Phenylketonuria in adulthood: exploring long-term consequences

PhD thesis

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Abbreviations

AA = Amino acid

AAS= Amino acid supplement

BBB= Blood brain barrier

BH4 = Tetrahydrobiopterin

BMD= Bone mineral density

BMI = Body Mass Index

DBS = Dried blood spot

DHPR = Dihydropteridine reductase

DNAJC12= DnaJ heat shock protein family (hsp40) member C12

DXA = Dual-energy X-ray absorptiometry

ETPKU = Early-treated PKU

FDA = Food and Drug administration

GMP = Glycomacropeptide

HDL = High-density lipoprotein

HPA = Hyperphenylalaninaemia

HPLC = High performance liquid chromatography

HRQoL = Health-related quality of life

IEM = Inborn error of metabolism

IQ = Intelligence quotient

IQR = Interquartile range

ISCD = International Society for Clinical Densitometry

LAT1 = L-type amino acid transporter 1

LDL = Low-density lipoprotein

LNAA = Large neutral amino acid

MF-BIA = Multifrequency bioelectrical impedance analysis

MPKUS = Maternal PKU syndrome

MSUD = maple syrup urine disease

NBS = newborn screening

PAH = Phenylalanine hydroxylase

PBF = Percent body fat

PET = Positron emission tomography

Phe = Phenylalanine

PKU = Phenylketonuria

PLP = Pyridoxal phosphate

PTPS = 6-pyruvoyl tetrahydropterin synthase

QoL = Quality of life

TH = Tyrosine hydroxylase

TpH = Tryptophan hydroxylase

Trp = Tryptophan

Tyr = Tyrosine

WHO = World Health Organization

WHR = Waist to hip ratio

1. Introduction

1.1. Inborn errors of metabolism

Genetic disorders of intermediary metabolism are called inborn error of metabolism (IEM) (1). The term IEM was first used in the first decade of the 20th century by the English physician Archibald Edward Garrod to group genetically inherited diseases in which there is a blockade of metabolic pathways (2). Thanks to more precise diagnostic methods, more than 1000 IEMs have been described so far. Single gene defects of proteins (enzymes, transporters, etc.) are the most frequent causative factors. Although the clinical picture is highly variable, these alterations mostly lead to accumulation of toxic substances or deficiencies of much needed end-products. Based on the pathophysiological classification of Saudubray et al. IEMs are grouped into defects in the metabolic pathway of complex molecules, diseases with features of intoxication and illnesses causing energy deficiency (3). Core characteristics of these illness-groups are shown in Table 1.

Table 1: Classification of inborn errors of metabolism based on the concept of Saudubray et al (3).

	Disorders involving energy metabolism	Disorders involving complex molecules	Disorders which give rise to intoxication
Core Pathomechanism	Energy production and utilization is damaged in different tissues.	Synthesis, trafficking and metabolism of complex molecules are disturbed in cellular organelles.	A metabolic block leads to intoxication due to accumulation of intermediary products.
Characteristics	Energy defects are of mitochondrial or cytoplasmic origin. Mitochondrial defects are more severe and may also affect embryo-fetal development and lead to malformations.	Symptoms are progressive, mostly permanent and unrelated to food intake.	A symptom-free interval and intermittent clinical expression is typical. Treatment is mostly available and emergency toxin removal is of utmost importance.
Common symptoms	Common symptoms include hypoglycemia, hyperlactatemia, hepatomegaly, severe generalized hypotonia, myopathy, failure to thrive, cardiac failure.	Multiple organs are affected: bone dysplasia, hepatomegaly, neurological consequences, cardiomyopathy.	Acute events can present with disturbed consciousness, liver failure, thromboembolic features. Long-term consequences are developmental delay, cardiac symptoms, etc.
Examples	Congenital lactic acidemias Mitochondrial respiratory chain disorders Fatty acid oxidation and ketone body defects.	Lysosomal storage disorders Peroxisomal disorders Congenital disorders of glycosylation	Inborn errors of amino acid catabolism Organic acidurias Congenital urea cycle defects

Although IEMs are considered to be rare one by one, cumulative prevalence is estimated to be around 1:1.000 (4). Main goal of treatment is to reach metabolic stability and to minimise complications. Therapeutic options range from dietary therapy with medical food supplements to pharmaceutical therapy. In rare cases liver transplantation may also be curative, whereas gene therapy is hoped to be the definitive solution in the future (5).

Although prognosis of different IEMs can vary widely, thanks to newborn screening (NBS) and widening range of therapeutic options, more and more patient reach adult care presenting novel challenges and long-term complications (6).

The most prevalent IEM is phenylketonuria. This is the most researched IEM and accumulated knowledge of this disease has led to a better understanding of all IEMs. And yet, several important details are still missing regarding its pathomechanism and long-term consequences.

1.2. Phenylketonuria

Phenylketonuria (PKU (OMIM # 261600)) is caused by pathogenic variants in the gene of the enzyme phenylalanine hydroxylase (PAH, EC 1.14.16.1). PAH is predominantly synthetised in the liver and converts Phe to Tyr in a reaction which involves tetrahydrobiopterin (BH4) as a co-factor. In PKU, hyperphenylalaninaemia (HPA) is seen as a consequence: blood Phe concentration is markedly higher than 120 μmol/l, which is the upper threshold of the normal range in healthy individuals. Phenylketone bodies are formed and get excreted with urine whereas blood Tyr levels are suboptimal. Untreated patients are intellectually disabled, experience seizures and have neuropsychiatric and executive symptoms. Light pigmentation of skin and body hair, skin rash and a musty odor is part of the clinical picture (7).

Neurocognitive outcome is closely related to blood Phe levels, so treatment primarily aims to decrease blood Phe levels. The disease was first described in 1934 by Følling (8), whereas the significance of dietary therapy was first reported in 1953 (9). The simple and cheap diagnostic method to detect HPA developed by Guthrie in the 1960's was a crucial revelation, which enabled newborn screening and prevention of mental retardation in this patient group (10).

Other causes of Hyperphenylalaninaemia: The widely used screening method detects HPA defined as blood Phe >120 μmol/L (6). Although 98% of HPA is caused by PKU, it needs to be distinguished from other causes like disorders of pterin metabolism, high protein intake or even liver disease (11). Specific patterns of excreted pterins enable diagnosis of most BH4 deficiencies whereas Dihydropteridine reductase (DHPR) activity is assessed using dried blood spot (DBS) analysis (12, 13). BH4 deficiencies were

historically called malignant HPA and treatment differs from PKU in many ways (14). The extent of HPA-related BH4 deficiency is variable but amine deficiency is generally more severe than in PKU (14), the most prevalent diseases are 6-pyruvoyl tetrahydropterin synthase (PTPS) deficiency and DHPR deficiency. Prognosis without treatment is poor, because of the progression of neurological symptoms leading to reduced life expectancy. Common symptoms include abnormal muscle tone, movement difficulties, seizures, lethargy, developmental delay (14).

1.2.1. Symptoms of untreated PKU

Antenatal development is not influenced by PKU of the fetus, infants are born phenotypically normal (15). Although PAH is mainly active in the liver and in the kidneys, PAH deficiency does not cause direct damage to these organs (16). Most severe consequences are on the developmental processes of the central nervous system: reduced dendrite arborisation and impaired myelination lead to reduced number of synapses (17). If untreated, PKU causes progressive neurological and psychiatrical disability: among others microcephaly, moderate to profound intellectual retardation (IQ<50), autism spectrum disorders, inadequate behavior, cortical blindness and seizures. Additional non-neurological symptoms include: lighter pigmentation (of skin, hair and iris), eczema and a characteristic „moldy" odor (3, 7). Growing number of studies show that in patients who receive treatment from birth, long-term therapy inadherence in later age can lead to muscle spasticity, cerebellar ataxia, tremors, and disturbed vision (18-20).

Thanks to NBS, therapy is usually initiated in the first weeks of life and irreversible complications are preventable. These so called „early-treated patients with PKU" (ETPKU) can live independently as adults and reach expected educational standards (6). Although PKU is considered to be a medical success, behavioral issues, neuropsychological symptoms occur in some, and patients in general have a mean neurocognitive level below siblings or peers of the general population (7, 21). Additionally, being adherent to lifelong therapy is burdensome in practice, especially in adolescence and adulthood (22).

