - 30 | 20-200 | 7 - 9 |
| 7–12 years (n = 8) | PKU | 81 ± 14 | 14 ± 6 | 73 ± 8 | 100 ± 42 | 33 ± 41 | 19 ± 6 |
| | Control group | 109 ± 18 | 10 ± 2 | 72 ± 11 | 99 ± 42 | 22 ± 11 | 11 ± 1 |
| | p-value | 0.838 | 0.429 | 1.000 | 1.000 | 0.949 | 0.296 |
| | Reference | 70-120 | 7 - 9 | 50-150 | 32 - 39 | 20-200 | 9 - 11 |
| 13–18 years (n = 7) | PKU | 71 ± 11 | 14 ± 6 | 67 ± 13 | 86 ± 35 | 39 ± 23 | 25 ± 10 |
| | Control group | 99 ± 15 | 7 ± 4 | 58 ± 8 | 90 ± 29 | 21 ± 24 | 9 ± 5 |
| | p-value | 0.120 | 1.000 | 0.563 | 1.000 | 0.995 | 0.462 |
| | Reference | 70-120 | 8–10 | 50–150 | 41–50 | 20–200 | 10–12 |

PKU – phenylketonuria, p-value < 0.05, Nutrient intake reference according MHRL, p-value < 0.05, calculated using Fisher's Exact Test one-tailed p-value

- Status of fatty acid concentration in erythrocytes in PKU patients

A deficiency of oleic acid, trans-palmitoleic acid, alpha-linoleic acid, EPA and DHA and total omega-3 levels and increased levels of elaidic acid and arachidonic acid (Ara) were observed in PKU patients. Trans-palmitoleic acid was significantly low in PKU patients – 0.06 ± 0.03 compared to 0.12± 0.03% in healthy controls ($p < 0.0001$). Eicosatrienoic acid was significantly higher in control group than in PKU patients ($p < 0.0001$). Elaidic acid was higher in PKU patients (0.09% ± 0.03) than in control group 0.04% ± 0.01 ($p < 0.0001$).

Significantly lower levels of EPA and DHA, total omega-3, and the omega-3 index were observed in PKU patients (Table 3.2). In healthy controls, deficiencies in oleic acid, arachidonic acid (ARA), EPA, DHA, and total omega-3 levels were also observed. In contrast, elaidic acid and trans-palmitoleic acid concentrations were within the normal range in healthy controls. No correlations were found between the status of fatty acids and cholesterol profile (Table 3.2).

Table 3.2

**Concentration of fatty acids in erythrocytes of PKU patients and healthy controls**

| Concentration of FA (%) | PKU (n = 40) | Control group (n = 40) | p-value |
|---|---|---|---|
| Myristic acid (0.16–0.4) | 0.34 ± 0.09 | 0.33 ± 0.08 | 0.539 |
| Palmitic acid (21.16–23.35) | 21.71 ± 0.75 | 21.09 ± 0.68 | **$7.7e^{-4}$** |
| Stearic acid (13.43–17.17) | 17.02 ± 0.85 | 16.93 ± 0.68 | 0.478 |
| Palmitoleic acid (0.15–0.49) | 0.18 ± 0.08 | 0.18 ± 0.04 | 0.583 |
| Cis-vaccenic acid (0.67–0.96) | 0.85 ± 0.15 | 0.79 ± 0.15 | 0.019 |
| Oleic acid (12–14.7) | 11.82 ± 1.16 | 11.14 ± 0.86 | **0.010** |
| Eicosatrienoic acid (1.51–1.87) | 1.59 ± 0.21 | 1.93 ± 0.21 | **0.001** |
| Trans-vaccenic acid (0–7) | 0.09 ± 0.04 | 0.12 ± 0.03 | 0.065 |
| Trans-palmitoleic acid (0.07–0.13) | 0.06 ± 0.03 | 0.12 ± 0.03 | **$1.22e^{-6}$** |
| Rumenic acid (0.05–0.12) | 0.06 ± 0.03 | 0.08 ± 0.02 | **0.011** |
| Elaidic acid (0–0.07) | 0.09 ± 0.03 | 0.04 ± 0.01 | **$1.29e^{-9}$** |
| CLA2 C18:2 (0.01–0.05) | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.118 |
| CLA3 C18:2 (0.01–0.06) | 0.04 ± 0.11 | 0.03 ± 0.01 | 0.178 |
| Linolenic acid (8.49–11.15) | 8.77 ± 1.4 | 9.06 ± 0.72 | 0.088 |
| Gamma-linolenic acid (0.04–0.09) | 0.07 ± 0.02 | 0.03 ± 0.01 | **$1.82e^{-7}$** |
| Dihomogammalinolenic acid (1.28–2.2) | 1.52 ± 0.4 | 1.41 ± 0.29 | **0.043** |
| Arachidonic acid (11–13.44) | 13.83 ± 1.2 | 14.32 ± 0.91 | **0.031** |
| Total omega-6 (21.9–25.4) | 23.78 ± 1.42 | 24.79 ± 1.01 | **0.007** |
| Alpha-linolenic acid (0.14–0.29) | 0.11 ± 0.04 | 0.1 ± 0.03 | 0.816 |
| EPA (0.75–2.34) | 0.32 ± 0.27 | 0.48 ± 0.3 | **$9.99e^{-6}$** |
| DHA (5.27–8.87) | 3.55 ±1.06 | 4.46 ± 1.05 | **0.002** |
| Total omega-3 (5–8.36) | 3.91 ± 1.22 | 4.98 ± 1.22 | **$4.17e^{-4}$** |
| Index Omega3 (7.5–10) | 4.15 ± 1.23 | 4.81 ± 1.23 | **0.003** |
| Omega6/Omega3 (1–4) | 6.1 ± 1.58 | 5.01 ± 1.19 | **0.043** |

Table 3.2 continued

| Concentration of FA (%) | PKU (n = 40) | Control group (n = 40) | p-value |
|---|---|---|---|
| Ara/EPA (5–10) | 43.94 ± 20.29 | 29.5 ± 10.18 | **1.21e-4** |
| LN/DGLA (0–4.4) | 5.5 ± 1.74 | 6.47 ± 2.13 | **0.008** |

FA% – fatty acid concentration, PKU – Phenylketonuria, CLA2 – conjugated linoleic acid-2 , CLA3 – conjugated linoleic acid-3, Ara – Arachidonic acid, EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, Ara/EPA – ratio of arachidonic acid to eicosapentaenoic acid , LN/DGLA – ratio of linoleic acid to dihomo-γ-linolenic acid, p-value calculated by Mann-Whitney U Test, statistically significant if $p < 0.05$.

Comparing FA status between PKU patients and the control group by sex, statistically significant differences were observed. Palmitic acid concentrations were higher in PKU patients of both sexes compared with the control group ($p = 0.012$ for males; $p = 0.001$ for females). A significant difference in stearic acid concentration was observed in female PKU patients, who exhibited higher levels than female controls ($p = 0.044$). No significant difference in stearic acid concentration was found in the male group.

Cis-vaccenic acid concentrations were within normal ranges in both sexes and groups; however, levels were significantly higher in male PKU patients compared with controls ($p = 0.012$). Although oleic acid concentrations were below reference ranges in both PKU patients and male and female controls, significantly higher concentrations were found in PKU boys (12.37 ± 1.23) compared with the control group (11.03 ± 0.89; $p = 0.005$). Ara levels were lower in PKU boys (13.45 ± 1.12) compared with controls (14.78 ± 1.23; $p = 0.026$).

Conversely, no statistically significant differences in arachidonic acid concentrations were observed between male PKU patients and male controls. However, in the female PKU group, statistically significant differences were observed in EPA, DHA, total omega-3, the omega-3 index, the omega-6 to omega-3 ratio, and the arachidonic acid to EPA ratio compared with female controls (see Table 3.3.)

Table 3.3

**Comparison of FA concentration between PKU and CG by gender**

| Concentration of FA % | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | PKU (n = 22) | CG (n = 22) | p-value | PKU (n = 18) | CG group (n = 18) | p-value |
| Myristic acid (0.16–0.4) | 0.33 ± 0.09 | 0.33 ± 0.07 | 0.322 | 0.35 ± 0.09 | 0.33 ± 0.09 | 0.347 |
| Palmitic acid (21.16–23.35) | 21.38 ± 0.88 | 20.7 ± 0.63 | **0.012** | 21.92 ± 0.47 | 21.21 ± 0.66 | **0.001** |
| Stearic acid (13.43–17.17) | 16.91 ± 0.94 | 17.07 ± 0.76 | 0.353 | 17.12 ± 0.72 | 16.75 ± 0.62 | 0.044 |
| Palmitoleic acid (0.15–0.49) | 0.2 ± 0.08 | 0.17 ± 0.03 | **0.043** | 0.17 ± 0.08 | 0.19 ± 0.05 | 0.474 |
| Cis-vaccenic acid (0.67–0.96) | 0.84 ± 0.15 | 0.78 ± 0.21 | **0.012** | 0.86 ± 0.15 | 0.79 ± 0.08 | **0.061** |

Table 3.3 continued

| Concentration of FA % | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | PKU (n = 22) | CG (n = 22) | p-value | PKU (n = 18) | CG group (n = 18) | p-value |
| Oleic acid (12–14.7) | 12.37 ± 1.23 | 11.03 ± 0.89 | **0.005** | 11.43 ± 0.97 | 11.32 ± 0.86 | 0.236 |
| Eicosatrienoic acid (1.51–1.87) | 1.57 ± 0.22 | 1.97 ± 0.2 | **3.86e$^{-4}$** | 1.67 ± 0.2 | 1.93 ± 0.22 | **4.11e$^{-4}$** |
| Trans-vaccenic acid (0–7) | 0.1 ± 0.04 | 0.11 ± 0.03 | 0.439 | 0.09 ± 0.04 | 0.12 ± 0.03 | **0.017** |
| Trans-palmitoleic acid (0.07–0.13) | 0.06 ± 0.03 | 0.12 ± 0.03 | **1.26e$^{-3}$** | 0.08 ± 0.03 | 0.12 ± 0.03 | **4.91e$^{-4}$** |
| Rumenic acid (0.05–0.12) | 0.06 ± 0.02 | 0.08 ± 0.02 | **0.08** | 0.08 ± 0.03 | 0.09 ± 0.03 | **0.035** |
| Elaidic acid (0–0.07) | 0.09 ± 0.02 | 0.04 ± 0.01 | **1.19e$^{-5}$** | 0.09 ± 0.03 | 0.04 ± 0.01 | **3.31e$^{-5}$** |
| CLA2 C18:2 (0.01–0.05) | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.374 | 0.04 ± 0.01 | 0.03 ± 0.01 | **0.024** |
| CLA3 C18:2 (0.01–0.06) | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.289 | 0.04 ± 0.16 | 0.03 ± 0.01 | 0.083 |
| Linolenic acid (8.49–11.15) | 8.37 ± 1.25 | 9.08 ± 0.75 | 0.108 | 8.87 ± 1.59 | 8.99 ± 0.7 | 0.102 |
| Gamma-linolenic acid (0.04–0.09) | 0.07 ± 0.02 | 0.03 ± 0.01 | **1.61e$^{-3}$** | 0.07 ± 0.02 | 0.03 ± 0.02 | **2.69e$^{-5}$** |
| Dihomogammalinolenic acid (1.28–2.2) | 1.5 ± 0.44 | 1.37 ± 0.32 | 0.128 | 1.56 ± 0.36 | 1.42 ± 0.27 | **0.04** |
| Arachidonic acid (11–13.44) | 13.45 ± 1.12 | 14.78 ± 1.23 | **0.026** | 13.97 ± 1.26 | 14.23 ± 0.62 | 0.224 |
| Total omega-6 (21.9–25.4) | 23.27 ± 1.45 | 25.28 ± 1.26 | **0.011** | 24.54 ± 1.34 | 24.78 ± 0.84 | 0.091 |
| Alpha-linolenic acid (0.14–0.29) | 0.12 ± 0.04 | 0.1 ± 0.02 | 0.066 | 0.09 ± 0.04 | 0.1 ± 0.04 | 0.141 |
| EPA (0.75–2.34) | 0.34 ± 0.34 | 0.45 ± 0.31 | 0.1 | 0.31 ± 0.15 | 0.52 ± 0.3 | **0.002** |
| DHA (5.27–8.87) | 4.02 ± 1.11 | 4.24 ± 1.34 | 0.09 | 3.46 ± 0.98 | 4.46 ± 0.84 | **0.002** |
| Total omega-3 (5–8.36) | 4.51 ± 1.35 | 4.78 ± 1.58 | 0.086 | 3.82 ± 1.02 | 4.98 ± 0.95 | **0.001** |
| Index Omega3 (7.5–10) | 4.49 ± 1.35 | 4.67 ± 1.59 | 0.108 | 3.73 ± 1.09 | 4.81 ± 0.95 | **0.007** |
| Omega6/Omega3 (1–4) | 5.27 ± 1.59 | 5.25 ± 1.44 | 0.193 | 6.52 ± 1.51 | 5.01 ± 1.03 | **0.008** |
| Ara/EPA (5–10) | 43.01 ± 14.96 | 34.47 ± 10.42 | **0.016** | 49.09 ± 24.08 | 27.37 ± 10.34 | **0.001** |
| LN/DGLA (0–4.4) | 5.19 ± 1.72 | 6.44 ± 1.36 | 0.082 | 5.62 ± 1.81 | 6.49 ± 2.56 | 0.097 |

FA% – fatty acid concentration, PKU – Phenylketonuria, CLA2 – conjugated linoleic acid-2 , CLA3 – conjugated linoleic acid-3, Ara – Arachidonic acid, EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, Ara/EPA – ratio of arachidonic acid to eicosapentaenoic acid , LN/DGLA – ratio of linoleic acid to dihomo-γ-linolenic acid, p-value calculated by Mann-Whitney U Test, statistically significant if $p < 0.05$.

FA status of PKU patients was also analysed based on BMI. No comparison was possible within the underweight group due to lack of data. However, comparisons were made in the obesity (> 97$^{th}$ percentile), overweight (> 85$^{th}$ percentile), and normal weight (50$^{th}$ percentile) groups, based on WHO growth charts.

Statistically significant differences were observed within the obese cohort. Palmitic acid was higher in PKU patients (21.42%) compared with the control group (20.49%, p = 0.01). Elaidic acid was also significantly higher in PKU patients (0.08%) than in controls (0.04%, p = 0.05). Additionally, the LN/DGLA ratio was significantly lower in obese PKU patients (4.82%, p = 0.04).

In the overweight group, eicosatrienoic acid was significantly lower in PKU patients (1.67%) compared with controls (2.05%, p = 0.04), and trans-palmitoleic acid was also lower (p = 0.02). Conversely, elaidic acid was significantly higher in overweight PKU patients (p < 0.001). Furthermore, significantly lower concentrations of EPA, DHA, total omega-3, and the omega-3 index were observed in PKU patients than in controls.

In the normal BMI group, significant differences in FA status were also observed. PKU patients had significantly lower concentrations of arachidonic acid, total omega-6, EPA, DHA, total omega-3, and the omega-3 index compared with controls.

A more detailed description of FA concentrations based on BMI is provided in Table 3.4.

