Olga Ļubina

ORCID 0000-0002-2775-7607

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

Summary of the Doctoral Thesis for obtaining the scientific degree "Doctor of Science (*PhD*)"

Sector Group – Medical and Health Sciences

Sector – Health Sciences

Sub-Sector – Nutrition

Riga, 2026

The Doctoral Thesis was developed at Rīga Stradiņš University, Latvia

Supervisor of the Doctoral Thesis:

MD, PhD, Associate Professor **Madara Auzenbaha**,
Rīga Stradiņš University, Latvia

Scientific Advisor:

MD, PhD **Linda Gailīte**,
Rīga Stradiņš University, Latvia

Official Reviewers:

MD, PhD, Professor **Iveta Dzīvīte-Krišāne**,
Rīga Stradiņš University, Latvia

MD, PhD, Professor **Ingrīda Rumba-Rozenfelde**,
University of Latvia

MD, PhD **Karit Reinson**,
Tartu University Hospital, Estonia

Chair of the Doctoral Committee:

Dr. med., Professor **Ilze Konrāde**,
Rīga Stradiņš University, Latvia

Members of the Doctoral Committee:

Dr. med., Professor **Inga Millere**,
Rīga Stradiņš University, Latvia

Dr. med., Associate Professor **Indra Zeltiņa**,
Rīga Stradiņš University, Latvia

Dr. med., Professor **Iveta Dzīvīte-Krišāne**,
Rīga Stradiņš University, Latvia

Dr. med., Associate Professor **Laila Meija**,
Rīga Stradiņš University, Latvia

Dr. med., Associate Professor **Inese Gobiņa**,
Rīga Stradiņš University, Latvia

Defence of the Doctoral Thesis will take place at the public session of the Promotion Council of Health Sciences on 9 December 2026 at 15.00 in the 2nd Auditorium, 16 Dzirciema iela, Rīga Stradiņš University

The Doctoral Thesis is available in RSU Library and on RSU website:
https://www.rsu.lv/en/dissertations





The research is carried out within the framework of the European Union Recovery and Resilience Mechanism Plan and the state budget funded project "RSU internal and RSU with LASE external consolidation" No. 5.2.1.1.i.0/2/24/I/CFLA/005, implemented under Doctoral Grant.

Table of Contents

Abbreviations used in the Thesis 5

Introduction 6
- Aim of the Thesis 7
- Objectives of the Thesis 7
- Hypotheses of the Thesis 7
- Novelty of the Thesis 8

Conclusions 9

Proposals 10

List of Publications on the Topic of the Thesis 11

References 12

Acknowledgments 13

Abbreviations used in the Thesis

PKU	phenylketonuria
Phe	phenylalanine

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.

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.

List of Publications on the Topic of the 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

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, 4th CEE Metabolic Roadshow Conference. 2025, May 7–9, Vilnius, Lithuania

References

1. Araújo, A. C. M. F., Araújo, W. M. C., Marquez, U. M. L., Akutsu, R., & Nakano, E. Y. (2017). Table of phenylalanine content of foods: Comparative analysis of data compiled in food composition tables. In *JIMD Reports* (Vol. 34, pp. 87-96). Springer. https://doi.org/10.1007/8904_2016_12

2. Blau, N., van Spronsen, F. J., & Levy, H. L. (2010). *Phenylketonuria* (Lancet, Issue. www.thelancet.com

3. Kaloteraki, E., Pardali, E. C., Poulimeneas, D., Mylona, V., Fotiadou, E., Papa, K., Gioxari, A., Spilioti, M., Bogdanos, D. P., & Grammatikopoulou, M. G. (2025). Nutritional Practices and Knowledge of Patients with Phenylketonuria. *Nutrients*, *17*(21). https://doi.org/10.3390/nu17213351