1.2.2. Pathomechanism

1.2.2.1. Disturbed function of PAH

PAH is an iron-containing monooxygenase enzyme that converts Phe to Tyr (23) (see Figure 1). Blockade of this conversion leads to HPA, moderate Tyr deficiency and transamination of Phe to phenylketones which are then excreted in the urine (hence the name Phenylketonuria). It is logical to presume that these 3 biochemical alterations may be the primary pathoetiological factors in PKU.

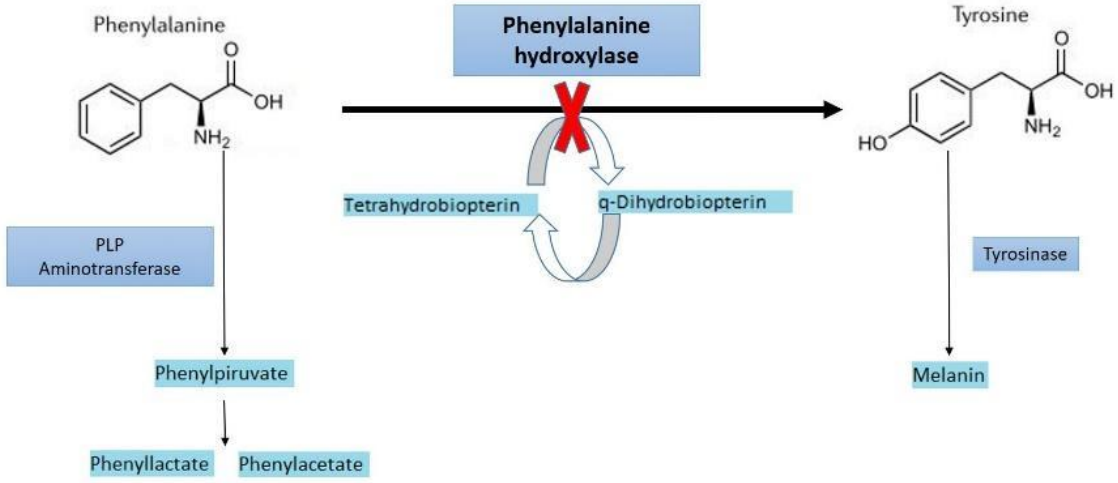

Figure 1: Phenylalanine metabolism in Phenylketonuria. *PAH catalyses the transformation of Phe to Tyr with ferrous iron, molecular oxygen and reduced tetrahydrobiopterin needed as cofactors. This pathway metabolises ca 90% of daily Phe intake (the remaining 10% is used in protein turnover). PAH activity is deficient in patients with PKU, which leads to HPA. At the same time Tyr levels are only mildly decreased because it can be substituted with dietary intake. In patients with PAH deficiency Phe follows an alternative metabolic pathway with deamination and forms phenylketones that are excreted in urine (24).*

Hypotyrosinaemia: Tyrosine is the precursor of several essential biologically active substances as neurotransmitters (dopamine, adrenaline, norepinephrine), melanin, thyroxine and can be utilised in complete catabolism as a source of energy. Although blood tyrosine concentrations are lower in PKU patients than in controls, usually are not below the lower-threshold of normal range. Additionally, the fact that tyrosine supplementation alone does not prevent severe consequences in patients prove that Tyr deficiency has a minor role in pathogenesis of PKU (25).

Accumulation of phenylketone bodies: These organic acids are rapidly excreted through the kidneys and studies suggest that tissue concentrations are not high enough to cause relevant damage even in patients off treatment (26).

Hyperphenylalaninaemia: The empirical hypothesis that restriction of Phe intake prevents major sequelae of PKU was confirmed in a study in 1960 (27). Now it is widely accepted that Phe itself is the main neurotoxin in Phenylketonuria (28).

1.2.2.2. Mechanisms of brain dysfunction

Although the precise cascade of brain damage is still incompletely known in PKU, several hypotheses are supported by evidence (29):

White matter disruption which is mostly reversible after restarting dietary therapy is seen in patients with long-term hyperphenylalaninaemia (30). It seems that high Phe levels influence the synthesis of lipids, alter dendritic connectivity (31) and may even change the characteristics of oligodendrocytes to non-myelinating in the central nervous system (32).

Both animal studies and in vivo PET studies on PKU patients showed that high Phe levels lead to reduced cerebral glucose metabolism in the frontal lobe (33, 34). It is assumed that Phe inhibits enzymes of glycolysis and oxidative phosphorylation (35).

Large neutral amino acids (LNAA-s) - as leucine, isoleucine, tryptophan, valine, phenylalanine, tyrosine - primarily move across the blood brain barrier through the L-type amino acid transport protein 1 (LAT1) (36). The transporter has a high affinity for large neutral amino acids, hence it is normally constantly saturated. High Phe levels competitively inhibit the flux of other LNAAs leading to relatively decreased concentrations in the central nervous system, which has a role in impaired protein synthesis and neurotransmitter deficiencies seen in PKU patients (37). However, crucial details still need to get clarified as there are additional sodium dependent amino acid (AA) transporters on the brain capillary endothelial cell membrane which have high affinity for LNAAs (38).

Some cognitive and behavioral symptoms are thought to be consequences of neurotransmitter deficiencies, primarily of serotonin and norepinephrine (39). Low tyrosine and tryptophan levels of the brain (40), impaired expression of tyrosine

hydroxylase and tryptophan hydroxylase and also direct inhibition of these enzymes caused by high Phe levels may all be responsible for neurotransmitter deficiency (41). Effects on the epigenome through influencing the methylation pattern of certain microRNAs are yet insufficiently identified, but possibly important consequences of hyperphenylalaninemia as well (42). Extreme high concentration of Phe can even aggregate into amyloid-like fibrils and may play a role in the cognitive impairments seen in some patients (43). A summary of these alterations is shown on *Figure 2*.

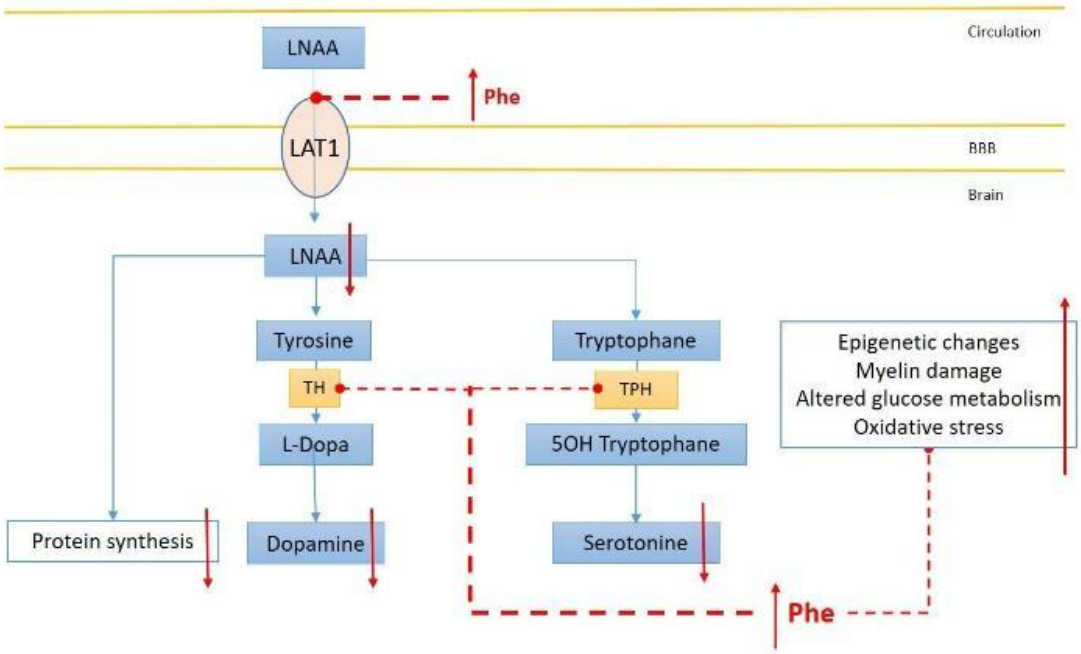

Figure 2: The most severe consequences of hyperphenylalaninaemia are on the central nervous system. *LAT1, a sodium dependent transport protein facilitates the crossing of LNAAs (Phe, Tyr, Trp, etc.) through the blood brain barrier (BBB). High blood Phe level competitively inhibits the transport of other LNAAs through LAT1 whereas high Phe level in the central nervous system competitively inhibits tryptophan hydroxylase (TpH) and tyrosine hydroxylase (TH). Low LNAA concentration in the brain causes impaired protein synthesis, while growing number of studies show that high Phe levels also cause epigenetic changes, impaired myelin synthesis, altered glucose metabolism in the frontal cortex, oxidative stress and aggregation as amyloid plaque – like fibrils (24).*

1.2.3. Classification

Proper definition of PKU phenotypes is a basic need to determine prognosis, treatment strategies and provide optimal patient care. Several classifications were historically used based on Phe concentrations, Phe tolerance, residual PAH activity or genotype (44).