Table 3.4

**FA status based on body mass index of PKU patients and CG**

| Concentration of FA % | 97pc. | | | 85pc. | | | 50pc. | | |
|---|---|---|---|---|---|---|---|---|---|
| | **PKU** | **CG** | **p-value** | **PKU** | **CG** | **p-value** | **PKU** | **CG** | **p-value** |
| Myristic acid (0.16–0.4) | 0.40 | 0.34 | 0.44 | 0.38 | 0.38 | 0.36 | 0.35 | 0.32 | 0.33 |
| Palmitic acid (21.16–23.35) | 21.42 | 20.49 | **0.01** | 21.38 | 21.16 | 0.15 | 21.94 | 21.14 | **4.08e$^{-4}$** |
| Stearic acid (13.43–17.17) | 17.79 | 17.23 | 0.37 | 17.64 | 16.75 | 0.12 | 16.98 | 16.93 | 0.42 |
| Palmitoleic acid (0.15–0.49) | 0.14 | 0.17 | 0.39 | 0.24 | 0.19 | 0.15 | 0.22 | 0.18 | **0.04** |
| Cis-vaccenic acid (0.67–0.96) | 0.87 | 0.77 | 0.26 | 0.83 | 0.75 | 0.16 | 0.88 | 0.80 | **0.03** |
| Oleic acid (12–14.7) | 12.58 | 11.00 | 0.23 | 11.43 | 10.69 | 0.14 | 12.15 | 11.32 | **0.02** |
| Eicosatrienoic acid (1.51–1.87) | 1.31 | 1.93 | 0.08 | 1.67 | 2.05 | **0.04** | 1.52 | 1.93 | **2.98e$^{-6}$** |
| Trans-vaccenic acid (0–7) | 0.10 | 0.11 | 0.34 | 0.10 | 0.12 | 0.27 | 0.11 | 0.12 | 0.20 |
| Trans-palmitoleic acid (0.07–0.13) | 0.06 | 0.11 | 0.26 | 0.07 | 0.13 | **0.02** | 0.08 | 0.12 | **3.91e$^{-4}$** |
| Rumenic acid (0.05–0.12) | 0.07 | 0.08 | 0.48 | 0.08 | 0.09 | 0.09 | 0.06 | 0.08 | 0.09 |

Table 3.4 continued

| Concentration of FA % | 97pc. | | | 85pc. | | | 50pc. | | |
|---|---|---|---|---|---|---|---|---|---|
| | **PKU** | **CG** | **p-value** | **PKU** | **CG** | **p-value** | **PKU** | **CG** | **p-value** |
| Elaidic acid (0–0.07) | 0.08 | 0.04 | **0.05** | 0.10 | 0.04 | **$7.71e^{-4}$** | 0.09 | 0.04 | **$2.53e^{-10}$** |
| CLA2 C18:2 (0.01–0.05) | 0.04 | 0.03 | 0.31 | 0.03 | 0.03 | 0.25 | 0.03 | 0.03 | 0.09 |
| CLA3 C18:2 (0.01–0.06) | 0.04 | 0.03 | 0.28 | 0.04 | 0.04 | 0.14 | 0.04 | 0.03 | **0.04** |
| Linolenic acid (8.49–11.15) | 9.21 | 8.93 | 0.45 | 8.80 | 9.27 | 0.46 | 8.25 | 9.04 | **0.03** |
| Gamma-linolenic acid (0.04–0.09) | 0.06 | 0.03 | **0.05** | 0.06 | 0.03 | 0.08 | 0.07 | 0.03 | **$3.35e^{-8}$** |
| Dihomogammalinolenic acid (1.28–2.2) | 1.91 | 1.30 | 0.11 | 1.45 | 1.41 | 0.19 | 1.74 | 1.42 | **0.004** |
| Arachidonic acid (11–13.44) | 13.20 | 15.23 | 0.07 | 14.55 | 14.14 | 0.40 | 13.66 | 14.23 | **0.02** |
| Total omega-6 (21.9–25.4) | 22.74 | 25.48 | 0.20 | 24.41 | 24.55 | 0.46 | 23.66 | 24.79 | **0.01** |
| Alpha-linolenic acid (0.14–0.29) | 0.11 | 0.10 | 0.43 | 0.09 | 0.09 | 0.47 | 0.13 | 0.10 | 0.15 |
| EPA (0.75–2.34) | 0.46 | 0.56 | 0.22 | 0.34 | 0.90 | **0.002** | 0.33 | 0.47 | **0.02** |
| DHA (5.27–8.87) | 4.01 | 4.67 | 0.15 | 3.85 | 6.07 | **0.004** | 3.44 | 4.43 | **0.01** |
| Total omega-3 (5–8.36) | 4.71 | 5.33 | 0.16 | 4.20 | 6.76 | **0.002** | 3.85 | 4.90 | **0.01** |
| Index Omega3 (7.5–10) | 4.60 | 5.23 | 0.16 | 4.15 | 6.62 | **0.002** | 3.86 | 4.80 | **0.05** |
| Omega6/Omega3 (1–4) | 4.83 | 4.93 | 0.31 | 6.10 | 4.25 | 0.19 | 6.24 | 5.01 | **0.03** |
| Ara/EPA (5–10) | 28.70 | 28.25 | 0.29 | 42.51 | 17.33 | **0.01** | 41.87 | 32.62 | **0.003** |
| LN/DGLA (0–4.4) | 4.82 | 6.89 | **0.04** | 5.62 | 6.02 | 0.15 | 5.04 | 6.44 | **0.02** |

FA% – fatty acid concentration, PKU – Phenylketonuria, CLA2 – conjugated linoleic acid-2 , CLA3 – conjugated linoleic acid-3, Ara – Arachidonic acid, EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, Ara/EPA – ratio of arachidonic acid to eicosapentaenoic acid , LN/DGLA – ratio of linoleic acid to dihomo-γ-linolenic acid, p-value calculated by Mann-Whitney U Test, statistically significant if $p < 0.05$.

As previously described, fat intake in PKU patients was below the MHRL recommendations, therefore, a comparative analysis of FA concentrations was conducted between the PKU group and the CG, focusing specifically on individuals with low dietary fat intake. The results revealed that the concentrations of palmitic acid, elaidic acid, and gamma-linolenic acid were higher in the PKU group compared with the CG with similarly low fat intake. In contrast, the levels of eicosatrienoic acid, trans-palmitoleic acid, and total omega-6 fatty acids were significantly lower in the PKU patients. A detailed comparison of FA concentrations between the groups is presented in Annex 6.

- Status of lipid profile, glucose and vitamin D in PKU patients

Significant differences were observed between the PKU and control group regarding total cholesterol, HDL cholesterol, and vitamin D levels ($p < 0.0001$) (Table 3.5). Total cholesterol, LDL cholesterol and glucose were within the normal range in both groups. Triglyceride concentrations were low in both groups ($0.87 \pm 0.53$ mmol/L and $0.69 \pm 0.35$ mmol/L respectively), but HDL cholesterol was increased in healthy controls and in normal range in PKU patients. Mild D vitamin deficiency was observed in control group ($27.9 \pm 9.89$ ng/ml), whereas in PKU patients it was in normal range ($39.39 \pm 14.71$ mmol/L).

Table 3.5

**Median levels of lipid profile, glucose and vitamin D levels of PKU patients and healthy controls**

| Parameters | Reference range | PKU | Control group | p-value |
|---|---|---|---|---|
| Total cholesterol | (2.72–5,77 mmol/L) | 3.54 ± 0.72 | 4.35 ± 0.42 | **2.75e-7** |
| HDL | (0.78–1,45 mmol/L) | 1,21 ± 0.31 | 1.5 ± 0.28 | **1.22e-5** |
| LDL | (0–2.59 mmol/L) | 1.99 ± 0.48 | 2.38 ± 0.57 | 0.068 |
| TG | (1,1–2.3 mmol/L) | 0.87 ± 0.53 | 0.69 ± 0.35 | 0.053 |
| Vitamin D | (30–50 ng/mL) | 39.39 ± 14.71 | 27.9 ± 9.89 | **1.44e-4** |
| Glucose | (3.33–5.55 mmol/L) | 4.68 ± 0.53 | 4.6 ± 0.47 | 0.308 |

PKU – phenylketonuria, HDL – high-density lipoprotein, LDL – low-density lipoprotein, TG – triglycerides, p-value calculated by Mann-Whitney U Test, statistically significant if $p < 0.05$.

## 3.3 Part II – Results of nutritional information in food

After analysis, all 34 products were divided into two groups: foods with a Phe content below 75 mg/100 g (Table 3.6), which is considered low Phe concentration and foods can be consumed freely, and foods containing more than 75 mg/100 g, so eaten amount of foods should be counted as the source of natural Phe in the diet (Table 3.7).

Among the analysed products, foods with the lowest Phe and protein content include frozen rhubarb (9 mg / 100 g of Phe; 0.9 g protein / 100 g), fresh celery stalk (21 mg Phe / 100 g; 0.5 g protein / 100 g), boiled celery root (28 mg Phe / 100 g; 0.9 g protein / 100 g), fresh leeks (37 mg Phe / 100 g; 1.2 g protein / 100 g), and Jumbo raisins (42 mg Phe / 100 g; 1.9 g protein / 100 g).

Highest concentration of Phe was observed in Spring onions – Phe was 71 mg and 2.2 g of protein per 100 g, although protein content was higher in black raisins (3.8 g grams of protein and 69 mg of Phe per 100 g).

Black and Jumbo raisins had a Phe content of ≤ 75 mg/100 g, so they can be included in a Phe-restricted diet without measurement. However, golden raisins had a higher Phe content of 86 mg/100 g meaning they cannot be eaten freely on a Phe-restricted diet.

All other amino acid analysis is given in Annex No 7.

Table 3.6

**Nutritional data of foods with Phe content below 75 mg/100 g**

| Foods | Energy (kcal/100 g) | Carbohydrates (g/100 g) | Fat (g/100 g) | Protein (g/100 g) | Phenylalanine (mg/100 g) |
|---|---|---|---|---|---|
| Spring onions, fresh | 18.2 | 1.9 | 0.2 | 2.2 | 71 |
| Raisins (black) | 329.2 | 74.9 | 1.6 | 3.8 | 69 |
| Shiitake mushroom, boiled | 20.5 | 2.3 | 0.5 | 1.7 | 64 |
| Sugar snap peas, fresh | 29.3 | 4.1 | 0.1 | 3 | 63 |
| Aquafaba | 28.6 | 3.1 | 1 | 1.8 | 57 |
| Chanterelle, frozen, tempered, cooked | 23.3 | 0.2 | 1.7 | 1.8 | 56 |
| *Boletus edulis*, frozen, tempered, cooked | 27.8 | 1.2 | 1.4 | 2.6 | 52 |
| Raisins (Jumbo) | 330.6 | 76.7 | 1.8 | 1.9 | 42 |
| Leeks, fresh | 29.3 | 5.9 | 0.1 | 1.2 | 37 |
| Celery root, boiled | 18.3 | 3 | 0.3 | 0.9 | 28 |
| Celery stalk, fresh | 16.5 | 3.4 | 0.1 | 0.5 | 21 |
| Rhubarb, frozen | 13.8 | 2.1 | 0.2 | 0.9 | 9 |

Microgreens (peas, 200 mg/100 g, and nettles, 210 mg/100 g) had the highest Phe content and can be consumed as exchange foods (50 grams of microgreens as 1 exchange) if not used as herbs in a minimal amount (See Table 3.7).

A high Phe content (> 75 mg/100 g) was also identified in wild garlic (140 mg/100 g), and sorrel (fresh and cooked 120 mg/100 g). Garden cress and sunflower seed microgreens contained 150 mg/100 g and 140 mg/100 g respectively, while radish and broccoli microgreens contained 100 mg/100 g and 97 mg/100 g. The highest concentration of Phe among the mushrooms was observed in cooked champignons (both brown and white) – 150 mg and 100 mg per 100 grams, respectively; Portobello mushrooms were also high in Phe, containing 120 mg/100 g.

Table 3.7

**Nutritional data of foods with Phe content above 75 mg/100 g**

| Foods | Energy (kcal/100 g) | Carbohydrates (g/100 g) | Fat (g/100 g) | Protein (g/100 g) | Phenylalanine (mg/100 g) |
|---|---|---|---|---|---|
| Nettle, frozen | 41.4 | 3.8 | 0.6 | 5.2 | 210 |
| Peas microgreens, fresh | 33.3 | 0.8 | 0.5 | 6.4 | 200 |
| Garden cress microgreens, fresh | 18.8 | 0.3 | 0.4 | 3.5 | 150 |
| Champignon mushroom (brown), cooked | 89.9 | 0.8 | 7.5 | 4.8 | 150 |
| Sunflower microgreens, fresh | 55.5 | 1.5 | 3.9 | 3.6 | 140 |
| Wild garlic, frozen | 34.2 | 4.0 | 0.6 | 3.2 | 140 |

Table 3.4 continued

| Foods | Energy (kcal/100 g) | Carbohydrates (g/100 g) | Fat (g/100 g) | Protein (g/100 g) | Phenylalanine (mg/100 g) |
|---|---|---|---|---|---|
| Portobello mushroom, cooked | 87.3 | 0.2 | 7.7 | 4.3 | 120 |
| Sorrel, fresh, frozen | 21.9 | 1.5 | 0.3 | 3.3 | 120 |
| Sorrel, boiled, frozen | 18.6 | 0.2 | 0.6 | 3.1 | 120 |
| Arugula, fresh | 13.4 | 0.2 | 0.2 | 2.7 | 110 |
| Coriander, fresh | 15.8 | 0.8 | 0.2 | 2.7 | 110 |
| Radish microgreens, fresh | 23.8 | 1.4 | 0.6 | 3.2 | 100 |
| Champignon mushroom (white), cooked | 67.1 | 1.1 | 5.5 | 3.3 | 100 |
| Broccoli microgreens, fresh | 21.1 | 0.7 | 1.1 | 2.1 | 97 |
| Raisins (golden) | 329.2 | 78.4 | 0 | 3.9 | 86 |
| Russula emetica, boiled, frozen | 29.2 | 2.3 | 1.2 | 2.3 | 81 |

Broths belong to a group of products for which opinions regarding their nutritional value and the necessity of their inclusion in the PKU diet remain controversial. Therefore, to reduce random error and account for methodological specificity, the nutritional analysis of the samples was conducted in two independent laboratories employing methods of differing sensitivity. During the first analysis of the foods, six types of broths were analysed, and it was found that protein and Phe levels in those dishes were very low, with less than 1 g of protein, and less than 75 mg of Phe. The results from the second laboratory revealed that the protein content of all broths (except the second test of venison broth) was below 1 g per 100 g. Similarly, the Phe levels in all broth samples were less than 75 mg per 100 g (Table 3.8). Macronutrients such as fat, CHO and total energy per 100 g of broth are shown in Annex 8. A comparative analysis of CHO and fat concentrations in broths between the two laboratories could not be conducted due to inconsistencies in the available data.

Table 3.8

**Nutrient profile of broths**

| Product | Protein (g/100 g) | | L1 difference of L2 | Phe (mg/100 g) | | L1 difference of L2 |
|---|---|---|---|---|---|---|
| | L1 | L2 | % | T1 | T2 | % |
| Chicken broth | 0.74 | 0.8 | 8 | 9 | 34.7 | 286 |
| Pork broth | 0.56 | 0.9 | 61 | 13 | 40.7 | 213 |
| Trout broth | 0.46 | 0.3 | −35 | 5 | 31 | 520 |
| Deer (venison) broth | 0.43 | 1.7 | 295 | 28 | 31 | 11 |
| Lamb broth | 0.53 | 0.5 | −6 | 8 | 31 | 288 |
| Turkey broth | 1.01 | 0.7 | −31 | 7 | 39.3 | 461 |

Phe – phenylalanine, L1 – Eurofins Laboratory, L2 – Hamilton Laboratory.

## 3.4 Part III – PKU patients' and their caregivers' knowledge of PKU and dietary treatment

### 3.4.1 Knowledge and self-reported dietary management among caregivers and patients with PKU

To evaluate knowledge of PKU, dietary treatment, and self-reported management practices, responses from main caregivers and patients were analysed separately and compared. In total, 30 caregivers and 21 patients with PKU completed the questionnaire.

Among caregivers, the majority were mothers (n = 29, 96.6%), while one respondent (3.4%) was the child's grandmother. Nine caregivers (30%) reported that responsibilities related to PKU dietary management – including adherence to the low-protein diet, meal preparation, and administration of the AA mixture – were shared equally between both parents. Regarding their place of residence, 10 caregivers (33%) lived in Riga, 11 (37%) in other state cities, four (13%) in the Riga suburban area, and five (17%) in small towns. In terms of their child's educational status, 11 caregivers (37%) reported that their child attended preschool, while 15 (50%) indicated that their child attended compulsory general education. Four caregivers (13%) reported that their child did not attend any educational institution, as preschool attendance is not mandatory in Latvia at this age.

In the patient group, 11 respondents (52%) were female, nine (43%) were male, and one participant (5%) preferred not to disclose their sex. Six patients (29%) were younger than 18 years, nine (42%) were aged 18–24 years, and six (29%) were older than 24 years; the oldest participant was 38 years old. In terms of residence, nine patients (43%) lived in Riga, three (14%) in other state cities, three (14%) in the Riga suburban area, and five (24%) in small towns, while one respondent (5%) preferred not to answer.

Caregivers demonstrated better knowledge of daily natural protein prescriptions than patients. None of the caregivers reported uncertainty regarding the permitted daily intake of natural protein. Most caregivers (n = 24, 80%) reported that the allowed intake was 5–6 g/day, while five (17%) reported ≤ 4 g/day and one (3%) reported > 6 g/day. In contrast, seven patients (33%) stated that they did not know their permitted daily natural protein intake. Four patients (19%) reported an allowed intake of ≤ 4 g/day, five (24%) reported 5–6 g/day, and another five (24%) reported > 6 g/day.