4. MacDonald, A., van Wegberg, A. M. J., Ahring, K., Beblo, S., Bélanger-Quintana, A., Burlina, A., Campistol, J., Coşkun, T., Feillet, F., Giżewska, M., Huijbregts, S. C., Leuzzi, V., Maillot, F., Muntau, A. C., Rocha, J. C., Romani, C., Trefz, F., & van Spronsen, F. J. (2020). PKU dietary handbook to accompany PKU guidelines. *Orphanet Journal of Rare Diseases*, *15*(1), 171-171. https://doi.org/10.1186/s13023-020-01391-y

5. Okano, Y., Hattori, T., Fujimoto, H., Noi, K., Okamoto, M., Watanabe, T., Watanabe, R., Fujii, R., & Tamaoki, T. (2016). Nutritional status of patients with phenylketonuria in Japan. *Molecular Genetics and Metabolism Reports*, *8*, 103-110. https://doi.org/10.1016/j.ymgmr.2016.08.005

6. van Wegberg, A. M. J., MacDonald, A., Ahring, K., Aitkenhead, L., Bélanger-Quintana, A., Beblo, S., Blau, N., Bosch, A. M., Burlina, A., Campistol, J., Coşkun, T., Feillet, F., Giżewska, M., Huijbregts, S. C., Leuzzi, V., Maillot, F., Muntau, A. C., Rocha, J. C., Romani, C.,…van Spronsen, F. J. (2025). European guidelines on diagnosis and treatment of phenylketonuria: First revision. *Molecular Genetics and Metabolism*, 109125-109125. https://doi.org/10.1016/J.YMGME.2025.109125

7. Witalis, E., Mikoluc, B., Motkowski, R., Sawicka-Powierza, J., Chrobot, A., Didycz, B., Lange, A., Mozrzymas, R., Milanowski, A., Nowacka, M., Piotrowska-Depta, M., Romanowska, H., Starostecka, E., Wierzba, J., Skorniewska, M., Wojcicka-Bartlomiejczyk, B. I., Gizewska, M., Car, H., & Polish Society of, P. (2017). Phenylketonuria patients' and their parents' knowledge and attitudes to the daily diet - multi-centre study. *Nutr Metab (Lond)*, *14*, 57. https://doi.org/10.1186/s12986-017-0207-1

8. Yagudina, R., Kulikov, A., Serpik, V., Protsenko, M., & Kopeyka, K. (2024). Factors Affecting Adherence to a Low Phenylalanine Diet in Patients with Phenylketonuria: A Systematic Review. *Nutrients*, *16*(18), 3119-3119. https://doi.org/10.3390/NU16183119/S1

Acknowledgments

First of all, I would like to express my profound gratitude to my doctoral supervisor, Professor **Madara Auzenbaha**, whose expertise, invaluable advice, and continuous support have guided me throughout this work. Your encouragement often exceeded my own self-belief, and for that I am deeply thankful. I am also sincerely grateful to Dr. **Linda Gailīte**, whose thoughtful consultations and assistance during every stage of this journey were of great importance.

I extend my heartfelt appreciation to all my colleagues at the Rare Disease Coordination Centre at the Children's Clinical University Hospital for their help and encouragement. In particular, I wish to acknowledge my colleague, **Alīna Kirillova**, whose dedication in assuming the primary responsibility for patient care allowed me to focus on completing this dissertation.

Above all, I owe my deepest thanks to my family: my husband **Aleksandrs** and daughter **Agata**, my parents **Jeļena** and **Jurijs**, my sister **Marina** and her family, and my parents-in-law **Natālija** and **Vjačeslavs**. Your love, support, strength, and inspiration have been the foundation of this achievement. Without you, none of this would have been possible.

I would also like to express my sincere gratitude to all **PKU patients** and their **caregivers** who agreed to participate in this study. Your willingness to participate, the time you devoted, and the answers you provided were highly valuable for the completion of this research. Thank you for your trust and contribution, which helped to improve the understanding of phenylketonuria care and the importance of knowledge in achieving metabolic control.