Using pre-treatment blood Phe concentrations for classification was invented in the 1980s and is still widely used in daily practice (45). Four phenotypes are generally classified with this method: Classic PKU (Phe > 1200 μmol/L); moderate PKU (Phe: 900-1200 μmol/L); mild PKU (Phe: 600-900 μmol/L) and mild HPA (Phe <600 μmol/L (46). However with early diagnosis and therapy initiation this method lost accuracy: patients in many cases do not reach peak pretreatment Phe levels (47). Calculating the safe amount of Phe intake individually that does not lead to blood Phe levels over the upper target range (daily Phe tolerance) is a different way for phenotyping PAH deficiency (47). Its major limitation is that Phe toleration is dependent on age, growth rate and concurrent diseases as well (24). A more detailed classification system is based on assessing the clinical course of PKU, this includes both laboratory results (fluctuation of Phe, Phe/Tyr ratios) and symptoms. Due to its complexity, this method is rarely used in practice (44).
Both the European and the American guideline stand for a simplified classification. The Complete European Guideline states, that PAH deficiency should be defined either as „mild HPA" (Phe: 120-360umol/l) where no therapy is necessary and „PKU" (Phe > 360 umol/L) which can be either BH4 responsive and non-responsive (6). The diagnosis and management guideline for Phenylalanine hydroxylase deficiency issued by the American College of Medical Genetics and Genomics suggests an unifying nomenclature as spectrum of PAH deficiency, although accepts to name the most severe form „classical PKU" (48).

1.2.4. Epidemiology

Prevalence of PKU is estimated to be 1:12.000 globally, although some ethnics and geographic regions are more often affected than others. PKU is generally more frequent in Caucasian populations and East Asian countries (49). In Europe the prevalence ranges from 1:2700 newborn in Italy to less than 1 in 100.000 in Finland (50). Turkey is a typical

example that higher prevalence of PKU can be seen than ethnic composition would suggest in countries, where consanguineous marriage is frequent (prevalence 1:4370) (51). The Russian republic of Karachay Cherkessia has the highest prevalence with 1:850 births (52).

Estimated prevalence in Hungary is 1:8500 newborn and carrier frequency is 1:50. Eight to twelve children are diagnosed with PKU in Hungary every year (53).

1.2.5. Genetics

There are 1150 known pathogenic variants of the PAH gene on chromosome 12 according to the ”Humane Gene Mutation Database” (http://www.hgmd.cf.ac.uk). Autosomal recessive inheritance of these mutations lead to disturbed production of the PAH monomers and consequently to impaired function or complete absence of the PAH protein. Less often the cause of functional PAH deficiency is disturbed BH4 metabolism or mutations in the chaperone DnaJ heat shock protein family (hsp40) member C12 (DNAJC12) (54).

58% of PAH variants are missense in-frame mutations (50). Some variants lead to overproduction of hypoactive monomers while others are associated with instability and fast degradation of the monomers (55). Due to the many existing variants and the autosomal recessive inheritance many patients are compound heterozygotes but some variants are more common than others. The R408W mutation (p.Arg408Trp) is the most common in Hungary and in most Eastern European populations (56). This variation is associated with extreme instability of PAH with 0% residual enzyme activity and causes classical PKU (55).

Genotype-phenotype prediction is particularly problematic in PKU (57): inter-allelic complementation may lead to changes in the activity and stability of the resulting tetramer (58). Correlation between pathogenic variants and clinical outcome is weak (14). However, genotyping is useful to suggest (or to rule out) potential BH4 responsiveness (59).

1.2.6. Screening

Obligatory NBS and early therapy initiation fundamentally changed the prognosis of PKU. It was the bacterial inhibition assay invented by R. Guthrie that enabled widespread newborn screening for hyperphenylalaninaemia in 1962 (10). Blood Phe concentrations are normal at birth, but reach toxic levels in the first days of life. Although precise timing is variable worldwide, blood sampling should happen in the 2nd or 3rd day of life (47). For analysis, a drop of blood is collected from the neonates heel using a standardized filter paper(44). If biochemical analysis is positive, confirmatory testing and further investigation is needed to provide precise diagnosis. The diagnosis of IEM has undergone revolutionary progress in recent decades enabling more sophisticated analytical methods than the Guthrie microbiological inhibition test: enzymatic techniques, High performance liquid chromatography (HPLC) and tandem mass spectrometry are widely used (3). In Hungary tandem mass spectrometry is preferred: this method reliably provides quantitative determination of concentration of multiple substances using small samples of blood (60).

As detailed above, genotyping is mainly used to exclude patients with BH4 unresponsive mutations (61). 24 or 48-hour BH4 loading tests may enable diagnosis of BH4 and DNAJC12 deficiency and are used to prove BH4 responsiveness in chosen PKU patients (24). Reassessing BH4 responsiveness may be justified as some patients who are initially unresponsive may still show response in older age (62). Although prenatal diagnosis is possible through chorion villus biopsy and genotyping, it is almost never sought for (14).

1.2.7. Therapy

Impairment in brain development starts from the first days of life: every 4 weeks delay in therapy initiation leads to 4 points decline in IQ score (63). In most PKU patients residual PAH activity cannot be stimulated so reducing Phe intake is crucial to achieve optimal metabolic control. The main aim of PKU treatment is to achieve adequate neurocognitive and somatic development with a subjectively perceived quality of life as normal as possible.

Recent American and European guidelines hold similar opinions regarding most aspects of PKU care, one of the major difference being that the US guideline suggests lower Phe

levels for patients older than 12 years (target Phe: 120-360 μmol/L (6, 48). The European guideline on PKU stands for maintaining Phe levels below 360 μmol/L to prevent severe cognitive sequelae only until patients reach the age of 12 years (64). For older patients controlled longitudinal studies would be much needed to describe the exact relationship between outcomes and increased Phe levels (6). Hence evidence is inconsistent regarding the consequences of Phe concentrations between 360 and 600 μmol/L in adults(65), the European guideline advises lifelong treatment with an upper Phe target value of 600 μmol/l. Regular follow-up is especially crucial in women of childbearing age because of potential maternal PKU syndrome of the fetus. Blood Phe concentrations should not exceed 360 μmol/L in pregnant women (6).

1.2.7.1. Dietary management

The concept of reducing certain amino acid intake was a huge step in understanding not only PKU but other IEMs as well (e.g. homocystinuria, maple syrup urine disease (MSUD)). Dietary therapy has been the mainstay of PKU therapy for several decades now. Dietary therapy consists of three major elements: limiting natural protein consumption, supplementation with amino acid supplements (AAS) and low-protein medical foods.

Limiting Phe intake trough natural protein restriction depends on the residual activity of PAH and on Phe necessity which is influenced by growth, age and concurrent illnesses as well. Different protein sources contain different amount of Phe: while animal sources usually provide about 50 mg Phe per 1 g protein, the same ratio is approximately 20-40 mg in most fruits and vegetables. As most patients on dietary therapy do not tolerate more than 500 mg Phe a day, most patients need to keep a strict, restrictive diet (66, 67).

Because it is not possible to selectively exclude Phe from nutrition, restriction of natural proteins alone can lead to deficiency of other essential amino acids, vitamins and micronutrients (68). Symptoms of protein and Phe deficiency are similar: abnormal growth, hair loss, lethargy or eczema among others (69, 70). To prevent malnutrition and hypovitaminosis, amino acid supplements enriched in Tyrosine and other micronutrients are given to all patients who keep a natural protein-restricted diet (6). Finally, low-protein foods which are high on fat and carbohydrates are available for patients to supplement energy and to enable a diet as normal as possible (66).