A similar pattern was observed for actual daily natural protein intake. Among caregivers, most respondents (n = 20, 67%) reported that their child consumed 5–6 g/day, seven (23%) reported ≤ 4 g/day, two (7%) reported > 6 g/day, and one caregiver (3%) was unsure of the exact amount. In the patient group, seven respondents (33%) were unable to estimate their actual daily natural protein intake. Three patients (14%) reported consuming ≤ 4 g/day, five

(24%) reported 5–6 g/day, and six (29%) reported > 6 g/day. These findings indicate that caregivers were generally more aware of both prescribed and actual natural protein intake than patients (See Table 3.9).

Table 3.9

**Prescribed and reported actual daily natural protein intake by caregivers and patients**

| Daily natural protein intake | Caregivers: allowed intake, n (%) | Caregivers: actual intake, n (%) | Patients: allowed intake n (%) | Patients: actual intake, n (%) |
|---|---|---|---|---|
| ≤ 4 g/day | 5 (17) | 7 (23) | 4 (19) | 3 (14) |
| 5–6 g/day | 24 (80) | 20 (67) | 5 (24) | 5 (24) |
| > 6 g/day | 1 (3) | 2 (7) | 5 (24) | 6 (29) |
| Unknown | 0 (0) | 1 (3) | 7 (33) | 7 (33) |

Regular intake of the prescribed protein substitute is an essential component of PKU dietary treatment. In the caregiver group, reported adherence was high: 26 caregivers (87%) stated that their child never forgot to take the protein substitute, three respondents (10%) reported forgetting approximately once every two weeks, and one caregiver (3%) reported forgetting 2–4 times per week. In contrast, responses among patients were more variable. Nine patients (43%) reported never forgetting to take the AA mixture, five (24%) reported forgetting once per week, three (14%) once every two weeks, one patient (5%) 2–4 times per week, one patient (5%) several times per day, and two patients (10%) reported that they did not take the protein substitute at all.

Patients were also asked about the recommended and actual frequency of AA mixture intake. Thirteen patients (62%) reported that the formula should be consumed three times per day, six (29%) reported four times per day, and two patients (9%) did not know the recommended frequency. In terms of actual intake, 12 patients (57%) reported taking the formula three times per day, five (24%) four times per day, one respondent (5%) did not know the frequency, and two patients (10%) reported not taking the protein substitute (See Table 3.10).

Table 3.10

**Protein substitute use and adherence among caregivers and patients**

| Parameter | Caregivers, n (%) | Patients, n (%) |
|---|---|---|
| Never forgets protein substitute | 26 (87) | 9 (43) |
| Forgets once every two weeks | 3 (10) | 3 (14) |
| Forgets once per week | – | 5 (24) |
| Forgets 2–4 times per week | 1 (3) | 1 (5) |
| Forgets several times per day | – | 1 (5) |
| Does not take protein substitute | – | 2 (10) |

Knowledge of recommended blood Phe target levels was also higher among caregivers than among patients. Twenty-eight caregivers (93%) correctly identified the appropriate Phe target range for their child, while two respondents (7%) did not know it. In the patient group, 12 respondents (57%) correctly identified their recommended Phe target level, six (29%) provided an incorrect answer, and three (14%) reported that they did not know their allowed Phe level.

Blood Phe monitoring frequency was assessed in the patient group. Four patients (19%) reported that they had not performed any blood Phe tests during the previous year, and another four (19%) reported testing less than twice per year. Three patients (14%) reported testing twice per year, three (14%) once every two months, and seven patients (33%) once per month (See Table 3.11).

Table 3.11

**Knowledge of Phe target levels and blood Phe monitoring**

| Parameter | Caregivers, n (%) | Patients, n (%) |
|---|---|---|
| Correctly identified Phe target level | 28 (93) | 12 (57) |
| Incorrect answer | – | 6 (29) |
| Did not know | 2 (7) | 3 (14) |
| **Blood Phe monitoring frequency among patients** | | |
| No blood tests during previous year | – | 4 (19) |
| Less than twice per year | – | 4 (19) |
| Twice per year | – | 3 (14) |
| Once every two months | – | 3 (14) |
| Once per month | – | 7 (33) |

Overall, caregivers demonstrated better knowledge of PKU dietary management, natural protein intake, protein substitute use, and recommended Phe target levels than patients. In the patient group, uncertainty regarding prescribed and actual natural protein intake, more frequent omission of protein substitute intake, and lower awareness of Phe targets were observed. These findings indicate a need to strengthen patient education, particularly during the transition from caregiver-led management to independent PKU self-management.

Comparison of correct responses between caregivers and patients revealed differences in knowledge related to PKU and its dietary management. The largest difference was observed for the question regarding the protein content of baked potatoes, where 77% of caregivers answered correctly compared to 19% of patients (a difference of 58 percentage points). A similarly large discrepancy was found for the question concerning whether protein intake should be reduced during illness (87% vs. 29%, difference 58%). Knowledge regarding the permissibility of sorbitol in the PKU diet also differed substantially between groups (67% of caregivers vs. 24% of patients, difference 43%).

Moderate differences were identified in questions related to illness and formula management. Ninety-three percent of caregivers and 62% of patients correctly answered that Phe levels increase during illness (difference 31%). Similarly, 93% of caregivers and 71% of patients correctly answered that a double portion of AA mixture should not be taken after a missed dose (difference 22%). Knowledge that irregular use of the AA mixture increases Phe levels differed by 12% (93% vs. 81%).

Understanding that Phe is the amino acid affected in PKU was high in both groups (100% caregivers vs. 81% patients, difference 19%). Knowledge that fasting increases Phe levels showed minimal difference (83% vs. 81%, difference 2%), and awareness regarding capsule-form medications differed by 6% (87% vs. 81%).

There was only one question where patients performed slightly better than caregivers: 48% of patients correctly answered that increased physical activity raises Phe levels compared with 40% of caregivers (difference –8%) (See Table 3.12).

Table 3.12

**Difference of correct answers in respondent's group**

| Questions | Caregivers | Patients | Difference |
|---|---|---|---|
| How many grams of baked potatoes contain 1 gram of protein? | 77% | 19% | **58%** |
| How many grams of sour cream does 1 gram of protein contain? | 70% | 52% | 18% |
| If your child / You forgot to drink the AA mixture, is it allowed to take double portion of AA amount next time? | 93% | 71% | 22% |
| Phenylalanine is: | 100% | 81% | 19% |
| Does fasting increase Phe levels? | 83% | 81% | 2% |
| Is it allowed to consume sorbitol in PKU diet? | 67% | 24% | **43%** |
| Is it allowed to take medications or supplements in capsule form? | 87% | 81% | 6% |
| Does irregular use of the AA mixture increase Phe levels? | 93% | 81% | 12% |
| Does increased physical activity raise Phe levels? | 40% | 48% | −8% |
| Does Phe level increase during illness ? | 93% | 62% | 31% |
| Should protein intake be reduced during illness? | 87% | 29% | **58%** |

PKU – phenylketonuria, Phe – phenylalanine, AA – aminoacid.

To compare knowledge regarding PKU and its dietary management, answers from both patients and caregivers were stratified according to metabolic control.

### 3.4.2 Metabolic control of PKU patients

The study included all available blood tests of Phe level of patients in the age group < 18 years in 2023 and 2025. In 2023, all patients younger than 4 years had good/average metabolic control (median Phe level per year < 360 µmol/l). However, in 2025, the annual median Phe value of 14% of these patients was above the recommended value (> 360 µmol/l). Metabolic control also deteriorated in the 4–6 years cohort, with the proportion of patients demonstrating good or acceptable control decreasing from 89% to 88%. Conversely, no change in metabolic control was observed in the 7–12 years age group in 2025. There were no statistically significant differences in metabolic control between 2023 and 2025, either in patients younger than 12 years ($p = 0.145$) or in those older than 12 years ($p = 0.493$) (See Figure 3.2).

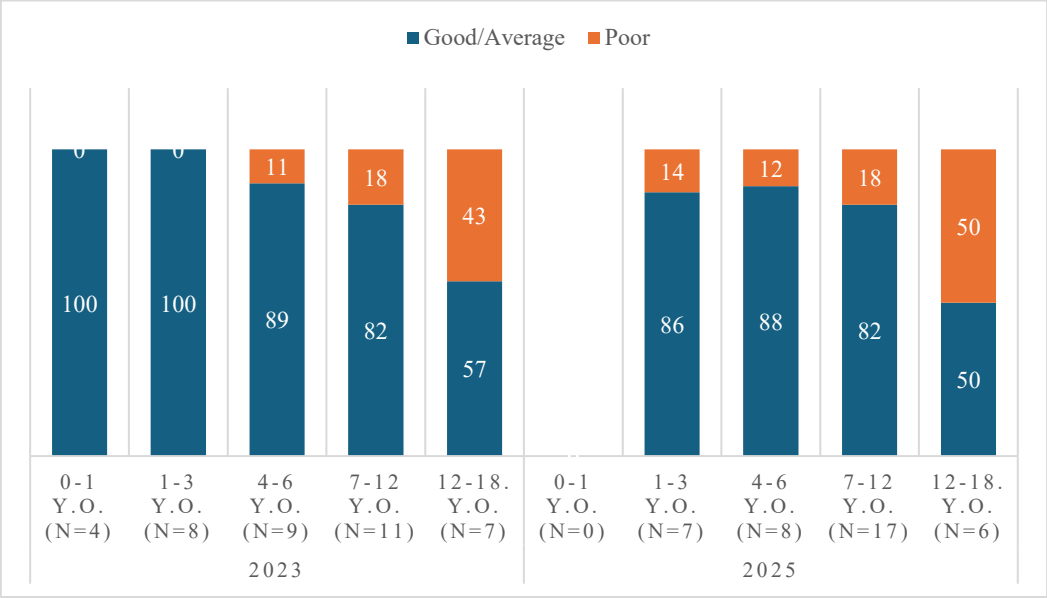



Figure 3.2 **Metabolic control of PKU patients (2023/2025)**

Table 3.13 presents the median Phe and Tyr concentrations, as well as the Phe/Tyr ratio, in different age groups of PKU patients during 2025. A clear age-related trend was observed, with higher Phe concentrations and Phe/Tyr ratios in older patients. In contrast, Tyr concentrations showed a slight decrease with age. The most pronounced impairment of metabolic control was observed in the adolescent group, which exhibited the highest median Phe concentration and Phe/Tyr ratio.

Table 3.13

**Metabolic control of patients in 2025 year**

| Median Parameter (2025) | PKU patient's age group | | | | |
|---|---|---|---|---|---|
| | 0–1 y.o. (n = 0) | 1–3 y.o. (n = 7) | 4–6 y.o. (n = 8) | 7–12 y.o. (n = 17) | 12–18 y.o. (n = 6) |
| **Phe (µmol/L)** | n/a | 190 | 193 | 260 | 510 |
| **Tyr (µmol/L)** | n/a | 66 | 68 | 54.5 | 52.5 |
| **Phe/Tyr ratio** | n/a | 2.48 | 3.03 | 4.33 | 9.53 |

Phe – phenylalanine, Tyr – tyrosine, Phe/Tyr ratio – phenylalanine/tyrosine ratio, n/a – no data available.

Respondents with good metabolic control demonstrated significantly higher PKU-related knowledge scores than respondents with poor metabolic control. The median knowledge score was 10/11 and 7/11 points, respectively. The difference was statistically significant (Mann–Whitney U test, $U = 528.0$, $p < 0.001$), with a large effect size (rank-biserial correlation = 0.64).

Furthermore, respondents with satisfactory metabolic control performed significantly better in questions related to protein counting, dietary management during illness, sorbitol use, and understanding of Phe and illness-related increases in blood phenylalanine levels (all $p < 0.05$). These findings suggest that better disease-specific knowledge is associated with improved metabolic control in PKU (Table 3.14):

Table 3.14

**Comparison of correct responses to individual PKU knowledge questions between respondents with good/average and poor metabolic control**

| Question | Good control (%) | Poor control (%) | p-value |
|---|---|---|---|
| How many grams of baked potatoes contain 1 g of protein? | 75.0 | 26.1 | 0.001 |
| Should protein intake be reduced during illness? | 85.7 | 34.8 | < 0.001 |
| Is sorbitol allowed in the PKU diet? | 64.3 | 30.4 | 0.025 |
| Does illness increase phenylalanine levels? | 92.9 | 65.2 | 0.030 |
| What is phenylalanine? | 100 | 82.6 | 0.035 |

PKU – phenylketonuria, p-value calculated by Mann-Whitney U Test, statistically significant if $p < 0.05$.

No statistically significant differences were found for the remaining questions ($p > 0.05$), although respondents with satisfactory metabolic control generally demonstrated higher proportions of correct answers.

In the caregiver group, a statistically significant difference in knowledge scores was observed between respondents with good and poor metabolic control. Caregivers with good metabolic control achieved higher overall knowledge scores than caregivers with poor metabolic control ($p = 0.018$).

Table 3.15

**Caregiver's and patient`s knowledge based on metabolic control**

| Group | Metabolic control | Mean score /11 | Median | p-value |
|---|---|---|---|---|
| Caregivers (n = 21) | Good | 9.45 | 10.0 | **0.018** |
| Caregivers (n = 9) | Poor | 7.38 | 7.5 | |
| Patients (n = 6) | Good | 7.50 | 7.5 | **0.143** |
| Patients (n = 15) | Poor | 5.80 | 5.0 | |

Question-specific analysis showed statistically significant differences in knowledge regarding the protein content of baked potatoes and whether protein intake should be reduced during illness.

In the patient group, knowledge scores were higher among respondents with good metabolic control; however, this difference did not reach statistical significance (p = 0.143). This may be explained by the relatively small number of patients, particularly in the good metabolic control group (See Table 3.16).

Table 3.16

**Correct response rates by metabolic control in caregivers and patients**

| Questions | Caregivers | | | Patients | | |
|---|---|---|---|---|---|---|
| | G, % | P, % | Difference, % | G, % | P, % | Difference, % |
| How many grams of baked potatoes contain 1 gram of protein? | 91 | 38 | 53 | 17 | 20 | 37 |
| How many grams of sour cream does 1 gram of protein contain? | 77 | 50 | 27 | 50 | 53 | −23 |
| If your child / You forgot to drink the AA mixture, is it allowed to take double portion of AA amount next time? | 95 | 88 | 8 | 83 | 67 | −75 |
| Phenylalanine is: | **100** | **100** | 0 | **100** | 73 | −100 |
| Does fasting increase Phe levels? | 86 | 75 | 11 | **100** | 73 | −89 |
| Is it allowed to consume sorbitol in PKU diet? | 77 | 38 | 40 | 17 | 27 | 23 |
| Is it allowed to take medications or supplements in capsule form? | 91 | 75 | 16 | 100 | 73 | −84 |
| Does irregular use of the AA mixture increase Phe levels? | 95 | 88 | 8 | 83 | 80 | −75 |
| Does increased physical activity raise Phe levels? | 36 | 50 | −14 | 83 | 33 | −97 |
| Does Phe level increase during illness ? | **100** | 75 | 25 | 67 | 60 | −42 |
| Should protein intake be reduced during illness? | 95 | 63 | 33 | 50 | 20 | −17 |

PKU – phenylketonuria, Phe – phenylalanine, AA – aminoacid

# 4 Discussion

## 4.1 Analysis of food diary results of Latvian PKU paediatric population

Although PKU was first described and diagnosed in the early 20th century, it continues to be a subject of active scientific investigation. Between 2019 and 2024, a total of 1190 publications related to PKU were indexed in the PubMed database, underscoring sustained research interest in this inherited metabolic disorder (PubMed, 2025). In 2025, new European guidelines addressing the screening, treatment, and management of PKU-related complications were published. This update reflects the ongoing progress in the field and emphasises the necessity of regularly revising clinical recommendations considering emerging scientific evidence and advances in therapeutic strategies (van Wegberg et al., 2025).

Numerous PKU centres worldwide, including ours, report challenges in ensuring long-term adherence to therapeutic diets, especially during adolescence and adulthood. This poor compliance complicates the collection of accurate dietary data through food diaries, as participants often struggle to record precise food quantities or fail to complete diaries altogether. In the study, nearly one-third of the primary caregivers of paediatric PKU patients either failed to submit their FDs or completed them incorrectly. These findings are consistent with previous research. For example, Garcia et al. presented data about Chilean PKU population (n = 75 patients) for 7 years and described a significant decrease in treatment compliance with age – the results showed a progressive increase in mean blood Phe concentrations as patients aged (García et al., 2017). In 2018, Cazzorla et al. confirmed that metabolic control and compliance with dietary treatments are poor in adult PKU patients They surveyed 111 Italian adults with early-treated cPKU, achieving a 94% response rateto assess dietary habits, disease perception, and factors influencing adherence to a low-Phe diet in Italy. (Cazzorla et al., 2018). Poor adherence in adolescent and adult PKU patients was also reported by Jurecki et al., who observed suboptimal adherence to the recommended Phe concentrations (Jurecki et al., 2017).

Currently, there is a significant lack of data regarding the nutritional status of Latvian adult patients with PKU aged over 18 years. This knowledge gap is primarily due to poor adherence to the 72-hour food diary (FD) method, which these patients often fail to complete. As dietary compliance tends to decline already during adolescence and worsens into adulthood, it is essential to adopt preventive strategies at an earlier stage. Until 2016, dietitians were not involved in the treatment of PKU in Latvia; patient management was led mainly by geneticists, and dietary monitoring relied almost exclusively on 24-hour dietary recall. Food diaries were not routinely collected, and patients were neither trained nor encouraged to use them. As a result, older patients are generally unfamiliar with this method, making it very difficult to persuade them to complete a food diary. This historical gap in dietary education and monitoring

further contributes to the scarcity of systematic nutritional data. Therefore, studying the nutritional habits of paediatric PKU patients is particularly important in order to identify and address potential risks before adherence begins to decrease. In our study, which focuses on a paediatric population, regular assessment using 24-hour dietary recall during outpatient visits serves as a practical and informative method to monitor dietary intake and promote long-term dietary compliance from an early age.

In the study, discrepancies were found in some of the information provided in the FD. For example, patients, whose BMI was above the 95$^{th}$ percentile provided a dietitian-filled FD and there was an observed lack of calories and nutrients, therefore real energy and nutrient intake was under-reported. Inconsistencies were seen with BMI and FD data on CHO and total energy intakes. Specifically, three obese patients reported CHO and total energy intakes that were lower than the values recommended by the MHRL. Additionally, other research groups have reported that children under-report their caloric intake by as much as 40%. (Bandini et al., 1990) (Jones et al., 2021).

Under-reporting of actual food and caloric intake is a well-documented issue among children and their parents. Numerous studies have demonstrated that both children and their caregivers tend to significantly underestimate the amount of food consumed, leading to inaccuracies in dietary assessments. Bandini et al.'s data is similar to Lioret et al.'s published article, where children may under-report their energy intake by as much as 40%, with some studies showing reported intakes as low as 38% of estimated energy requirements (Bandini et al., 1990; Jones et al., 2021; Lioret et al., 2011). This misreporting can result from several factors, including social desirability bias, memory limitations, and difficulties in estimating portion sizes. Parents, who often serve as proxies for reporting their children's diets, are similarly prone to these biases and may unintentionally minimise or omit certain foods, particularly snacks or energy-dense items. Such underestimation complicates the evaluation of nutritional status and dietary interventions, especially in clinical populations like children with PKU, where precise monitoring of protein and Phe intake is critical. Addressing this challenge requires the use of more objective dietary assessment methods, enhanced education on portion estimation, and supportive tools to improve reporting accuracy (Bel-Serrat et al., 2016).

### 4.2 Anthropometric parameters of PKU paediatric population

Our findings indicate an increased prevalence of overweight and obesity in the Latvian paediatric PKU population, particularly among adolescent girls, compared to the general paediatric population in Latvia. This observation aligns with results from several international studies across diverse ethnic and cultural contexts. In a retrospective cohort study by

Walkowiak et al. in 2019, which included 63 children with classical PKU (40 girls and 23 boys, aged 5–16 years), 31.7% were classified as overweight or obese. Notably, 37.5% of girls were affected compared to 21.7% of boys, with the difference reaching statistical significance when compared to national reference data ($p = 0.0031$) (Walkowiak et al., 2019). Ahmadzadeh et al. in 2022 reported even higher rates in an Iranian cohort of 94 PKU patients aged 4–18 years, where 37.5% were overweight and 43.8% were obese.(Ahmadzadeh et al., 2022) These values considerably exceed those observed in our Latvian cohort, highlighting possible contributions from regional dietary customs, religious food restrictions, or lower dietary supervision. In contrast, a study conducted by Silveira et al. in Brazil involving 101 PKU patients found that 27.7% of adolescents were either overweight or obese – rates comparable to the general population – yet still emphasising the need for weight monitoring within this metabolic disorder (Silveira et al., 2022). Supporting this trend, a large multicentre study by Gokmen-Ozel et al. (2014) included 947 PKU patients (783 children under 19 years and 164 adults ≥19 years) from eight European and Turkish centres. While childhood obesity rates in boys were mostly consistent with national norms, 57% of centres (4 out of 7) reported higher obesity rates in girls. (Gokmen Ozel et al., 2014) The study also demonstrated a progressive increase in the prevalence of overweight and obesity with age, indicating that weight-related risks in the PKU population accumulate over time. While the dietary management of PKU – characterised by low protein intake and reliance on carbohydrate-rich medical and low-protein foods – may contribute to this trend, the impact is modulated by additional factors such as sex, age, lifestyle, and sociocultural context. Notably, adolescent girls appear particularly vulnerable, aligning with findings from other studies reporting higher obesity rates in female patients. Although our own data did not show a statistically significant difference in CHO intake between PKU patients and controls, evidence from previous research consistently highlights a disproportionately high contribution of CHO to total energy intake in individuals with PKU. This dietary imbalance, combined with possible behavioural patterns (e. g., reduced physical activity or emotional eating) and metabolic adaptations, may further exacerbate the risk of excess weight gain. These insights underscore the need for early, tailored interventions that address not only macronutrient distribution but also individual behavioural and developmental needs. Regular monitoring of nutritional status and body composition, along with culturally sensitive dietary education, should be prioritised – particularly during adolescence, when adherence to dietary recommendations often begins to decline.

### 4.3 Nutrient intake and biochemical parameters status

It was observed that most of the patients reported a CHO intake that was above the MHRL's recommended level. This is in line with the findings of Sailer et al., where children with PKU consumed a substantially higher proportion of their energy from CHO compared to CG. Specifically, male subjects with PKU derived approximately 67% of their total energy intake from CHO, significantly higher than the 54.6% reported in male controls ($p = 0.0005$). A similar trend was noted among female subjects with PKU, who obtained 67.5% of energy from carbohydrates versus 58.3% in their CG, although this difference did not reach statistical significance ($p = 0.06$). This elevated CHO intake likely reflects the dietary adaptations required to maintain low Phe levels, including severe restrictions on natural protein (Sailer et al., 2020). Couce et al. also showed that PKU patients had a higher energy intake from CHO comparing with patients with mHPA – the caloric intake in the form of carbohydrates was also higher in PKU than mHPA patients ($p = 0.038$) (Couce et al., 2018).

In contrast, the fat intake of the vast majority of our PKU patients was below the MHRL's recommended level. A low intake of fat is a characteristic finding of many PKU patient studies. For example, a study by Rose et al. in 2005 concluded that the youngest children (< 5 years of age) in their group of PKU patients appeared to be especially vulnerable to inadequate fat intake due to a lack of diversity in their diets ($p < 0.05$) (Rose et al., 2005) These results are in accordance with our data showing that the biggest disparity between recommended and actual fat intake was observed in the 1–3 age group. Furthermore, data presented by Schulpis et al. and van Calcar et al. revealed that PKU diets tend to contain more CHO and less fat (20–25% energy) compared to a typical omnivore diet which provides 30–35% energy from fat (Schulpis et al., 2005; van Calcar et al., 2009). It has been proposed that all PKU patients diagnosed with a nutrient imbalance should undergo dietary corrections, increasing fat intake and supplementation with PUFA (Demirdas et al., 2017).

In the early decades of dietary treatment for PKU, low-Phe AA substitutes were initially formulated with a primary focus on restricting Phe intake and preventing severe neurological damage. However, these early mixtures often lacked adequate amounts of essential micronutrients and vitamins, which led to deficiencies in trace elements such as Se, Zn and Fe among patients. Over time, the formulations have been substantially improved to address these nutritional gaps. Phe-free formula is fortified with many micronutrients, including Se, Zn and iron (Daly, Evans, et al., 2021).

Another issue that has been discussed lately is the concentration of micronutrients in protein substitutes. According to a recent review by Tummolo et al. (2025) on protein substitutes available in Italy, many products were found to provide excessive amounts of several

micronutrients, particularly in infants (0–6 months). Extremely high intakes were reported for vitamin K (up to 1060% of RDA), manganese (8140%), chromium (5180%), iron (1960%), and molybdenum (970%). Iron intake remained consistently above RDA values across age groups, raising concerns about potential adverse effects such as liver damage and mineral imbalance. A similar pattern has been observed in the Latvian paediatric PKU population, where iron intake also exceeded the RDA. Selenium content was generally within recommended ranges (12.8–13.9 μg), while zinc levels decreased slightly with age (4.1–3.2 mg). While these levels complied with general recommendations, Tummolo et al. pointed out that cumulative intake from all dietary sources could still exceed tolerable upper intake levels, increasing the risk of interference with copper metabolism and weakening of the immune system. Additionally, the study revealed substantial variability in micronutrient content among different protein substitute brands and across age groups, with coefficients of variation for some nutrients exceeding 100% (Tummolo et al., 2025). These findings are similar with our results, despite blood levels were in normal range, intake of Se and Zn was higher than recommended, particularly among patients who reported regular use of AA formula. This suggests that medical foods substantially contribute to the overall micronutrient intake in PKU patients, highlighting both – their importance for meeting nutritional requirements and the potential need for monitoring to avoid excessive intake.

Despite a high concentration of minerals and vitamin overdoses of such elements as Zn, Se, and iron, it is not common, while deficiency has been reported in many studies of patients with PKU. For example, Okano et al. concluded that Se intake in Japan was low and suggested that this was due to a lack of Se in Phe-free protein substitutes available in the country (Okano et al., 2016; Pena et al., 2015) Thus, the bioavailability of Se derived from the Phe-free formula requires further investigation.

In study published by Crujeiras et al. where aim of this study was to determine the vitamin and mineral status in PKU patients according to the Phe tolerance, age, diet, BH4 supplementation and adherence to diet, participated 156 patients of them 78.2% of the patients were diagnosed with PKU and 21.8% with MHPA. From PKU patients, 70.5% were diagnosed with cPKU and 29.5% patients with mPKU. It was observed that Zn levels were found to be in the normal range in almost all patients. Se was found to be diminished in 25% of patients, 76.9% of them with classic PKU but only in 2.56% of patients under BH4 treatment. Vitamin D was found altered in 14% of total patients (Crujeiras et al., 2015). These findings differ from our results, where no deficiencies in either Se or vitamin D were observed among Latvian paediatric PKU patients. This is particularly noteworthy given that vitamin D insufficiency is commonly reported in the general Latvian population due to geographic latitude and limited sun exposure

during winter months. A natural source of many essential nutrients, including Zn, Se, and iron, is protein-rich food. However, such foods must be strictly excluded or used in very limited amounts in the diet of patients with PKU. For example, egg yolk, which is rich in Se, is permitted in Latvia only in quantities of 6 grams per meal (equivalent to one protein exchange), which is insufficient to provide an adequate intake of Se. (MacDonald et al., 2020)

Although protein substitutes are fortified and contain excessive amounts of certain micronutrients – iron, zinc, selenium, manganese, copper, and various vitamins (e. g., vitamin B12, folate, and vitamin K, but, deficiencies may still occur. These deficiencies might result from irregular or inappropriate use of protein substitutes or from insufficient bioavailability of vitamins and minerals derived from Phe-free formulas. This issue requires further investigation.

As discussed previously, the intake of fat in Latvian paediatric PKU patients was significantly lower compared to the control group, and it was lower than the recommended amount by the MHRL. Low-fat intake can impact fatty acid status; one of the findings in our study was that PKU patients had significantly different fatty acid profile in erythrocytes compared to the control group. Altered fatty acid status is a metabolic consequence in children with PKU, resulting from lifelong adherence to a Phe-restricted diet with severely limited intake of natural protein sources. We also examined whether differences in FA concentrations existed between the PKU and control groups among participants whose fat intake fell below the MHRL recommendation. Previous studies have suggested that low-fat diets may positively influence FA profiles by reducing saturated fat intake and improving the omega-6 to omega-3 ratio (Giroli et al., 2021). However, in our study, low-fat intake did not lead to an increase in omega-3 concentrations. In fact, omega-3 FA levels were low across all fat intake categories (deficient, adequate, and excessive), with the lowest concentrations observed in patients with PKU who had insufficient fat intake.

Several factors may explain this finding. The PKU diet is characterised by a strict restriction of natural protein sources such as nuts, seeds, fish and seafood, which are the primary sources of preformed omega-3 FA – EPA and DHA. Consequently, even in the context of reduced total fat intake, the absolute intake of omega-3 FA remains extremely low or absent in this patient group. Second, the special low-protein foods and just several AA mixtures typically consumed by PKU patients are generally not enriched with omega-3 fatty acids (Giroli et al., 2021; Koletzko et al., 2009). Without targeted supplementation, there is limited opportunity for these patients to obtain sufficient omega-3s through diet alone. In contrast to general populations, where a reduction in total fat may lead to a relatively higher proportion of omega-3s in blood lipids, PKU patients appear unable to achieve this due to the overall deficiency in dietary omega-3 sources. Third, endogenous conversion of ALA to long-chain

omega-3 fatty acids is known to be inefficient in humans and may be further impaired in PKU due to metabolic disturbances. Elevated Phe and its metabolites may inhibit key enzymes in the desaturation and elongation pathway, such as Δ6-desaturase, thereby reducing the synthesis of EPA and DHA from ALA. Although total fat intake may be low, the types of fats consumed in the PKU diet are often disproportionately high in omega-6 fatty acids, particularly from vegetable oils such as sunflower or corn oil (Guerra et al., 2020). There was a statistically significant difference in HDL cholesterol levels comparing PKU and CG, although it was in the normal range, but in CG it was significantly higher than in PKU. The reason for this may be lower intake of fat, including lower PUFA intake.

During the analysis of FA status in PKU patients by gender, statistically significant differences were identified in the levels of palmitic acid, oleic acid, and in the arachidonic acid to EPA ratio. These results contrast with previous studies, which have generally reported no significant gender-based differences in these fatty acids. Other investigations have documented higher concentrations of DHA in females (Giltay et al., 2004; Tabassum et al., 2023). In a meta-analysis presented by Lohner et al. in 2013, significantly lower values of both Ara and DHA were observed in total plasma lipids (32 and 33 studies) and in plasma phospholipids (23 studies) in men compared to women (S. Lohner et al., 2013). However, a key distinction is that our study focused exclusively on a paediatric population, whereas many earlier studies were conducted in adults. Given the dynamic metabolic changes during growth and development, as well as differences in hormonal profiles before and after puberty, it is plausible that age plays a significant role in modulating FA metabolism and status, suggesting that sex-related differences in FA profiles may be context-dependent and influenced by various metabolic or dietary factors.

In our study, PKU patients showed significantly lower levels of essential omega-3 fatty acids: low levels of EPA and DHA, reduced total omega-3 content and a lower omega-3 index, when compared to CG. These findings are consistent with previous studies that have demonstrated depletion of DHA and EPA in PKU population. Koletzko et al. in 2009 conducted two related studies involving 36 paediatric patients with classical PKU under good metabolic control (plasma Phe levels < 360 μmol/L). At baseline, these patients exhibited markedly reduced concentrations of the LC-PUFAs, EPA, DHA in plasma phospholipids, with DHA averaging only 2.4% and EPA 0.4% of total fatty acids. These levels were substantially lower compared to omnivorous controls and reflect the near absence of dietary sources of preformed DHA and EPA in the highly protein-restricted PKU diet. In contrast, levels of arachidonic acid were relatively higher (mean 9.3%) and decreased after omega-3 supplementation, suggesting possible competitive effects in PUFA metabolism. The same cohort was subsequently

supplemented with refined fish oil capsules providing 15 mg DHA/kg body weight per day over a 3-month period. This intervention resulted in a significant increase in plasma DHA to 7.1% and EPA to 3.3% of total fatty acids ($p < 0.001$ for both), while AA decreased to 6.8%. Importantly, plasma Phe levels remained unchanged, indicating that the supplementation was metabolically safe and well tolerated (Koletzko et al., 2009).