Although palatability of low-protein foods and amino acid mixtures improved in the last decade (71), it is still an impeding factor in therapy adherence (72). Novel inventions as glycomacropeptide (GMP) and LNAAs may serve as viable alternatives in the near future (73, 74).

1.2.7.2. Pharmacological treatment

Although dietary treatment is the golden standard in PKU care, two drugs were approved in recent years to lower Phe levels. The synthetic analogue of the cofactor BH4 (sapropterin dihydrocloride) was approved in 2007 (74). Sapropterine dihydrocloride is a widely used medication in BH4 deficiency and which later proved to be useful in some PKU patients as it has an additional role in stabilising PAH (74). Only a minority of PKU patients profit from BH4 treatment however, responsiveness is most often seen in those who have mild PKU with higher residual PAH activity (24). Although some mutations determine BH4 unresponsiveness, in most cases a BH4 loading test is needed to prove usefulness (59). Sapropterine dihydrocloride is available in Hungary based on individual evaluation. The BIOPKU database lists all alleles that are proved to be responsive to BH4 treatment (http://www.biopku.org/home/biopku.asp). According to the complete European guidelines on PKU: patients with non-BH4-responsive mutations should not be tested for BH4 responsiveness whereas patients with 2 responsive alleles may start instantly with a trial of BH4 instead of a loading test (6).

Recent development of the injectable pegylated Phenylalanine-ammonia lyase (pegvaliase) is a huge milestone in PKU therapy. Pegvaliase was approved in 2018 by the FDA and is increasingly available for patients in Europe. This enzyme replacement therapy is administered as a subcutaneous injection and reduces blood Phe levels independently of PAH or BH4 (75). Pegvaliase was able to normalise blood Phe level and therefore completely replace diet in the majority of patients (75). The disadvantage of pegvaliase is the unfavorable adverse reaction profile: severity ranges from skin rash to joint pain and in some cases to anaphylaxis (75). Although it can take more than a year to achieve the aimed reduction in Phe levels, adverse reactions show a tendency to wear off in the first 6 month (75).

1.2.8. Special considerations of patient care in different stages of life

Complications of PKU and difficulties associated with therapy present different challenges at different ages.

Infancy: Dietary adherence is best in infancy (14). Until the exact protein composition of breastmilk was not described, breastfeeding was not allowed for babies with PKU (24). Fortunately, breastfeeding is now possible in PKU patients and with adequate metabolic control, a mother with PKU can breastfeed her affected child too (76).

Childhood: Fonnesbeck et al reported in their meta-analysis, that having high Phe levels in young age predicts low IQ score (<85) more precisely than high levels in older patients. Based on this finding, a critical (<6 years) period was defined (77). Intensified care is needed in childhood as uneven growth rates and concurrent illnesses may lead to highly variable Phe requirements (6). There is great room for improvement in this aspect because the majority of patients younger than 10 years struggle to achieve optimal metabolic control (78).

Adolescence: Walter et al reported that after 10 years of age the majority of analysed samples exceed the recommended threshold for Phe (22). Adolescence is characterised by a decrease in dietary adherence for several reasons: A strict low-protein diet is very different from the diet of peers, making optimal dietary adherence difficult (72, 79). Another challenge for patients is the timing of taking over responsibility of their own diet from parents. Van Spronsen et al. in a recent paper emphasize the ponderosity of living with a chronic disease were therapy inadherence does not lead to acute consequences (24). Finally, suboptimal metabolic control itself may impair compliance: adequate higher cerebral functions (e.g. strategic planning) are needed to maintain target Phe levels (80).

Adulthood: There is increasing evidence regarding the long-term consequences seen in adult, early treated PKU patients. Symptoms include executive function deficits, disturbances in attention and psychosocial impairment. Correlation is proven between concurrent/past Phe levels and brain damage in adult PKU patients (81).

The oldest early-treated PKU patients are still middle-aged adults: we can only speculate which novel complications may surface when these patients advance in age. Regular outpatient visits and close follow-up should help to determine individual risks for

complications. The complete European guideline on Phenylketonuria stands for life-long, regular follow-up of all patients and contains specific recommendations for patient care. Principles regarding follow-up of adult patients are shown on *Table 2* (6).

Table 2: Recommendations for optimal patient care of adult PKU patients based on the complete European guideline on Phenylketonuria (6). *Special recommendations for pregnant women are not included.*

Systematic follow-up	Frequency	Recommendations
Outpatient visit	Every year	Additional visits are recommended if metabolic control is suboptimal.
Clinical nutritional assessment	Every 12-24 months	Dietary assessment with food diary Assessment of anthropometric characteristics with particular emphasis on clinical features of micronutrient and Phe deficiency
Metabolic control	Every month	Monthly determination of plasma Phe levels and yearly AA profiling
Bone density	If needed	Bone mineral density (BMD) measurement in case of increased risk or clinical suspect of metabolic bone disease
Neurocognitive functions	Once at age 18 years with additional tests if needed	Testing should include IQ, perception and visuospatial functioning, executive functioning and motor controls
Adaptive/behavioral issues	Every year	Screening of adaptive issues is needed at age 18 and than annual reevaluation is recommended.
Neurological complications	Every year	As part of the clinical examination
Psychosocial functioning, Quality of Life (QoL)	Every year	Screening with the PKU-QoL adult questionnaire with annual reassessment is suggested
Psychiatric examination	If needed	If psychiatric disturbances occur
White matter abnormalities	If needed	If clinical outcome is unexpected

Maternal PKU Syndrome (MPKUS): Suboptimal metabolic control of PKU in pregnant female patients may lead to MPKUS of the fetus (while not causing symptoms in the mother). The association between high maternal blood Phe levels and the syndrome was first described in 1957 (24). Several studies proved (82, 83) that even moderately elevated Phe levels (300-400 μmol/L) in maternal blood may increase the risk for MPKUS.

Typical symptoms of MPKUS are developmental delay, microcephaly, cardiac malformations, intrauterine growth restriction, dysmorphia (84). The European guideline on Phenylketonuria not only sets a stricter upper target value of Phe to 360 μmol/L but also recommends intensified follow-up already while planning childbirth (6).

1.2.9. Patient care in Hungary

In Hungary, newborn screening of 3 inborn errors of metabolism was introduced nation-wide in 1976: galactosaemia, biotinidase-deficiency and PKU. These 3 diseases were later expanded to 25 (with congenital hypothyroidism being the 26th) altogether.
Two metabolic centers are responsible for PKU patients in Hungary:[1] Department of Pediatrics and Pediatric Health Center of the University of Szeged and [2] 1st Department of Pediatrics of the Semmelweis University.
In the Budapest region, adult PKU patients are treated at the Department of Internal Medicine and Oncology of Semmelweis University. With around 200 patients attending annual check-ups, this metabolic center belongs to the larger ones in Europe (85).

1.2.10. Complications in adulthood

1.2.10.1. Neurological alterations

Several neurological symptoms have been described by patients who discontinued treatment: leukoencephalopathy, parkinsonism and even loss of vision (81). Restarting therapy can partially or fully reverse these complications and frank neurological disease is uncommon (3, 19). Even patients who adhere to continuous dietary treatment may experience brisk reflexes and tremors (86). Previously published results of this research group showed that several domains of executive function (coordination of fine movement, spatial planning and problem solving) are affected in both adherent and partially-adherent adult patients (87). We also reported structural changes of the retina in early-treated adult PKU patients (88, 89).

1.2.10.2. Neuropsychiatric alterations

Loss of metabolic control in adulthood and adolescence leads to anxiety, social withdrawal and irritability by some patients (90, 91). On the other hand, optimal therapy adherence does not hinder the occurrence of all psychiatric complications either: attention-deficit hyperactivity disorder and learning disabilities are more common in early-treated PKU patients than their healthy peers (92).

1.2.10.3. Alterations in bone metabolism

It is widely known that risk for osteopenia (and even severely decreased bone mineral density) is higher in a subgroup of early treated PKU patients (93, 94). However, the lack of articles of appropriate quality and methodology makes it difficult to examine these alterations. Even the definitions of osteoporosis and osteopenia are used heterogeneously between articles and often do not meet WHO and International Society for Clinical Densitometry (ISCD) standards on reporting BMD (93).