The study by Drzymała-Czyż et al. published in 2018, also described deficiencies of LC-PUFA in PKU. In a cohort of 40 PKU patients, the serum phospholipid profile revealed significantly lower levels of several omega-6 and omega-3 LC-PUFAs, including Ara, docosapentaenoic acid, and DHA, compared to matched healthy controls. Interestingly, the study reported elevated levels of the precursor α-linolenic acid and γ-linolenic acid along with altered desaturase activity indices, suggesting impaired endogenous conversion pathways. The observed discrepancies in FA ratios suggest a potential downregulation of Δ5- and Δ6-desaturase activities in PKU, possibly influenced by Phe or its metabolites. Importantly, Drzymała-Czyż et al. found no significant correlation between dietary fatty acid intake and serum levels (Drzymała-Czyz et al., 2018).

Our finding is similar with data presented in scientific review by Montoya P et al. where significantly lower levels of DHA was observed, also low cholesterol was observed in PKU patients compared to control group. Montoya Parra et al. published a systematic review of the literature to compare the status of nutrients known to be important for brain development and functioning between individuals with PKU and healthy controls and it observed significantly lower levels of DHA ($p = 0.0005$;), EPA ($p = 0.003$) and cholesterol ($p < 0.0001$) were suggested in patients with PKU compared to healthy controls. (Montoya Parra et al., 2018) Low levels of EPA and DHA were also previously described by Lohner S et al (Szimonetta Lohner et al., 2013) where the primary analysis showed significantly lower EPA and DHA values in patients as compared with controls, but Ara acid levels in Latvian PKU patients were statistically higher than in controls and it differs from results that were described by Lohner S et al. ALA levels in Latvian PKU patients were lower than in the control group, and they also vary with the findings of Lohner et al. (Szimonetta Lohner et al., 2013). The inability of PKU patients to adequately convert ALA to longer-chain derivatives further exacerbates this deficiency.

But there are also studies, where no differences were found in FA status. For example, in a study performed by Gramer in 2016, no significant differences were found in DHA or EPA concentrations between PKU patients and healthy controls. The study included 36 well-controlled PKU patients and assessed LC-PUFA levels in multiple lipid fractions, including plasma phospholipids, cholesteryl esters, and erythrocyte phospholipids. EPA and

DHA levels did not differ significantly between patients and control subjects in plasma and erythrocyte fractions. There was a significant negative correlation between standard deviation scores of DHA-levels in erythrocyte parameters from the respective age-matched control group and patients' concurrent and long-term Phe levels for erythrocyte phosphatidylethanolamine and erythrocyte phosphatidylcholine. Patients with lower (higher) Phe levels had positive (negative) DHA- standard deviation scores. These results suggest that in PKU patients with good dietary adherence and stable Phe concentrations, the LC-PUFA status can remain within the normal range, potentially reducing the need for routine LC-PUFA supplementation in all cases (Gramer et al., 2016).

Interesting finding in our study – PKU patients was statistically low level of trans-palmitoleic acid comparing to references and healthy controls. Mozzafarian et al. presented data about trans-palmitoleic acid impact on metabolic risk and incident of diabetes type 2. It has found that higher trans-palmitoleic acid was associated with slightly lower adiposity and, independently, higher HDL-cholesterol (across quintiles: +1.9%, $p=0.04$), lower TG (−19.0%, $p < 0.001$), lower total: HDL-cholesterol (−4.7%, $p < 0.001$), lower C-reactive protein (−13.8%, $p=0.05$), and lower insulin resistance (−16.7%, $p < 0.001$). (Mozaffarian et al.) It means, that patients with PKU due to low levels trans-palmitoleic acid may have higher risk of cardiometabolic disorders, including diabetes type 2.

Interestingly, we observed higher concentrations of elaidic acid in PKU patients compared with controls, despite similar overall low fat intake. Elaidic acid is the predominant industrial trans fatty acid, typically derived from partially hydrogenated vegetable oils, although it is also present in smaller amounts in ruminant fats. Elevated levels of elaidic acid are clinically important, as they have been associated with adverse lipid profiles, systemic inflammation, and increased cardiovascular risk in the general population. In their study, Hadj Ahmed and colleagues demonstrated that trans fatty acids were elevated in patients with coronary artery disease, and that elaidic acid specifically correlated with an unfavourable LDL-C/HDL-C ratio, increased apolipoprotein B, enhanced lipid peroxidation, and higher concentrations of inflammatory markers such as high-sensitivity C-reactive protein, interleukin-6, and tumour necrosis factor-α.(Hadj Ahmed et al., 2018)

The higher elaidic acid concentrations observed in PKU patients may reflect differences in the composition of special low-protein processed foods, many of which contain refined vegetable oils or additives to improve texture and palatability. Although trans fatty acid levels in the general food supply have declined in recent years due to regulatory measures, even modest increases in circulating elaidic acid could be concerning in a patient group already predisposed to an unfavourable fatty acid profile. These findings emphasise the need to evaluate

the quality of dietary fat sources used in low-protein foods for PKU and to further investigate the potential contribution of trans fatty acids to long-term cardiovascular risk in this population.

It was observed that APA/EPA ratio significantly higher in PKU patients than in control group. The ratio of arachidonic acid to EPA in the blood has been investigated for its potential impact on various health conditions. A higher Ara/EPA ratio has been associated with an increased risk of cardiovascular events (Shibata et al., 2018). It was described before by Tutino V et al. that high levels of the AA/EPA ratio were detected in tumor tissue of patients with metastatic colorectal cancer (Tutino et al., 2019). So PKU patients may be in higher risk of cardiovascular diseases due to not only high Ara/EPA, but also trans-palmitoleic acid and changed omega-3.

The ratio of LN/DGLA was higher in both groups; what is interesting is that in healthy controls, the ratio is higher than in PKU. The ratio of LN/DGLA increases when the Δ6-desaturase enzyme is inhibited by Zn and magnesium deficiency, elevated insulin, or dietary excess of saturated, monoenoic or trans fatty acid. Under these conditions, the enzyme cannot convert the substrate (LA) to its product (DGLA) fast enough. The production of all desaturation products is affected, including GLA, EPA and arachidonic acids (Knez et al., 2017). As we observed before there was no Zn deficiency in PKU patients and HC, but no levels of mg or insulin were investigated in Latvian PKU population.

The ratio of LN/DGLA could be higher due to increased concentrations of saturated fat and trans FA in the diet. When comparing our results with previously published data, we observed both similarities and differences. Consistent with earlier studies, PKU patients demonstrated lower levels of long-chain omega-3 fatty acids (EPA and DHA) and a higher Ara/EPA ratio, suggesting an unfavourable FA profile that may contribute to increased cardiovascular risk. At the same time, certain findings - such as alterations in trans-palmitoleic acid and other minor fatty acids – have been less frequently reported and therefore warrant cautious interpretation.

It should also be emphasised that while most of the discussion has focused on EPA and DHA due to their well-established clinical relevance, our results indicate that the FA profile in PKU patients is altered more broadly. Changes in FA such as arachidonic acid, trans-palmitoleic acid, and others may carry physiological significance, but current evidence is limited. Many of these associations remain inconsistent across studies, and knowledge gaps persist regarding their clinical implications.

In summary, our findings largely support previous reports of disturbed omega-3 status in PKU patients, while also highlighting additional changes in other fatty acids that have been less systematically studied. These results should be interpreted with caution, as variability

between studies and methodological differences may influence outcomes. Further research with larger cohorts and standardised methodologies is needed to clarify the role of these FA in long-term health outcomes of PKU patients.

The longitudinal analysis of metabolic control in Latvian paediatric PKU patients between 2023 vs 2025 revealed several noteworthy trends. Overall, the total number of blood samples within the recommended Phe range increased across all age groups. The age-dependent pattern of blood Phe control in our cohort is consistent with observations reported in the broader PKU literature. In our study, good metabolic control (median Phe < 360 μmol/L) was maintained in younger children (< 12 years) in 2023 and 2025 and adolescents' blood Phe levels (12–18 years) exhibiting the highest median Phe levels (510 μmol/L). In 2025, when it was possible to check regularly not only Phe level, bet also Tyr it was observed although median Phe level in all patients in the age of 12 till 18 was < 600 μmol/L, Phe/Tyr ratios were above recommended value (< 6). This age-related decline in metabolic control aligns with previously reported trends indicating that metabolic control tends to worsen with increasing age in PKU populations.

International studies consistently show that adherence to the PKU diet decreases with age, particularly during adolescence, when increased autonomy, social influences, and treatment fatigue reduce compliance. A study from Estonia by Lilleväli et al. performed in 2018, which included 67 patients aged 1–18 years, reported that younger children maintained better metabolic control than school-aged children, a trend consistent with our findings in Latvia. However, the Estonian cohort demonstrated a stabilisation of metabolic control after 12 years of age, whereas the Latvian patients showed a gradual decline continuing into adolescence. The data from Lilleväli et al. differ from the opinion published by E.S.PKU guidelines, which discuss worse compliance with the diet during adolescence (Lillevali et al., 2018; van Wegberg et al., 2025).

Similar discrepancies are seen in other regions. In Portugal, Kanufre et al. analysed 87 paediatric PKU patients and found that 83% of Phe measurements were within the recommended range, with somewhat better control in older children, particularly those ≥ 12 years. This contrasts with both Latvian, where metabolic control worsened with age (Kanufre et al., 2021). In Canada, a study by Yuskiv et al. based on data from the Canadian Inherited Metabolic Diseases Research Network included 191 patients and found that while early childhood Phe levels were generally well controlled (mean ≈ 331 μmol/L), only 28% of classic PKU patients maintained target levels by 8–9 years of age (Yuskiv, 2020). These variations across countries illustrate how patient adherence is shaped not only by age but also

by system-level factors such as access to low-protein foods, frequency of monitoring, and the availability of specialised dietary support.

In summary, the Latvian data align with the global trend of declining metabolic control with age but indicate that adherence challenges may emerge earlier and that the youngest patients may also be at risk of inadequate or overly restrictive intake. Comparisons with data from Estonia, Portugal, and Canada suggest that structural healthcare factors – such as the availability of specialised dietary support, regular follow-up, and patient education – play a crucial role in maintaining optimal metabolic control. Future strategies should therefore prioritise reinforcing structured, multidisciplinary follow-up programs, expanding access to low-protein food options, and developing educational tools tailored to both caregivers and older patients.

### 4.4 Nutritional profiling of foods appropriate for PKU

The scarcity of reliable AA nutritional composition data, particularly for fruits, vegetables, and emerging plant-based foods, continues to pose a significant limitation for dietary planning in PKU. One of the primary reasons for this gap is the high cost and technical complexity of amino acid analysis, which restricts the frequency and scope of food testing. Historically, comprehensive AA data of the foods has been limited; in the United Kingdom, for instance, no major update to Phe content listings occurred between the 1980 edition of McCance and Widdowson's Composition of Foods and the 2006 initiative by the National Society for Phenylketonuria, which analysed 172 foods – mainly fruits and vegetables – specifically for Phe content. More recently, Ford et al. expanded this work by analysing the amino acid profiles of 73 plant-based foods, including many novel items, and provided detailed data on AA content.

In Latvia, however, the situation remains challenging. There is no publicly available national food composition database that includes AA profiles or even full macronutrient content for commonly consumed foods. As a result, healthcare professionals and dietitians must rely on international food composition databases such as FRIDA (Denmark), Fineli (Finland), and USDA (United States) ("Etusivu - Fineli," ; "FoodData Central," ; "Frida,"). While these databases are robust and widely used, there is often lack of information about specific to local and culturally significant foods, including wild plants like nettles and wild garlic or more recently popularised products such as microgreens or manufactured foods like broths. Additionally, discrepancies between different databases regarding protein and Phe content further complicate clinical decision-making.

For example, microgreens, that became popular "super-food" in the last years, contain an abundant number of different phytonutrients, varying according to the nature of the plants

that are selected to produce the microgreen. They are source of micronutrients such as magnesium, potassium, calcium, iron, vitamin A, vitamin B, C and vitamin K (De Pascale et al., 2019). They also have reported antioxidant activity, anti-obesity, diabetic and cholinergic activity (Dereje et al., 2023; Wojdyło et al., 2020; Wojdyło et al., 2007). Unfortunately, information about the protein and amino acid content of microgreens is limited. For example, in the FRIDA, there is no available information ("Frida,"), but in the USDA, only data about the protein content of broccoli microgreens is available. This information is controversial because they contain 0g/100 g according to the USDA, which is different from our data – the protein content is 2.1 g/100 g, and Phe content is 97 mg/100 g. Our data describing the protein content of broccoli and radish microgreens is similar with data that was published by Dereje et.al in 2023, who described the protein content as 2.23–3g/100 g and 1.81–2.58g/100 g respectively. (Dereje et al., 2023) Recently published data by Boyle et al., where 165 samples of fruits, vegetables and starchy roots were analysed to determine their protein and AA content, aim of that study was similar with ours - to describe a further analysis of the protein and AA content of fruits, vegetables and starchy roots, specifically focusing on AAs most relevant to IMDs. Also Boyle et al. reported that broccoli microgreens contained Phe > 75 mg/100 g (Boyle et al., 2024).

Traditional or regionally specific foods are often excluded from international datasets. In Latvia, for instance, wild edible plants such as nettles and wild garlic are frequently used in seasonal and culturally significant dishes. However, these items are either absent or lack complete macronutrient and AA profiles – Phe – in FRIDA, Fineli, and USDA databases. This omission poses a disproportionate challenge for dietary planning in regions where such foods are traditionally used. As we report, the Phe content of leeks is 37 mg and protein is 1.2 g per 100 g; in the FRIDA, Phe concentration is 50 mg/100 g, and protein content there is also reported higher – 2 g per 100 g, there is also data available about protein content in USDA about leeks – 1.57 g per 100 g, but there is no data about Phe concentration. Although data of protein in our study is closer to USDA information.

There is a small number of publications of protein and amino acid content in broths. Comparing data on protein and amino acid content in broths, Shaw M. et al. in 2019 presented data about commercially available broths. The content of Phe in beef broth was 58,6 mg, in chicken broth – 58,9 mg, and turkey broth – was 61 mg amino acid per gram of bone broth. It is a bit higher concentration of Phe than in our study, but however Phe concentration in all broths was below 75 mg per 100 g. Average protein content of bone broths was 2,3 g per 100 g product and it is significantly higher than our data (Shaw & Flynn, 2019). Challenges arise when evaluating foods like broths, which are rarely standardised in composition. The nutritional

content of broth can vary widely depending on preparation factors, including the type and quantity of meat, bones, or vegetables used, cooking duration, fat removal techniques, and dilution with water. Consequently, two broths labelled identically may differ markedly in their macronutrient and amino acid profiles, making direct comparison difficult unless standardised recipes and analytic methods are employed.

Moreover, variability can be introduced at the stage of laboratory analysis itself. Differences in analytical methods, instrument calibration, and sample preparation (e. g., filtration, centrifugation, or freeze-drying) can lead to measurable discrepancies between laboratories. According to EU guidance Regulation No 152/2009 on nutrient analysis, such variability is addressed by setting acceptable tolerance ranges that account for analytical uncertainty, natural variation in food composition, and nutrient stability. For protein determination in foods, deviations within ±20% from the declared value are generally considered acceptable when analytical uncertainty is included. For AA, particularly in complex matrices like broth, repeatability (within-laboratory variation) is expected to fall within 2–6% for most AA, while inter-laboratory reproducibility can range from 6% up to 15–20% depending on the AA and its concentration. For AA present in very low amounts, reproducibility may reach 30–50%, as noted in European inter-laboratory validation studies. Thus, small differences between studies may reflect both genuine variation in the product and the inherent limitations of the analytical process (Regulation, 2009).

Another study done by Mar-Solis L et.al presented data of AA and protein content in bone broth obtained from bovine femur and their data is similar to our results. Phe content was 7 mg per 100 g and protein content 248 mg per 100ml of broth, so also in this study Phe content was below 75 mg per 100 g, so it can be considered as exchange free in low protein diet. (Mar-Solís et al., 2021)

Introducing any new food that has traditionally not been allowed in the diet such as a ‘meat’ or fish-based broth brings a juxtaposition. On the one hand it will allow dietary variability, letting the child or adult with PKU to participate in a family meal using a broth as part of the meal. On the other hand, there is a potential to provoke liking a taste for meat and fish, which is too high in Phe content. It may also lead to frustration about meat restrictions, because patients want to eat for meat-based foods. Therefore, education and teaching and consensus among professionals is needed before introducing such foods.

A similar challenge was observed previously in Latvia, when foods such as bananas were listed in the “yellow” category – products permitted only in limited quantities. Subsequent analytical studies demonstrated that bananas contain less than 75 mg of Phe per 100 g and can therefore be classified as exchange-free in a low-protein diet (MacDonald et al., 2020; Rohde

et al., 2012). However, changing the classification is only the first step; convincing patients and their families to accept that a food once considered restricted can now be consumed freely is often difficult. This resistance can be partly explained by food neophobia, which has been reported in children with PKU at a higher prevalence than in the general population. Long-standing avoidance of certain foods may lead to reluctance or anxiety when trying new or reclassified foods, even if robust biochemical evidence supports their safety.

Surprisingly, a high protein and Phe content was observed in golden raisins, but a mean for 3 different types of raisins was protein content 3.2 g/100 g and mean Phe concentration 65 mg/100 g. Similar results were reported by Boyle et al, where mean Phe content of raisins (all types) was 59 mg/100 g (Boyle et al., 2024) This is also similar to the USDA, with available information describing their Phe content as 65 mg/100 g and protein content 3.07 g/100 g. Previously, it was reported that protein quantity was 2.2 g/100 g and Phe content was 97 mg/100 g (Bolin & Petrucci) ("Food - Fineli,") although in the PKU dietary handbook, raisins are included in the list of fruits and vegetables that are allowed without measurement in PKU (MacDonald et al., 2020). The PKU diet is already very challenging, so it is important that any dietary advice should be practical and straightforward. It may cause confusion if there are different rules for different types of raisins.

Given these discrepancies, there is a need to decide on the most appropriate recommendation for clinical practice. One option would be to treat all raisins as a source of phenylalanine and require them to be counted towards the daily Phe allowance, thereby eliminating the risk of underestimating intake in patients who consume higher-Phe varieties such as golden raisins. Alternatively, following current European guidelines for the classification of “exchange-free” foods – where products containing less than 75 mg Phe per 100 g are considered free and do not require measurement (MacDonald et al., 2020; van Wegberg et al., 2025) – all raisins could remain in the “green” list of exchange-free foods, considering that the mean Phe content across varieties is below this threshold and occasional consumption is unlikely to significantly affect blood Phe control in most patients. A third, more tailored approach could involve keeping standard raisins in the green list but recommending that golden raisins be counted as a measurable source of Phe, especially for patients with stricter tolerance or poorer metabolic control. Each approach has implications for patient adherence, dietary flexibility, and the clarity of dietary guidelines, and consensus among professionals is required before implementing changes.

Mushrooms represent a unique food group of both culinary and potential nutritional importance for patients with PKU. In many European countries, including Latvia, wild and cultivated mushrooms are part of traditional diets and are consumed seasonally in various

dishes. For the general population, mushrooms are valued for their flavour, low energy content, and bioactive compounds such as ergothioneine, polyphenols, and vitamin D, mushrooms also provide a good amount of B vitamins and various minerals, making them a powerhouse of nutrients for human physiological functions (Cardwell et al., 2018; Rathore et al., 2017; Samsudin & Abdullah, 2019; Yadav & Negi, 2021). However, for individuals with PKU, the inclusion of mushrooms in the diet is more complex due to their protein and Phe content, which varies considerably across species and preparation methods.

Existing food composition databases often contain incomplete or inconsistent data on the macronutrient AA profile of mushrooms. A we found Phe content can range from below 50 mg / 100 g in some varieties to over 150 mg / 100 g in others, making it unclear whether certain species can be classified as "exchange-free" or should be counted towards daily Phe allowance. This uncertainty can lead to either overly restrictive exclusion of potentially safe mushroom types or unintentional overconsumption of higher-Phe varieties. For example, boiled shiitake mushrooms contain only 64 mg of Phe/100 g, while cooked Portobello champignon mushrooms, white and brown, as based in our results, contain 120 mg, 100 mg, and 150 mg per 100 g, respectively, and should be counted in a low-Phe diet according to E.S.PKU guidelines (van Wegberg et al., 2025). Traditional mushrooms for Latvian PKU patients, such as Boletus edulis and chanterelles, are low in Phe. However, there is limited information on the Phe content of Boletus edulis. Our data indicate a protein content of 2.6 g/100 g, which aligns with findings published by Jaworska et al (Jaworska et al., 2015). As of January 2025, there was no published information about the Phe content of Boletus edulis mushrooms, White mushrooms, and brown mushrooms in USDA and FRIDA databases. Previously, in Latvia, mushrooms like shiitake and white and brown Champignons were allowed without measurement, but Boletus edulis and Chanterelles were allowed in limited amounts in the PKU diet. The latest data on the Phe content of mushrooms suggests that repeated testing is needed to verify the Phe content in different types of mushrooms. A low-protein diet can lead to a reduced feeling of satiety, which may cause individuals to consume larger quantities of other foods, such as potatoes, potentially impacting metabolic control. Incorporating various mushrooms into the diet could help individuals with PKU experience a greater sense of fullness due to their fibre content. Many people with PKU often express that it is challenging to achieve satiety with low-protein foods and vegetables (Daly et al.; Li et al., 2020; Moon & Koh, 2020; Pezeshki et al., 2016).

Unfortunately, patients with PKU often exhibit neophobia, which makes introducing new products and flavours into their diets a challenging task. Therefore, it is crucial to incorporate as many allowed products as possible early in life, particularly during the first few years. This strategy aims to expand the child's menu to include items from both the allowed

and in limited amount consumed food lists (Evans et al., 2018; Evans et al., 2016; Tonon et al., 2019). At present, there is no clear stepwise approach for introducing new products into the low-protein diet for PKU, particularly for foods that were previously categorised as exchange products but are now considered suitable for free use (i. e., not counted as a source of natural Phe or protein). Although there are studies and guidelines describe general strategies for transitioning protein substitutes or integrating novel low-protein foods, standardised protocols for reclassifying and gradually introducing these products into daily practice are lacking (MacDonald et al., 2020; Yilmaz Nas et al., 2024). This creates uncertainty for both clinicians and families and highlights the need for structured recommendations to ensure safe, consistent, and practical use of such foods in PKU dietary management.

By actively involving patients with PKU and their caregivers, our study ensures that the research is based on the real-life experiences of patients and the needs of the PKU community. It also helps develop practical dietary recommendations that can improve the quality of life for individuals with PKU.

### 4.5 Knowledge and awareness of the PKU diet among patients and their caregivers

The present study demonstrates clear differences in PKU-related knowledge between caregivers and patients, with caregivers consistently achieving higher rates of correct responses across the questions. These findings are consistent with previously published literature indicating that caregivers, particularly parents of younger children, generally possess stronger knowledge of dietary guidelines and practical PKU management than patients managing their treatment independently.

In the study performed by Witalis et al. was reported that only 45% of PKU patients were able to correctly state the recommendations if their daily Phe intake, and fewer than one-third accurately identified Phe content in selected foods (Witalis et al., 2017). Similarly, in our cohort, a substantial proportion of patients were unable to report their permitted natural protein intake or Phe target levels, and performance in practical dietary calculation questions such as estimating protein content of common foods, was particularly low.

In contrast, caregivers in our study demonstrated high accuracy in identifying Phe targets and prescribed protein allowances, which aligns with findings from Ramos-Álvarez et al., who observed that caregiver knowledge is generally strong regarding restricted foods but may vary in more complex dietary assessments (Ramos-Álvarez et al., 2023). The study performed by Ramos-Álvarez et al. in the United States (n = 178) in 2023 using an online or paper-based survey, it included a validated food selection questionnaire designed to assess whether respondents could correctly identify foods requiring restriction versus those permitted

freely in the PKU diet. The study found that misclassification of foods was more common among caregivers with lower overall knowledge, thereby potentially compromising dietary adherence. Most participants were mothers of young children, many of whom had a high level of education and were members of a PKU association, suggesting they were a particularly motivated and well-informed group. This highlights that even within relatively knowledgeable caregivers, significant misconceptions about food categorisation persist, and that knowledge gaps may be even greater in less engaged or indirect caregivers (Ramos-Álvarez et al., 2023).

Our data demonstrate that knowledge differences are associated with metabolic control. Among patients only 29% achieved good or average metabolic control, whereas 70% of children represented by caregivers were within this range. This pattern is consistent with the study made by Rovelly et al., where was documented age-related deterioration in metabolic control, particularly during adolescence and young adulthood when responsibility for disease management shifts from caregiver to patient. Rovelli et al. highlighted that transition to self-management is frequently accompanied by informational gaps and unmet educational needs, especially regarding practical aspects of dietary implementation. The knowledge deficits observed in our patient group, particularly in relation to illness management, formula adherence, and protein calculation may contribute to poorer metabolic outcomes (Rovelli et al., 2024).

The emotional and practical burdens on caregivers must also be considered. As it was previously noted by Van Rijn et al. and MacDonald et al., the demands of lifelong dietary adherence can become overwhelming, especially in adolescence, leading to a decline in compliance (MacDonald et al., 2016; van Rijn et al., 2015). Eijgelshoven et al. further quantified this burden, showing that PKU caregiving consumes over 19 hours per week and involves substantial out-of-pocket costs (Eijgelshoven et al., 2013). These pressures can lead to chronic stress, which may in turn affect how caregivers process or retain medical information. Walkowiak emphasised that parents' emotional well-being, professional responsibilities, and gender dynamics all influence their caregiving experience (Walkowiak et al., 2023). Unfortunately, our study did not include questions that could directly assess or provide specialists with insights into the emotional state of patients or caregivers. Given the significant role that emotional health plays in dietary adherence and long-term management, future research should integrate validated tools for assessing psychological well-being. This would allow for earlier identification of emotional strain, enabling timely interventions and the development of supportive measures tailored to the needs of both patients and their families.

Several studies support the role of targeted interventions to improve caregiver understanding. It was by Zamani et al. demonstrated the effectiveness of educational

programmes based on behavioural models, especially when paired with the provision of low-protein foods (Zamani et al., 2021). Similarly, Poole et al. called for structural changes in public settings such as restaurants to accommodate PKU-friendly diets, showing how education can extend beyond the home and healthcare environment (Poole et al., 2022).

Adherence-related findings in our study also parallel published evidence. While most caregivers reported consistent administration of the amino acid formula, patient responses indicated more variable adherence, including missed doses and, in some cases, complete discontinuation of formula intake. Teruya et al. have suggested that knowledge gaps may interact with psychosocial barriers, such as treatment fatigue and perceived dietary burden, resulting in suboptimal adherence (Teruya et al., 2025). The reduced frequency of blood Phe monitoring observed in a proportion of our patient cohort further supports concerns regarding incomplete self-management.

Importantly, our results highlight that knowledge deficits were particularly pronounced in practical, calculation-based dietary questions and illness-related management, rather than in general conceptual understanding of PKU. This suggests that educational interventions should prioritise applied dietary skills, real-life decision-making scenarios, and structured transition training programs rather than focusing solely on theoretical disease knowledge. Moreover, the results indicate that while patients and their parents are generally aware of which foods are allowed and which are prohibited, they often lack the skills – or do not apply them in daily life – to weigh foods accurately or to use the exchange system for dietary management. This gap between theoretical knowledge and everyday practice highlights the need for targeted interventions that focus not only on information delivery but also on the consistent application of dietary principles in real-world settings. By bridging this gap, healthcare providers can improve dietary adherence, optimise metabolic control, and enhance the long-term health outcomes of individuals with PKU.

### 4.6 Limitations of this study

We acknowledge several limitations of this study. The most significant limitation of this study was the small number of PKU patients included, which is primarily explained by Latvia's small overall population and declining birth rate in recent years and consequently resulted in a limited amount of usable data. Nevertheless, the patients and their families did their best to participate and contribute, which allowed to collect valuable information despite the small cohort size. Consequently, our evaluation of the nutritional status of PKU patients was constrained by the small number of completed food diaries.

Our analysis was further hindered by the absence of a unified database on the composition of Latvian foods and by the lack of detailed information on the nutritional composition of special low-protein foods, as reported previously. In addition, we were unable to calculate fatty acid intake (saturated, mono and polyunsaturated) from the dietary records. This limitation was due to the fact that the Nutritics software used for dietary analysis did not contain fatty acid composition data for many of the foods consumed by participants.

During laboratory analysis of foods, we acknowledged another limitation that nutrient analysis was conducted during a single season, which may have been influenced by climatic variation. Future studies should therefore include sampling of the same local products across multiple seasons. The nutrient content of foods can also be affected by storage methods, storage duration, and cooking techniques. For example, sorrel dehydrates rapidly and could not be delivered fresh to the laboratory. To ensure transportation, it was frozen, a process likely to have altered its water, protein, and Phe content.

In the final part of the study, which examined the knowledge of PKU patients and their caregivers, it should be noted that although the sample included a relatively large proportion of the total PKU patient population in Latvia, the dataset lacked sufficient representation from patients themselves. Furthermore, the questionnaire primarily focused on theoretical knowledge, what respondents knew should be done – while it did not capture whether these practices were actually applied in daily life. Including such questions in future studies would allow for a better understanding of the gap between knowledge and practical implementation, which is crucial for effective disease management.

### 4.7 Key Strengths and Practical Contributions of the Study

This study has several notable strengths. It provides one of the first detailed analyses of locally available Latvian food products with regard to protein and phenylalanine content, an aspect highly valuable for clinical practice, where region-specific food composition data are scarce but essential for accurate dietary management in PKU. To ensure analytical reliability, laboratory testing of products requiring high precision was performed twice, thereby minimizing error and strengthening confidence in the results.

Another strength lies in the close collaboration with patients and their families, who actively contributed food diaries despite the challenges of a small cohort size, enhancing the validity and real-world applicability of the findings.

The study also highlights practical barriers, such as the complexity of interpreting food labels and counting protein exchange, underscoring the urgent need for targeted patient education and improved dietary resources. By combining clinical data with nutritional analyses,

this research addresses a significant knowledge gap and provides a comprehensive perspective on dietary adherence, nutrient intake, and biochemical outcomes in the Latvian PKU population.

# Conclusions

1 The prevalence of overweight and obesity is higher among patients with PKU compared with the general population. Although patients with PKU consumed less dietary fat than the control group, their intake did not meet the recommendations established by the Ministry of Health, which are intended for healthy children and therefore may not fully reflect the specific nutritional requirements of children with PKU. Micronutrient intakes exceeded the recommended levels across all age groups.

2 PKU patients demonstrated alterations in lipid metabolism, including an increased intake of trans-fatty acids, a reduced intake of polyunsaturated fatty acids, and an altered lipid profile characterised by significantly lower high-density lipoprotein concentrations ($p < 0.001$).

3 A total of 16 food products were identified as requiring restriction and inclusion in the Phe counting system, while 18 products with a Phe content below 75 mg per 100 g were identified as suitable for free consumption within the PKU diet.

4 Caregivers generally have better knowledge of PKU dietary management than patients, particularly regarding the practical aspects of the diet and illness-related management.

5 No statistically significant differences in metabolic control were observed among PKU patients between 2023 and 2025. However, metabolic control tended to worsen with age, as reflected by increasing median phenylalanine levels.

## Proposals

Based on the findings of this study, several key recommendations can be proposed to address the identified gaps in knowledge, dietary adherence, and nutritional adequacy among PKU patients in Latvia. These recommendations aim to strengthen patient education, improve metabolic control, enhance dietary quality, and provide healthcare professionals with better tools for individualised management. It is important to educate and encourage families to complete food diaries. A structured approach to teaching PKU patients and their caregivers – especially parents and grandparents – how to accurately record food intake is essential. Regular use of food diaries should be actively encouraged as a tool for improving dietary adherence, identifying nutritional risks, and enabling more precise dietary adjustments during clinical visits.

Creating targeted educational materials for PKU patients and their families is essential. Design and implement clear, visually engaging, and age-appropriate educational resources aimed at improving the knowledge of PKU patients and their family members regarding the disease and its dietary management. These materials should cater to different age groups and learning preferences, thereby enhancing patient adherence and supporting the long-term success of dietary treatment.

Introduce structured, regular monitoring of biochemical parameters in PKU patients, combined with individualised dietary adjustments based on real-time results. This approach would help optimise metabolic control, prevent long-term complications, and enhance patient quality of life.

Develop a publicly accessible Latvian food composition database – establish a comprehensive, regularly updated, and publicly available database of the nutritional composition of foods commonly consumed in Latvia. Such a resource would enable more accurate calculation of nutrient intake for the general population as well as for patients with specific dietary needs. This would also facilitate the daily work of dietitians in Latvia, ensuring evidence-based dietary planning and monitoring.

Another proposal that could improve patient's adherence to the diet is developing a dedicated mobile application for PKU management. A user-friendly mobile app tailored specifically for PKU patients could integrate food diary functions, a searchable food composition database, and automatic Phe/protein calculations. Additional features, such as reminders for blood tests, tracking biochemical results, and communication with healthcare providers, would further support adherence, empower patients, and streamline routine care.