In contrast to previous results (21, 95), Demirdas et al published a meta-analysis in which only early-treated PKU patients were involved. They found lower BMD Z-scores in patients with ETPKU compared to healthy controls at both the lumbar spine and the femoral hip but mean BMD was not out of the reference range (93). A recent review confirms that mean BMD is decreased in patients with PKU, although only a minority of patients had results below normal range (96). Detailed assessment of bone metabolism proved that bone turnover is shifted towards bone removal, especially in adults (97-100). One study found a 2.6 times higher risk for fracture in patients compared to healthy controls, although this aspect is insufficiently examined (96, 101). All in all, international literature on bone health in adult PKU is still scarce, and publications often report contradictory results.

The underlying pathomechanism is only partially described, with several etiological factors hypothesized: It seems that high Phe concentrations may alter osteoclast and osteoblast function (102). A restrictive diet low in natural protein and consuming medical formulae may also add to role in altered bone formation (103). Calcium, phosphorus and vitamin D intake may be disturbed, and excessive ureagenesis and suboptimal amino acid composition may also contribute (98, 100). As regular physical exercise and sport is crucial in maintaining healthy bone structure, patients suffering from neurological and psychiatric complications are at greater risk (104, 105). Additionally, fluctuations in

blood Phe concentrations and genetic polymorphisms linked to Phenylketonuria may have a role in altered bone health (96).

The European guideline on PKU stresses the importance of adequate vitamin D and calcium intake, regular physical activity and optimizing natural protein intake. BMD should be assessed in adolescence, and further follow-up may be necessary by all patients at risk (6).

1.2.10.4. Quality of life outcome

Both PKU itself and its therapy is expected to influence Quality of Life (QoL) of early-treated PKU patients and their families. Living with a chronic disease, avoiding high protein foods as meat or eggs and consuming medical foods (that often have unpleasant tastes) presents social, emotional and financial burden (72). This results in suboptimal compliance to therapy (especially in adolescence and adulthood), and an increasing frequency of somatic complications (79, 106) As new therapies emerge, new goals are set for patient care and improving quality of life of patients (and their loved-ones) became a new therapeutic goal (107, 108).

Quality of life can be defined using general or disease specific Health-Related Quality of Life (HRQoL) questionnaires. The concept is based on subjective patient- report about disease and therapy impact on daily life. Target domains include: somatic, psychological, social and general well-being (109). Several authors investigated HRQoL in adult ETPKU patients, but most of them utilised generic QoL questionnaires., Although most articles found a HRQoL comparable to healthy controls (110-115), some studies report unfavorable outcome in adult PKU patients (116, 117). A recent, generic questionnaire based study conducted by Aitkenhead et al with a large sample of adult PKU patients (154 patients involved) reported, that - in case of adequate therapy adherence in childhood – relaxing diet in adulthood did not lead to worse educational, occupational outcomes or greater prevalence of anxiety or depression (118). The same research concluded that partially therapy adherent patients had significantly worse HRQoL scores than those on- or off-diet.

Generic questionnaires are widely used to compare certain patient groups to an unaffected population but tend to miss specific areas of interest which are not relevant for the general

population (difficulties of „eating-out", financial burden of consuming medical foods, etc.) (119). The European guideline on Phenylketonuria recommends the use of the recently developed, population specific „PKU-QoL" questionnaire (6), which addresses the most important issues that affect PKU patients: symptoms, disease impact, diet and supplements (120). Research using specific quality-of-life questionnaires is essential to identify patients' problems, but few studies have been carried out so far in PKU, and most of these included either children or their parents only: Morawska et al. found that in families where the parenting strategy of over reactivity is often used, children with PKU tend to have lower lifetime Phe-levels but higher scores in the impact of PKU module (121). Recently, a Hungarian study was published as well: Becsei et al. focused on the effects of metabolic control using the PKU-QoL version for parents and children. In general, patients and parents showed good HRQoL with most domains minimally affected but children with suboptimal metabolic control had worse HRQoL (122).

1.2.10.5. Alterations in body composition

Although growth restriction in treated children was hypothesized earlier (123), recent results disproved this (124-126). Authors found contradictory evidence regarding weight (125, 127-131) but there is growing body of work suggesting that overweight is more prevalent in PKU (132, 133), especially in females (134, 135). Pathogenesis of obesity is not known in PKU patients. It is assumed that taking calorie-rich AAS, premature transition to adult products and lack of physical exercise all add to unfavorable body composition (132, 136).

Obesity as a major risk factor for metabolic syndrome and various diseases receives increasing attention in adult PKU population. Indeed, some recent articles reported accumulation of several cardiovascular risk factors in patients with ETPKU. Preventing - and if needed, treating - obesity has become a necessity (137, 138), but before appropriate care and prevention plans can be optimised, carefully designed studies are much needed.

2. Objectives

Early treated PKU patients reach adulthood without major impairments, but long-term outcome is only partially explored (139). Potential consequences are neuropsychiatric symptoms, nutritional deficiencies, suboptimal psychosocial outcomes, altered body and bone composition. Our research team aims to help detect and prevent long-term complications of early treated PKU patients: previously published work covered ophthalmological (88, 89); neurocognitive (87) and endocrinological (140) fields of interest. My doctoral work and this thesis focus on three areas which have recently received increasing attention. Precise assessment of health-related quality of life is an essential need to improve patient care, while impaired bone health and altered body composition may lead to secondary consequences as patients reach older ages.

2.1. Assessment of Health Related Quality of Life outcomes in early-treated adult PKU patients using the PKU-QOL adult questionnaire

Patients' subjective perception of quality of life may be affected by the disease as well as by the strict therapy. Few studies have investigated the relationship between adherence to therapy and quality of life (all of these using generic methods) and results are contradictory (117, 129, 141, 142). Prior to our study, there was no results available at all about differences in health-related quality of life between patients on good and suboptimal therapy using the recently developed PKU-QoL (143). The aim of our study was to assess the quality of life of Hungarian adult ETPKU population and to compare adult diet adherence and health-related quality of life outcomes.

2.2. Body composition assessment of early-treated, adult Phenylketonuria patients using Multifrequency bioelectrical impedance analysis (MF-BIA)

Traditional, simplified methods (e.g. Body mass index) can be misleading when assessing body composition. A detailed assessment is essential for obtaining precise results. Most studies use Dual-energy X-ray absorptiometry (DXA), which has the disadvantage of exposing the patient to ionising radiation. The mutifrequency Bioelectrical Impedance analysis (MF-BIA) method is able to measure the differences in the impedance of the electronic currents flowing through the cells of the body, calculating regression models and performing accurate body composition measurements. The method has the advantage of having no side effects and is inexpensive, but its use is limited by changes in water homeostasis (144, 145). Bioelectrical Impedance analysis has been well tested in the general population (146).

In this single-center, cross-sectional study we examined the detailed body composition of adults with ETPKU using MF-BIA. Main goals were the following: 1) to reveal potential correlations between body composition characteristics and therapy adherence, 2) to clarify sex differences and 3) to compare MF-BIA results of patients with healthy age and gender matched adults.

2.3. Bone mineral density in early-treated, adult PKU patients using dual-energy x-ray absorptiometry

Although decreased mean BMD is reported in patients with PKU, data regarding adult patients is insufficient and methodology of publications is highly heterogeneous (93, 94). In Hungary, there has been no comprehensive study of bone health in adult PKU patients so far. We aimed to assess bone mineral density of the Hungarian early-treated adult Phenylketonuric population.

Some authors hypothesize that the restrictive dietary therapy may play a significant role in altered bone health, although there is no consensus on the pathoetiology in PKU (103). We analysed the change in bone mineral density longitudinally over several years and simultaneously assessed blood amino acid concentrations to reveal possible correlation between metabolic control and changes in bone density.

3. Results

Following the guidance of the Doctoral school, in this thesis I excluded or only briefly described the methods which I have already published in previous articles with my authorship.

3.1. Assessment of Health Related Quality of Life outcomes in early-treated adult PKU patients using the PKU-QoL adult questionnaire

Eighty-eight adult ETPKU patients (with classical, moderate and mild phenotype) were recruited in this single-center, cross sectional, observational study in which we assessed 1) the HRQoL of Hungarian PKU patients and 2) the relation of HRQoL scores and dietary adherence.