# Publications and reports on topics of Doctoral Thesis

## Publications:

1. **Lubina, O**., Daly, A., Auzenbaha, M. *et al*. Nutritional profiling of foods for Phenylketonuria. *Sci Rep* 15, 22538 (2025). https://doi.org/10.1038/s41598-025-06633-2
2. **Lubina, O**., Gailite, L., Borodulina, J., Auzenbaha, M. Nutrient Status among Latvian Children with Phenylketonuria. *Children* (Basel). 2023 May 26;10(6):936. doi: 10.3390/children10060936
3. Kreile, M, **Lubina, O**., Ozola-Zalite, I., Lugovska, R., Pronina, N., Sterna, O., Vevere, P., Konika, M., Malniece, I., Gailite, L. Phenylketonuria in the Latvian population: Molecular basis, phenylalanine levels, and patient compliance. *Mol Genet Metab Rep*. 2020 Oct 20; 25:100671. doi: 10.1016/j.y mgmr.2020.100671. PMID: 33101986; PMCID: PMC7578541

## Other publications related to topics of Doctoral Thesis:

1. Pinto, A., Adams, S., … **Ļubina, O.**… McDonald, A. Early feeding practices in infants with phenylketonuria across Europe, Molecular Genetics and Metabolism Reports Volume, Volume 16, September 2018, Pages 82-89;
2. Pinto, A., Adams, S., … **Ļubina, O.**… McDonald, A. Weaning practices in phenylketonuria vary between health professionals in Europe, Molecular Genetics and Metabolism Reports Volume 18, March 2019, Pages 39-44

## Reports and theses at international congresses and conferences:

1. **Lubina**, **O.**, Lugovska, R., Pronina, N., Sterna, O., Kvasova, A., Gailite, L., Vevere, P., Kreile M., Metabolic Control and Anthropometric Parameters of Phenylketonuria Patients in Latvia in 2018, Rīga Stradiņš University International Conference on Medical and Health Care Sciences Knowledge for Use in Practice; 594.lpp
2. Kreile, M., **Lubina**, **O.**, Lugovska, R., Sterna, O., Gailite, L. Polymorphisms in CD36 and leptin gene could modify obesity risk in Latvian phenylketonuria patients. Society for the Study of Inborn Metabolic 2019 Symposium,P-174
3. **Lubina**, **O.**, Kreile, M., Lugovska, R., Gailite, L. Status of nutrient among Latvian children with classical phenylketonuria. Rīga Stradiņš University International Research Conference on Medical and Health Care Sciences "Knowledge for Use in Practice": Abstracts, 24–26 March, 2021
4. **Lubina**, **O.** Fatty acid status of Latvian pediatric PKU patients. Nordic Baltic Meeting on Inborn Errors of Metabolism 2023, November 13–14, Stockholm, Sweden
5. **Lubina**, **O.**, Gailite, L., Auzenbaha, M., Fatty acid status in Latvian pediatric PKU patients. Nutrition and Health 2024, December 5–6, Riga, Latvia
6. Borodulina, J., Gailite, L., , Auzenbaha, M., **Lubina**, **O.**, Knowledge of pku dietary treatment among caregivers and patients with phenylketonuria in Latvia, Nutrition and Growth 2025, February 20–22, Athens, Greece
7. **Lubina**, **O.**, Daly, A., Laktina, S., Gailite, L., Auzenbaha, M., MacDonald, A., Nutritional Profiling of PKU foods, 4$^{th}$ CEE Metabolic Roadshow Conference. 2025, May 7–9, Vilnius, Lithuania

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# Annexes

Annex 1

**Ethical approval**

**Genoma izpētes padome**

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Rīgā

08.03.2019. Nr.A-2/19-03-08

Bērnu Klīniskajai Universitātes Slimnīcai
Dr. Olgai Ļubinai

Atzinums par pētījumu: *"Fenilketonūrijas bērnu vecuma pacientu dietoterapijas novērtējums un tās ietekme uz pacienta veselību"*

Genoma izpētes padome izskatīja Bērnu Klīniskās Universitātes Slimnīcas Dr. Olgas Ļubinas iesniegumu par pētījumu *"Fenilketonūrijas bērnu vecuma pacientu dietoterapijas novērtējums un tās ietekme uz pacienta veselību".*

Pamatojoties uz Genoma izpētes padomes locekļu balsojumu, tiek izsniegts atzinums, ka Genoma izpētes padome atbalsta Bērnu Klīniskās Universitātes Slimnīcas Dr. Olgas Ļubinas pētījuma *"Fenilketonūrijas bērnu vecuma pacientu dietoterapijas novērtējums un tās ietekme uz pacienta veselību"* īstenošanu.

Genoma izpētes padomes
Priekšsēdētājs J. Kloviņš

Annex 2

**Informative material for pacients**

**Informācija par iesaistīšanos pētījumā**

Cienījama Kundze! / Godātais Kungs!

Mēs aicinām Jūsu bērnu piedalīties pētījumā "Fenilketonūrijas bērnu vecuma pacientu dietoterapijas novērtējums un tās ietekme uz pacienta veselību", kas tiks realizēts sadarbībā ar VSIA "Bērnu klīniskā universitātes slimnīcas" Reto slimību koordinācijas centru un Rīgas Stradiņa universitātes Molekulāras ģenētikas zinātnisko laboratoriju. Lūdzu, izlasiet informāciju par pētījumu, pirms parakstāt piekrišanas formu.

Pētījuma mērķis

Izvērtēt klīniskos, laboratoros rādītājus un citus faktorus (organoleptiskās īpašības, ķermeņa kompozīcija, garšas gēni), kas saistīti ar fenilketonūrijas pacientu dietoterapiju un kuri var ietekmēt pacienta veselību bērna vecumā.

Projekta apraksts

Pētījuma laikā Jums būs nepieciešams aizpildīt trīs dienu uztura dienasgrāmatu, kā arī Jūsu bērnam jānogaršo specifiskus ar zemu fenilalanīna saturu produktus un jānovērtē tos pēc Jūsu garšas izjūtām. Papildus tam vienu reizi Jūsu bērnam, kopā ar rutīnas analīzēm paņems asins analīzes, lai veiktu garšas gēnu ģenētisko analīzi

Projekta ilgums

Projekts plānots uz 5 gadiem. Jūsu piekrišanas dokuments tiks uzglabāts 75 gadus. Saņemot Jūsu piekrišanu, ģenētiskais materiāls var tikt izmantots arī citos projektos pēc Jūsu ieskatiem. Ja Jūs pārdomājat par dalību projektā, tad, sazinoties ar pētījuma koordinatoru, ir iespējams pārtraukt dalību projektā, šādā gadījumā Jūsu bērna ģenētiskais materiāls tiks iznīcināts. Atteikšanās no dalības projektā neietekmēs ārstēšanas gaitu.

Iespējamie riski

Piedalīšanas pētījumā nav saistīta ar riskiem Jūsu bērna veselībai. Jūsu bērnam tiks paņemts asins vai siekalu paraugs. Retos gadījumos parauga ņemšanas vietā var izveidoties asins izplūdums vai iekaisums.

Konfidencialitāte un projekta dalībnieka tiesības

Latvijas Republikas Saeimas pieņemtais "Cilvēka genoma izpētes likums", kā arī "Fizisko personu datu aizsardzības likums" garantē Jūsu personas datu, veselības un iedzimtības datu, kā arī ģenētiskās izpētes rezultātā iegūtās informācijas konfidencialitāti. Informācija tiks uzglabāta vietā ar ierobežotu pieejamību un netiks izsniegta ne Jūsu ģimenes locekļiem, ne apdrošināšanas firmām, ne darba devējam. Iegūtie dati un audu paraugi netiks izmantoti komerciāliem mērķiem un nelikumīgi nodoti neiesaistītām trešajām personām. Dati, kas par jums tiks uzglabāti Rīgas Stradiņa universitātes Molekulārās ģenētikas zinātniskajā laboratorijā, tiks izsniegti tikai sekojošos gadījumos - Jums personīgi, pēc Jūsu rakstiska pieprasījuma. Ziņas par Jūsu bērna veselības stāvokli un ģenētisko pētījumu rezultāti (bet ne personu identificējoši dati!) būs pieejami arī pētniekiem, kas piedalās

pētījumā. Tāpat ar Jūsu rakstveida piekrišanu Jums atrastās klīniski nozīmīgās ģenētiskās variācijas drīkstēs izsniegt Jūsu bērna ārstam pēc viņa pamatota pieprasījuma. Parakstot piekrišanas dokumentu, Jums ir tiesības arī aizliegt sava bērna veselības stāvokļa apraksta papildināšanu, atjaunošanu vai pārbaudi projekta ietvaros vai ierobežot sava genoma izpētes apjomu. Jums ir tiesības jebkurā laikā atsaukt savu piekrišanu piedalīties šajā projektā, šādā gadījumā Jūsu bērna audu paraugi, veselības stāvokļa un iedzimtības apraksts, un jebkura ar personas identificēšanu saistīta informācija tiks iznīcināta.

Brīvprātīga piedalīšanās

Jūsu piedalīšanas projektā ir brīvprātīga. Atteikšanas no piedalīšanas nerādīs Jums nekādas nevēlamas sekas.

Iespējamie ieguvumi

Piedalīšanās projektā iespējams nedos Jums tūlītēju labumu, bet var uzlabot Jūsu bērna slimības kontroli, mazināt slēptos veselības riskus, un izprast Jūsu bērna garšas īpatnības, kas ļaus nedaudz piemērot ēdienkarti

Kontaktpersonas:

Ja Jums rodas jautājumi par šo pētījumu, lūdzu, sazināties ar

Olga Ļubina, e-pasts: ljubina.olga@gmail.com, mob. tālr. +371 26077812

**Pacienta piekrišana dalībai pētījumā**

1. Esmu saņēmis/usi un iepazinusies ar rakstisku informāciju par pētījumu "Fenilketonūrijas bērnu vecuma pacientu dietoterapijas novērtējums un tās ietekme uz pacienta veselību". Uz visiem maniem jautājumiem esmu saņēmis/usi atbildes. Bija pietiekami daudz laika, lai pārdomātu savu lēmumu atļaut bioloģisko materiālu izpētei.
2. Piekrītu bez atlīdzības nodot no bioloģiskā materiāla paraugu – ģenētiskiem pētījumiem. Es saprotu, ka materiāla paraugu nodošana nerada risku manai/mana bērna veselībai, kas man ir izskaidrots.
3. Es saprotu, ka jebkura mani/manu bērnu identificējoša informācija būs konfidenciāla, un ka visi paraugi būs kodēti. Es apzinos, ka es jebkurā brīdī bez paskaidrojumiem varu pārtraukt piedalīšanos pētījumā, zinot, ka tas neietekmēs manu/mana bērna turpmāko ārstēšanos. Zinu, ka šādā gadījumā bioloģiskais materiāls un jebkura identificējoša informācija tiks iznīcināta.

Veselības stāvokļa apraksti:

□ Es ATĻAUJU medicīniskās dokumentācijas apraksta papildināšanu, atjaunošanu vai pārbaudi.

□ Es AIZLIEDZU medicīniskās dokumentācijas apraksta papildināšanu, atjaunošanu vai pārbaudi.

□ Es ATĻAUJU izmantot medicīniskās dokumentācijas attēlus, fotogrāfijas zinātniskiem mērķiem, ievērojot Eiropas Parlamenta un Padomes regulas (ES) 2016/679 par fizisko personu aizsardzību attiecībā uz personas datu apstrādi un šādu datu brīvu apriti.

□ Es AIZLIEDZU izmantot medicīniskās dokumentācijas attēlus, fotogrāfijas zinātniskiem mērķiem.

Gadījumā, ja bioloģiskā materiāla izpētē tiks atklāta informācija par kādu man līdz šim nezināmu apdraudējumu mana/mana bērna veselībai (vajadzīgo atzīmēt):

□ Es PIEKRĪTU, ka man tiek paziņota šī informācija;

□ Es PIEKRĪTU, ka man tiek paziņota šāda informācija tikai tādā gadījumā, ja risks veselībai ir novēršams;

□ Es NEVĒLOS saņemt nekādu papildus informāciju.

Bioloģiskā materiāla uzglabāšana:

□ Es PIEKRĪTU, ka bioloģiskais materiāls tiks uzglabāts un izmantots citos pētījumos pēc pētnieku ieskatiem bez ierobežojuma

□ Es PIEKRĪTU, ka bioloģiskais materiāls tiks uzglabāts

□ Es PIEKRĪTU, ka bioloģiskais materiāls tiek izmantots tikai šajā pētījumā un pēc tam ir jāiznīcina

**Pacients/ viņa pilnvarotā persona**

_____
Vārds, uzvārds (drukātiem burtiem)

_____
Personas kods

_____
Kontaktinformācija

_____
Datums, paraksts

**Pētnieks**

_____
Vārds, uzvārds (drukātiem burtiem)

_____
Personas kods

_____
Datums, paraksts

**Questionnaire about knowledge of PKU and dietary treatment**

**Questionnaire for Caregivers of Patients with PKU**

Dear respondent,

You are invited to participate in a survey to assess your knowledge about phenylketonuria and the specific aspects of its dietary treatment. The aim of this questionnaire is to evaluate both strong and improvable areas of knowledge so that the PKU care team can provide more effective support to patients and families in everyday disease management.

Completing the questionnaire takes approximately 10 minutes.

If your family has a child with PKU younger than 12 years, we ask that one main caregiver complete the questionnaire.

All responses will be processed anonymously and used only in an aggregated form.

1. Please select:

□ Male

□ Female

□ Prefer not to say

2. Your relationship to the PKU patient:

□ Mother

□ Father

□ Other _____

3. Are responsibilities of managing the PKU diet shared equally in your family?

□ Yes

□ No (please indicate the main caregiver) _____

4. How many children under 18 live in your household? _____

   Your's child's age (years): _____

5. Which educational institution does your child attend?

□ Preschool / kindergarten

□ General education school

□ Special education school

□ Distance learning

□ Homeschooling

6. Place of residence:

□ Riga

□ Riga suburb

□ State city

□ Other _____

7. How many grams of protein is your child allowed to consume per day? _____
8. How many grams of protein does your child consume per day? _____
9. How often should your child take the amino acid mixture (special formula)? _____
10. How many times per day does your child take the amino acid mixture? _____

11. How often does your child forget to drink the amino acid mixture?
□ Several times per day
□ Once per day
□ 2–4 times per week
□ Once per week
□ Once every two weeks
□ Never
□ My child does not drink the amino acid mixture
12. What is the allowed blood phenylalanine level for your child?
□ < 120 µmol/L
□ 120–360 µmol/L
□ 120–600 µmol/L
□ < 600 µmol/L
□ < 1000 µmol/L
□ I do not know
13. How often does your child perform a phenylalanine blood test? _____
14. What is phenylalanine?
□ An amino acid that forms proteins
□ A monosaccharide that forms carbohydrates
□ A fatty acid that forms lipids
□ I do not know
15. Does fasting increase phenylalanine levels?
□ Yes
□ No
□ I do not know
16. Does illness increase phenylalanine levels?
□ Yes
□ No
□ I do not know
17. Does increased physical activity increase phenylalanine levels?
□ Yes
□ No
□ I do not know
18. Does irregular use of the amino acid mixture increase phenylalanine levels?
□ Yes
□ No
□ I do not know
19. Is your child allowed to take medications in capsule form?
□ Yes
□ No
□ I do not know

20. Is your child allowed to consume the sweetener sorbitol?

□ Yes

□ No

□ I do not know

21. How many grams of baked potatoes contain 1 gram of protein?

□ 30 g

□ 45 g

□ 55 g

□ 80 g

□ I do not know

22. How many grams of sour cream contain 1 gram of protein?

□ 10 g

□ 30 g

□ 50 g

□ 60 g

□ I do not know

23. If the child forgets to take the amino acid mixture, can they take a double portion next time?

□ Yes

□ No

□ I do not know

24. During illness, should the child's protein intake be reduced?

□ Yes

□ No

□ I do not know

25. Do you have any questions about PKU?

□ Yes, there are things I do not know / feel uncertain about

□ No, I know everything

**Questionnaire for Patients with PKU**

Dear PKU patient,

You are invited to participate in a survey to assess your knowledge about phenylketonuria (and the specific aspects of its dietary treatment. The aim of this questionnaire is to evaluate both strong and improvable areas of knowledge so that the PKU care team can provide more effective support to patients and families in everyday disease management.

Completing the questionnaire takes approximately 10 minutes.

This survey is intended for PKU patients aged 12 years and older.

All responses will be processed anonymously and used only in aggregated form.

1. Please select:

□ Male

□ Female

□ Prefer not to say

2. Age: _____

3. How many children under 18 currently live in your household? _____

4. Where do you live?

□ Riga

□ Riga suburb

□ State city

□ Other _____

5. How many grams of protein are you allowed to consume per day? _____

6. How many grams of protein do you actually consume per day? _____

7. How often should you take the amino acid mixture (special formula)? _____

8. How many times per day do you take the amino acid mixture? _____

9. How often do you forget to drink the amino acid mixture?

□ Several times per day

□ Once per day

□ 2–4 times per week

□ Once per week

□ Once every two weeks

□ Never

□ I do not drink the amino acid mixture

10. What is your allowed phenylalanine level in blood?

□ < 120 µmol/L

□ 120–360 µmol/L

□ 120–600 µmol/L

□ < 600 µmol/L

□ < 1000 µmol/L

□ I do not know

11. How often do you perform a phenylalanine blood test? _____

12. What is phenylalanine?
□ An amino acid that forms proteins
□ A monosaccharide that forms carbohydrates
□ A fatty acid that forms lipids
□ I do not know
13. Does fasting increase phenylalanine levels?
□ Yes
□ No
□ I do not know
14. Does illness increase phenylalanine levels?
□ Yes
□ No
□ I do not know
15. Does increased physical activity increase phenylalanine levels?
□ Yes
□ No
□ I do not know
16. Does irregular use of the amino acid mixture increase phenylalanine levels?
□ Yes
□ No
□ I do not know
17. Are you allowed to take medications in capsule form?
□ Yes
□ No
□ I do not know
18. Are you allowed to consume the sweetener sorbitol?
□ Yes
□ No
□ I do not know
19. How many grams of baked potatoes contain 1 gram of protein?
□ 30 g
□ 45 g
□ 55 g
□ 80 g
□ I do not know
20. How many grams of sour cream contain 1 gram of protein?
□ 10 g
□ 30 g
□ 50 g
□ 60 g
□ I do not know

21. If you forget to take the amino acid mixture, can you take a double portion next time?

□ Yes

□ No

□ I do not know

22. During illness, should protein intake be reduced?

□ Yes

□ No

□ I do not know

23. Do you have any questions about PKU?

□ Yes, there are things I do not know / feel uncertain about

□ No, I know everything

**Nutrient intake based on FD in PKU patients and the control group compared with suggested values from Ministry of the Health of Republic of Latvia**

| Age group | Group | Total energy intake (kcal/day) | Total protein intake (g/day) | Total CHO intake (g/day) | Total fat intake (g/day) |
|---|---|---|---|---|---|
| | | median ±SD | median ±SD | median ±SD | median ±SD |
| 1–3 years (n = 5) | PKU | 1236 ± 403 | 42 ± 12 | 210 ± 40 | 27 ± 16 |
| | Control group | 973 ± 206 | 44 ± 8 | 122 ± 49 | 40 ± 4 |
| | MHRL | 960–1290 | 24–48 | 108–194 | 32–57 |
| | p-value * | 0.738 | 0.738 | 0.738 | 0.004 |
| 4–6 years (n = 11) | PKU | 1142 ± 371 | 41 ± 14 | 191 ± 90 | 29 ± 14 |
| | Control group | 1461 ± 276 | 55 ± 11 | 184 ± 48 | 49 ± 7 |
| | MHRL | 1200–1433 | 30–54 | 135–215 | 40–56 |
| | p-value * | 0.705 | 0.964 | 0.923 | 0.063 |
| 7–12 years (n = 8) | PKU | 1963 ± 541 | 60 ± 29 | 260 ± 83 | 55 ± 43 |
| | Control group | 1870 ± 241 | 75 ± 61 | 232 ± 79 | 66 ± 7 |
| | MHRL | 1400–1830 | 35–92 | 158–275 | 47–71 |
| | p-value * | 0.175 | 0.657 | 0.238 | 0.657 |
| 13–18 years (n = 7) | PKU | 1682 ± 394 | 64 ± 17 | 213 ± 93 | 48 ± 17 |
| | Control group | 2051 ± 565 | 59 ± 16 | 229 ± 68 | 76 ± 39 |
| | MHRL | 1920–2283 | 48–114 | 216–342 | 64–89 |
| | p-value * | 0.462 | 0.269 | 0.086 | 0.217 |

PKU – phenylketonuria, SD – standard deviation, BMI – body mass index, MHRL – Ministry of Health of the Republic of Latvia, * p-value – calculated using Fisher's Exact Test one-tailed p.

**Nutrient share in energy intake in analysed case control group**

| Age group | Group | Fat share in energy intake | vs MHRL | Protein equivalent share in energy intake | vs MHRL | CHO share in energy intake | vs MHRL |
|---|---|---|---|---|---|---|---|
| 1–3 years | PKU, % | 20 | −50 | 13 | −10 | 68 | 13 |
| | Control group, % | 37 | −7 | 18 | 20 | 50 | −16 |
| | *p-value** | *0.012* | | *0.125* | | *0.004* | |
| 4–6 years | PKU, % | 23 | −35 | 14 | −4 | 67 | 12 |
| | Control group, % | 30 | −14 | 15 | 0 | 50 | −16 |
| | *p-value** | *0.026* | | *0.019* | | *0.213* | |
| 7–12 years | PKU, % | 25 | −28 | 12 | −39 | 53 | −12 |
| | Control group, % | 32 | −10 | 16 | −20 | 50 | −17 |
| | *p-value** | *0.323* | | *0.124* | | *0.078* | |
| 13–18 years | PKU, % | 26 | −26 | 15 | −24 | 51 | −16 |
| | Control group, % | 33 | −4 | 12 | −42 | 45 | −26 |
| | *p-value** | *0.026* | | *0.002* | | *0.363* | |

PKU – phenylketonuria, MHRL – Ministry of the Health of the Republic of Latvia, CHO – carbohydrate, * p-value – calculated using Fisher's Exact Test one-tailed p-value comparing each group with MHRL suggested values.

**Comparasion of FA concentration in individuals with low fat intake**

| FA (%) | Fatty acid concentration in individuals with low fat intake in the diet | | |
|---|---|---|---|
| | PKU | Control | p-value |
| Myristic acid (0.16–0.4) | 0.34 | 0.29 | 0.16 |
| Palmitic acid (21.16–23.35) | 21.92 | 20.60 | **0.01** |
| Stearic acid (13.43–17.17) | 17.02 | 16.95 | 0.48 |
| Palmitoleic acid (0.15–0.49) | 0.19 | 0.15 | 0.17 |
| Cis-vaccenic acid (0.67–0.96) | 0.87 | 0.76 | 0.07 |
| Oleic acid (12–14.7) | 12.39 | 11.44 | 0.20 |
| Eicosatrienoic acid (1.51–1.87) | 1.52 | 1.99 | **0.002** |
| Trans-vaccenic acid (0–7) | 0.09 | 0.12 | 0.11 |
| Trans-palmitoleic acid (0.07–0.13) | 0.07 | 0.12 | **0.03** |
| Rumenic acid (0.05–0.12) | 0.06 | 0.09 | 0.17 |
| Elaidic acid (0–0.07) | 0.09 | 0.04 | **1.92e-4** |
| CLA2 C18:2 (0.01–0.05) | 0.03 | 0.03 | 0.38 |
| CLA3 C18:2 (0.01–0.06) | 0.04 | 0.04 | 0.33 |
| Linolenic acid (8.49–11.15) | 8.60 | 9.47 | 0.19 |
| Gamma-linolenic acid (0.04–0.09) | 0.07 | 0.03 | **0.01** |
| Dihomogammalinolenic acid (1.28–2.2) | 1.56 | 1.43 | 0.14 |
| Arachidonic acid (11–13.44) | 13.62 | 15.02 | **0.05** |
| Total omega-6 (21.9–25.4) | 23.43 | 25.94 | **0.03** |
| Alpha-linolenic acid (0.14–0.29) | 0.12 | 0.13 | 0.39 |
| EPA (0.75–2.34) | 0.33 | 0.43 | 0.16 |
| DHA (5.27–8.87) | 3.46 | 3.75 | 0.44 |
| Total omega-3 (5–8.36) | 3.88 | 4.31 | 0.41 |
| Index Omega3 (7.5–10) | 3.85 | 4.18 | 0.48 |
| Omega6/Omega3 (1–4) | 6.14 | 6.12 | 0.49 |
| Ara/EPA (5–10) | 42.21 | 36.36 | 0.24 |
| LN/DGLA (0–4.4) | 5.19 | 6.64 | 0.09 |

FA% – fatty acid concentration, PKU – Phenylketonuria, CLA2 – conjugated linoleic acid-2 , CLA3 – conjugated linoleic acid-3, Ara – Arachidonic acid, EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, Ara/EPA – ratio of arachidonic acid to eicosapentaenoic acid, LN/DGLA – ratio of linoleic acid to dihomo-γ-linolenic acid, p-value calculated by Mann-Whitney U Test, statisticaly significant if $p < 0.05$

**Amino acid content in foods**

| Foods | Phe | Tyr | Gln | Leu | Lys | Met | Pro | Ser | Thr | Asp | Arg | Ala | Cys | Gly | His | Ile | Val |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aquafaba | 57 | 43 | 350 | 73 | 140 | 21 | 48 | 71 | 59 | 120 | 160 | 73 | 23 | 73 | 46 | 36 | 49 |
| Arugula, fresh | 110 | 40 | 280 | 140 | 100 | 5 | 75 | 77 | 80 | 110 | 110 | 100 | 21 | 93 | 36 | 71 | 100 |
| Boletus edulis, frozen, tempered, cooked | 52 | 51 | 170 | 100 | 68 | 35 | 69 | 85 | 72 | 120 | 80 | 180 | 17 | 100 | 46 | 56 | 73 |
| Broccoli microgreens, fresh | 97 | 42 | 180 | 150 | 74 | 42 | 86 | 51 | 63 | 81 | 57 | 114 | 12 | 110 | 48 | 90 | 120 |
| Celery root, boiled | 28 | 29 | 150 | 56 | 44 | 16 | 36 | 34 | 26 | 120 | 32 | 37 | 13 | 35 | 16 | 38 | 42 |
| Celery stalk, fresh | 21 | 16 | 46 | 24 | 18 | 5 | 15 | 17 | 14 | 52 | 16 | 18 | 5 | 19 | 6 | 18 | 17 |
| Champignon mushroom brown, cooked | 150 | 110 | 590 | 250 | 200 | 60 | 140 | 170 | 170 | 280 | 180 | 250 | 24 | 170 | 100 | 140 | 180 |
| Champignon mushroom white, cooked | 100 | 70 | 420 | 160 | 130 | 36 | 94 | 110 | 120 | 160 | 100 | 170 | 14 | 110 | 68 | 90 | 110 |
| Chanterelle, frozen, tempered, cooked | 56 | 46 | 160 | 100 | 57 | 13 | 63 | 76 | 62 | 93 | 74 | 78 | 5 | 65 | 36 | 57 | 65 |
| Coriander, fresh | 110 | 70 | 200 | 150 | 100 | 29 | 82 | 110 | 88 | 350 | 75 | 110 | 25 | 100 | 41 | 91 | 120 |
| Garden cress microgreens, fresh | 150 | 110 | 490 | 200 | 180 | 57 | 120 | 120 | 140 | 200 | 170 | 170 | 18 | 140 | 90 | 130 | 170 |
| Leeks, fresh | 37 | 30 | 160 | 68 | 63 | 18 | 39 | 45 | 35 | 79 | 44 | 54 | 13 | 46 | 15 | 36 | 45 |
| Nettle, frozen | 210 | 150 | 380 | 350 | 260 | 91 | 190 | 180 | 190 | 150 | 250 | 270 | 36 | 230 | 79 | 180 | 240 |
| Peas microgreens, fresh | 200 | 68 | 340 | 280 | 240 | 67 | 160 | 110 | 150 | 820 | 130 | 220 | 130 | 280 | 110 | 210 | 230 |
| Portobello mushroom, cooked | 120 | 90 | 580 | 200 | 150 | 43 | 120 | 150 | 150 | 240 | 130 | 220 | 16 | 150 | 90 | 110 | 140 |
| Radish microgreens, fresh | 100 | 80 | 520 | 140 | 130 | 44 | 91 | 100 | 100 | 170 | 140 | 120 | 31 | 100 | 78 | 87 | 120 |
| Raisins, black | 69 | 27 | 210 | 100 | 81 | 28 | 280 | 74 | 51 | 83 | 450 | 110 | 11 | 97 | 70 | 65 | 93 |
| Raisins, golden | 86 | 31 | 290 | 110 | 100 | 27 | 350 | 100 | 76 | 120 | 520 | 130 | 30 | 95 | 78 | 67 | 93 |
| Raisins, Jambo) | 42 | 15 | 100 | 67 | 59 | 16 | 540 | 51 | 40 | 47 | 80 | 48 | 8 | 61 | 31 | 44 | 57 |
| Rhubarb, frozen | 9 | 9 | 37 | 23 | 18 | 5 | 13 | 15 | 11 | 23 | 15 | 17 | 5 | 16 | 7 | 15 | 16 |
| Russula emetica, boiled, frozen | 81 | 63 | 190 | 150 | 91 | 30 | 80 | 90 | 73 | 130 | 100 | 120 | 15 | 90 | 49 | 86 | 100 |
| Shiitake mushroom, boiled | 64 | 50 | 240 | 110 | 82 | 24 | 63 | 93 | 69 | 100 | 76 | 86 | 6 | 74 | 46 | 59 | 68 |

| Foods | Phe | Tyr | Gln | Leu | Lys | Met | Pro | Ser | Thr | Asp | Arg | Ala | Cys | Gly | His | Ile | Val |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sorrel, boiled, frozen | 120 | 90 | 310 | 190 | 150 | 50 | 110 | 100 | 100 | 180 | 140 | 130 | 20 | 120 | 60 | 100 | 130 |
| Sorrel, fresh, frozen | 120 | 90 | 430 | 190 | 150 | 50 | 110 | 120 | 120 | 200 | 150 | 140 | 20 | 130 | 60 | 110 | 150 |
| Spring onions, fresh | 71 | 53 | 330 | 120 | 110 | 33 | 75 | 92 | 67 | 310 | 100 | 110 | 32 | 84 | 29 | 66 | 80 |
| Sugar snap peas, fresh | 63 | 33 | 180 | 86 | 94 | 23 | 57 | 150 | 71 | 340 | 110 | 120 | 21 | 220 | 31 | 83 | 92 |
| Sunflower microgreens, fresh | 140 | 99 | 450 | 240 | 150 | 62 | 150 | 140 | 140 | 230 | 220 | 190 | 11 | 170 | 80 | 140 | 170 |
| Wild garlic, frozen | 140 | 92 | 400 | 220 | 180 | 55 | 120 | 120 | 110 | 220 | 150 | 170 | 27 | 150 | 52 | 110 | 140 |

Annex 8

**Difference in broth nutritional profiling among different laboratories**

| Product | CHO (g/100 g) | | Fat (g/100 g) | | Protein (g/100 g) | | T1 difference of T2 | Phe (mg/100 g) | | T1 difference of T2 | Energy (kcal/100 g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T1 | T2 | T1 | T2 | % | T1 | T2 | % | T1 | T2 |
| Chicken broth | < 0.8 | 0 | 0.32 | 1.3 | 0.74 | 0.8 | 8 | 9 | 34.7 | 286 | 6 | 15 |
| Pork broth | < 0.8 | 0.2 | < 0.3 | 0.2 | 0.56 | 0.9 | 61 | 13 | 40.7 | 213 | < 5 | 4 |
| Trout broth | < 0.8 | 0.2 | < 0.3 | 0 | 0.46 | 0.3 | −35 | 5 | 31 | 520 | < 5 | 1 |
| Deer (venison) broth | < 0.8 | 0 | < 0.3 | 0.1 | 0.43 | 1.7 | 295 | 28 | 31 | 11 | < 5 | 4 |
| Lamb broth | < 0.8 | 0 | 1.1 | 0 | 0.53 | 0.5 | −6 | 8 | 31 | 288 | 12 | 20 |
| Turkey broth | < 0.8 | 0 | < 0.3 | 0 | 1.01 | 0.7 | −31 | 7 | 39.3 | 461 | < 5 | 3 |

Phe – phenylalanine, T1 – Eurofins Laboratory, T2 – Hamilton laborator.