All participants attended our metabolic outpatient clinic for at least 10 years at the time of filling out the standardized and validated PKU-QoL adult questionnaire (120). The 65 questions of the PKU-QoL adult questionnaire cover the main issues related to living with PKU and PKU therapy. Questions are grouped in domains which form the 4 main modules (*Table 3*). Actual Phe concentration was measured at the time of completing the questionnaire. We assessed long- and medium-term adherence by calculating the mean Phe concentration from the last 10 years and last year. Lifetime Phe levels from birth were available for 47 patients.

Table 3: Modules and domains of the adult PKU-QOL questionnaire. (120)

PKU Symptoms module	PKU in General module	Administration of Phe-free Protein Supplements module	Dietary Protein Restriction module
Self-health rated status	Emotional impact of PKU	Adherence to supplements	Food temptations
Headaches	Practical impact of PKU	Practical impact of supplements	Adherence to dietary protein restriction
Stomach aches	Social impact of PKU	Guilt if poor adherence to supplements	Social impact of dietary protein restriction
Tiredness	Overall impact of PKU	Impact of supplements on family	Practical impact of dietary protein restriction
Lack of concentration	Anxiety-blood test	Taste - supplements	Overall impact of dietary protein restriction
Slow thinking	Anxiety-blood Phe levels		Taste of low protein food
Trembling hands	Anxiety-blood Phe levels during pregnancy		Guilt if dietary protein restriction not followed
Irritability	Financial impact of PKU		Overall difficulty following dietary protein restriction
Aggressiveness	Information on PKU		Food enjoyment
Moodiness			
Sadness			
Anxiety			

Detailed description about calculation of scores is published in our previous article, here I highlight core elements: All domain scores were transformed to scores 0-100 for better clarity. In general, higher scores mean greater impact (or worse adherence / more frequent symptoms). Severity of scores in the domains should be interpreted according to the following: 0-25 indicates little/no; 26-50 indicates moderate; 51-75 indicates major and scores over 75 indicates severe impact / frequent symptoms (143). Similarly, self-rated health status was rated subjectively by the patient between 4 – 0: Poor (4), fair (3), good (2), very good (1) or excellent (0).

3.1.1. Descriptive results

Baseline demographics and blood Phe results are shown in *Table 4.* Gender distribution was balanced (46 female/42 male), median age was 31 (25-40) years. Mean Phe over the

last decade was 588±197 μmol/l. Seventy-five percent of all patients had classical PKU, 14% had moderate and 11% had mild PKU or HPA. Seventy-seven percent of all patients declared their own overall health status as good or excellent (with a median score of 50 (25-50)). The subgroup of patients by whom lifetime Phe was available („Lifetime Phe group") had a median age of 30 (25-37) and gender distribution in this group was almost equal (23 female and 24 male). Mean lifetime Phe was 543±200 μmol/l.

Table 4: Baseline demographics and blood Phe measurements of patients grouped based on disease severity. *Tests are two-tailed, using independent samples t-test. Significance: *p<0.001. SD=standard deviation, IQR=(25th–75th percentile). (147)*

		Non-classical PKU (n=22)	**Classical PKU (n=66)**	**All patients (N=88)**
Age	median, IQR (years)	30 (24–38)	33 (26–41)	31 (25–40)
Gender	male (n)	7	35	42
	female (n)	15	31	46
Blood Phe last measurement	mean±SD (μmol/L)	421±175*	660±236*	600±236
Blood Phe last year	mean±SD (μmol/L)	427±176*	637±198*	584±212
Blood Phe last 10 years	mean±SD (μmol/L)	441±185*	636±176*	588±197

Median domain scores did not reach „major or severe impact/frequent symptom" category (median score larger than 50) in any domain in the total patient group. Domains where moderate impact/symptom severity was noted are shown in *Table 5.* We highlighted „guilt of poor adherence to supplements" domain as well because of the relatively high number of patients who experienced major impact.

Table 5: Most severely affected domains among all patients. *Median (25th-75th percentile). (147)*

Affected domains	**Median (IQR)**
Tiredness	50 (25–50)
Anxiety - Phe levels during pregnancy	50 (25–100)
Emotional impact of PKU	35 (15–50)
Taste - supplements	50 (25–50)
Guilt if poor adherence to supplements	25 (25–75)
Guilt if dietary protein restriction not followed	50 (25–75)

Symptoms like stomach aches (median, IQR (0, 0–25)), slow thinking (0, 0–25), trembling hands (0, 0–25), aggressiveness (0, 0–25) and sadness (0, 0–50) were especially rarely reported by patients. Other spared aspects of living with PKU were food enjoyment (0, 0–25), practical impact of supplements (0, 0–19), anxiety about blood tests (0, 0–25) and financial impact of PKU (0, 0–25).

3.1.2. Association between metabolic control and quality of life scores

We assessed the correlation between PKU-QoL scores and blood Phe levels in three time periods among all patients: concurrent, last year mean and last 10 year mean Phe concentrations. Although no strong correlation was found, we report weak to fair positive correlation in various domains. Significant correlations are shown in *Table 6.*

Table 6: Correlation analysis of QoL scores and metabolic control. *Tests are one-tailed, for positive correlation using Spearman test (except three domains). Alternative hypothesis states that there is a positive correlation between domain score and Phe level in the examined time frames. Pearson's test was used in 3 domains because the pairs of variables followed a bivariate normal distribution in the study population. Rs=Spearman's Rho, Rp=Pearson's Correlation. Significance: *p<0.05; **p<0.01. (147)*

Domain name		Blood Phe last measurement	Blood Phe last year	Blood Phe last 10 years
Trembling hands	Rs	0.145	0.114	**0.213***
Taste - Low-protein food	Rs	**0.284****	**0.258***	0.172
Food temptation	Rs	0.173	**0.248***	0.161
Social impact of dietary protein restriction	Rs	**0.263***	**0.231***	**0.203***
Adherence to supplements	Rs	**0.235***	**0.290****	**0.344****
Adherence to dietary protein restriction	Rs	**0.264***	**0.350****	**0.268***
Practical impact of dietary protein restriction	Rp	**0.198***	**0.202***	0.17
Overall impact of dietary protein restriction	Rp	**0.265***	**0.252***	**0.228***
Anxiety - Phe levels	Rs	**0.186***	**0.209***	0.163
Practical impact of PKU	Rs	**0.253***	**0.210***	**0.219***
Overall impact of PKU	Rs	**0.203***	**0.206***	0.165
Emotional impact of PKU	Rp	**0.306****	**0.251***	0.172

3.1.3. QoL scores and lifetime Phe levels

Forty-seven patients were eligible for analysis based on lifetime metabolic control. We assessed the correlation between QoL scores and lifetime mean Phe levels and we found that trembling hands affected patients with higher lifetime Phe levels more often (Rs=0.468, p<0.001) and we report positive correlation in the emotional impact of PKU (Rs=0.291, p=0.025) domain as well.

3.4. Classical versus Non-classical PKU group

Classical PKU patients need a much stricter therapy to maintain optimal metabolic control so we compared QoL scores of patients with classical and with non-classical PKU. Patients with classical PKU reported significantly greater financial burden in conjunction with their illness (p=0.039; U=840; Rank-Biserial=0.231) but there was no apparent difference in other domains.

3.5. Differences between classical PKU patient groups based on therapy adherence

Patients who are less adherent to therapy are freer to organize their daily lives but suffer more from complications of PKU. We performed subgroup analysis in the classical PKU group: two subgroups were formed in all time frames based on therapy adherence: Classical PKU patients with optimal therapy adherence (Phe or mean Phe < 600μmol/l) and with suboptimal therapy adherence (Phe or mean Phe > 600 μmol/l). We compared HRQoL of these subgroups to evaluate the importance of therapy adherence on subjectively rated quality of life and found significantly greater scores in patients with suboptimal therapy adherence in several domains (*Figure 3*).

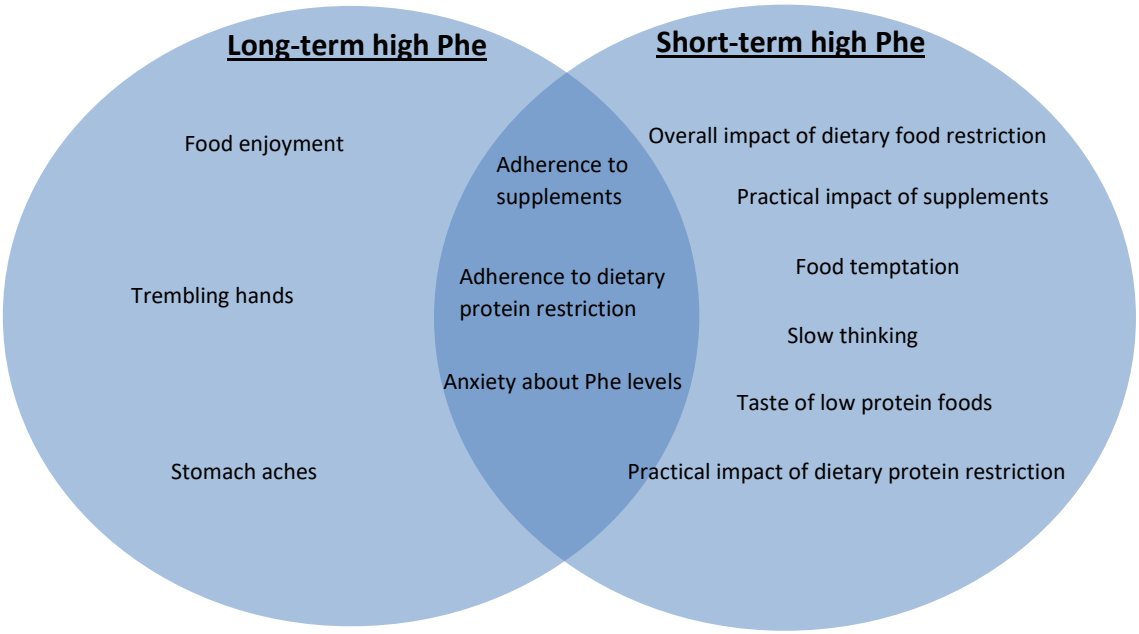

Figure 3: Patients with classical PKU on short- and/or long-term suboptimal therapy adherence had significantly worse outcomes in the above listed domains. *Long-term Phenylalanine is calculated based on mean Phe in the last 10 years whereas short-term phenylalanine was measured at the time of filling out the questionnaire. For statistical analyses Mann-Whitney U test or T-test were used based on the distribution of variables. domains are only included if difference was significant ($p<0.05$). (147)*

Long-term mean phenylalanine was 636±176 μmol/L (270-1174) in the classical PKU patients group, whereas 30 (45%) patients were able to keep good therapy adherence (mean Phe levels below 600 μmol/L) over 10 years prior to the investigation. Similar number of patients adhered to diet optimally, independently from examined time frames.

Classical PKU patients who maintained good therapy adherence for at least 10 years, rated their general health status significantly better than patients with suboptimal adherence ($p=0.043$; $U=616$; Rank-Biserial=0.245). It should be highlighted however, that this difference was not apparent in the short-term (*Figure 4*).

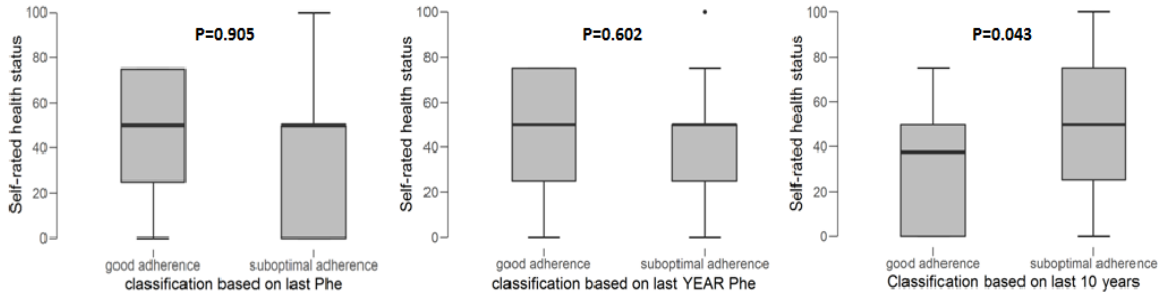

Figure 4: Comparison of self-rated health status. PKU-QoL scores related to diet adherence among classical PKU patients in different time frames. Lower scores represent better self-rated health status. *Boxplots should be interpreted as follows: interquartile range (Q1-Q3); -: median; bottom and top bars: observed minimum and maximum values; ○: outliers*. Tests are one-tailed Mann-Whitney tests. Significance: *p<0.05. (147)*

3.2. Body composition assessment of early-treated, adult Phenylketonuria patients using MF-BIA

Among the fifty patients with ETPKU (age range 18–42 years), forty-five had classical PKU and five (four female and one male) had moderate PKU. Forty healthy age and gender matched controls were recruited for body composition comparison. Detailed description of methodology and inclusion criteria are available in the original paper of our study.

3.2.1. Baseline characteristics of PKU patients

Relevant anthropometric findings, AAS intake and Phe levels are presented in *Table 7*, whereas lipid and protein markers are presented in *Table 8.*

Table 7: Differences in baseline characteristics and anthropometry between female and male patients with PKU. *Daily amino acid supplement intake (AAS) is calculated as total AAS intake per day divided with ideal body weight. Tests are two-tailed, using independent samples t-test (results reported as mean±SD) or Mann-Whitney test (results reported as median (25^{th}--75^{th} percentile)) based on the distribution of variables. Significance p<0.05. (148).*

	Female n=27	**Male n=23**	**p-value**
Age	31 ± 7.8	26.6 ± 7.6	0.052
Height (cm)	160 ± 6.4	173 ± 7.4	**<0.001**
Weight (kg)	67.3± 11.2	76.1 ± 15.8	**0.035**
Body mass index	26.3 ± 4.4	25.2 ± 4.9	0.410
Waist to hip ratio	0.92 ± 0.06	0.90 ± 0.09	0.570
Percent body fat (PBF)	36.7 (30.6-42.2)	18.7 (14.3-29.8)	**<0.001**
AAS (g/kg)	0.85 (0.01-0.93)	0.94 (0.82-1.12)	**0.014**
Mean Phe over last 10 years (µmol/l)	676 ± 174	579 ± 146	**0.041**

Table 8: Differences in laboratory findings between female and male patients with PKU. *Tests are two-tailed, using independent samples t-test (results reported as mean±SD) or Mann-Whitney test (results reported as median (25^{th}-75^{th} percentile)) based on the distribution of data. Significance p<0.05. (148)*

	Female (n=27)	**Male (n=23)**	**p-value**
Triglycerides (mmol/L)	1.12 (0.81-1.5)	1.37 (0.91-1.7)	0.362
Total cholesterol (mmol/L)	4.58 ± 0.83	3.96 ± 0.79	**0.010**
HDL-cholesterol (mmol/L)	1.29 ± 0.29	1.06 ± 0.19	**0.002**
LDL-cholesterol (mmol/L)	2.90 ± 0.65	2.54 ± 0.66	0.061
LDL/HDL	2.19 (1.71-2.95)	2.36 (1.77-3.08)	0.629
Albumin (g/L)	47.56 ± 3.37	48.20 ± 3.56	0.514
Prealbumin (mg/dL)	27.94 ± 3.88	30.86 ± 3.39	**0.008**
Total protein (g/L)	75.80 ± 4.93	74.68 ± 4.77	0.396

Therapy adherence was significantly worse in female patients when compared to males: Long-term (10 years interval) mean Phe levels were higher (p=0.041; Cohen's D=0.59) whereas AAS intake (per ideal body weight) was less (p=0.014; Cohen's D= −0.61).

Thirty-five percent of males and 67% of female patient had mean Phe levels over the 600 µmol/l threshold during the examined 10 years interval.

While we found Percent Body Fat below target range by one male patient, PBF was above target range (given by the MF-BIA machine) by 48% of male and 86% of female patients. Although Body mass index (BMI) was similar in both sexes (p=0.4; Cohen's D=0.23), women had significantly higher PBF than men (p<0.001; Cohen's D=1.39). Eighty-nine percent of women had a waist to hip ratio (WHR) over 0.85 whereas only 26% of male patients had WHR above the upper threshold (>0.90). Mean WHR, which is normally higher in males was similar in both sexes in the PKU group (p=0.571; Cohen's D=0.23). Overweight and obesity was frequently seen in the PKU patient group, detailed distribution is shown in *Figure 5.*

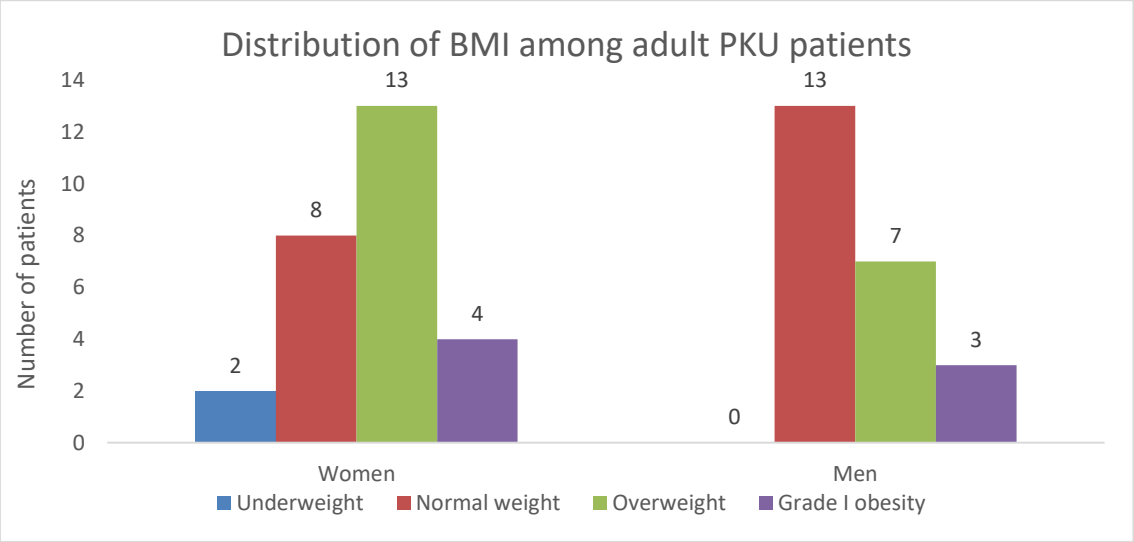

Figure 5: Distribution of body mass index among adult PKU patients. *Patients with Grade II or Extreme obesity were not included in this study. (148)*

Laboratory findings suggested that hypercholesterinemia (total cholesterol >5.2 mmol/L) is prevalent in the patient group: 4% of males and 15% of females had high total cholesterol. Elevated LDL (>3.3 mmol/L) was seen in 9% and 19% of men and women respectively. Female patients had significantly higher HDL (p=0.002; Cohen's D= 0.9) and total cholesterol (p=0.002; Cohen's D=0.76) when compared with males. We do not report hypalbuminaemia, subnormal protein or prealbumin levels. Prealbumin of male patients was significantly higher than females however (P=0.008; Cohen's D= −0.79).

3.2.2. Correlation analyses of laboratory findings, body composition analysis and therapy adherence in the PKU patient group

We performed extensive correlation analyses in the PKU patient group (n=50) between serum protein (albumin, total protein, prealbumin), serum fat (triglyceride, total Cholesterol, HDL-, LDL-cholesterol) and long-term metabolic control of PKU (mean Phe over the last 10 years). Significant correlation between therapy adherence and prealbumin was observed: AAS intake correlated positively with prealbumin (p=0.012; Spearman's Rho=0.358) but negatively with mean Phe levels in the last 10 years interval (p=0.021; Pearson's R=-0.329).

Markers of therapy adherence (Phe levels or AAS intake) did not show significant correlation with body composition parameters in PKU patients, detailed results are shown in *Table 9.*

Table 9: Correlational analyses between body composition results and dietary compliance or AAS intake by male and female patients with PKU. *For calculations, amino acid supplement (AAS) intake intake was given as daily AAS intake per ideal body weight, and body composition parameters were given as percentages of total weight. For correlational analyses, two-tailed Pearson's or Spearman's correlation methods were used based on the distribution of variables. Significance: p<0.05. (148)*

	Mean Phe over the last 10 years				**AAS intake**			
	Male (n=23)		Female (n=27)		Male (n=23)		Female (n=27)	
	p-value	Pearson's R	p-value	Pearson's R	p-value	Spearman's Rho	p-value	Spearman's Rho
Fat free mass (%)	0.47	0.15	0.27	-0.21	0.47	-0.15	0.24	0.23
Skeletal Muscle Mass (%)	0.58	0.12	0.31	-0.20	0.56	-0.12	0.29	0.20
Percent Body Fat	0.47	-0.15	0.27	0.21	0.47	-0.15	0.23	-0.23
Protein (%)	0.52	0.14	0.25	-0.22	0.46	-0.16	0.26	0.22

3.2.3. Comparison of body composition between PKU patients and healthy controls

We compared the results of MF-BIA examination of 40 PKU (F/M=20/20) patients with 40 gender-matched healthy adult controls. We performed statistical analysis in women and men separately.

Similar weight and age distribution was seen between patients and controls *(Table 10).* Female patients differed from their healthy controls in several aspects, but this was not seen in males. Female controls showed a tendency to have lower BMI (p=0.055; Rank biserial effect size: −0.357). whereas WHR (p=0.007; Rank biserial effect size: −0.502) and PBF (p=0.028; Rank biserial effect size: −0.407) was significantly higher in women with PKU. In contrast, female controls had higher mineral content, fat-free mass, protein content and skeletal muscle mass percentage per body weight than PKU patients (*Table 10*).

Table 10: Anthropometric and body composition results of the case-control investigations. *Female patients with PKU were compared with female control subjects and male patients with PKU were compared with male control subjects. Tests are two-tailed, using independent samples t-test (results are reported as mean±SD) or Mann-Whitney test (results are reported as median (25^{th}-75^{th} percentile)) based on the distribution of variables. Significance: p<0.05. (148)*

	Male			**Female**		
	PKU (n=20)	**Control (n=20)**	**p--value**	**PKU (n=20)**	**Control (n=20)**	**p-value**
Age	26 (20-30.25)	24 (21.75-28.25)	0.37	29.00 (24.00-35.25)	26.5 (23.25-34)	0.92
Height	175.4±5.9	178.8±5.0	0.060	162.3±6.4	164±4.8	0.379
Weight	69.85 (63.32-84.67)	81.10 (76.55-89.92)	0.10	71.10 (59.55-78.85)	56.45 (53.30-67.50)	0.06
Protein (%)	16.29 (14.53-17.13)	15.97 (14.25-16.72)	0.59	12.71 (11.49-13.82)	14.77 (13.51-15.46)	0.03
Mineral (%)	5.39±0.54	5.49±0.56	0.61	4.6±0.6	5.1±0.6	0.01
Fat free mass (%)	81.96 (73.05-85.25)	80.61 (72.52-84.90)	0.71	64.20 (58.45-70.24)	75.19 (69.25-77.83)	**0.03**
Skeletal Muscle Mass (%)	46.12 (41.55-48.55)	45.47 (41.11-47.92)	0.72	35.56 (32.18-38.40)	40.69 (37.52-43.11)	**0.03**
Percent Body Fat	18.05 (13.67-26.95)	19.4 (15.07-27.52)	0.70	35.80 (29.80-41.45)	24.70 (22.17-30.75)	**0.03**
Body Mass Index	22.80 (20.72-27.22)	25.40 (23.25-27.20)	0.13	26.00 (23.17-29.30)	21.50 (19.65-24.50)	0.06
Waist to Hip ratio	0.87 (0.84-0.96)	0.88 (0.84-0.93)	0.65	0.92 (0.87-0.98)	0.84 (0.81-0.88)	**0.01**

3.3. Bone mineral density in early-treated, adult PKU patients using DXA

We performed a retrospective analysis among young-adult ETPKU patients who had at least two DXA examinations between 2010 and 2016 and by whom concurrent, regular blood Phe measurement results were available. Fifty-nine ETPKU patients were included in this study, detailed methodology and exclusion criteria are available in the previously published article. The mean interval between the two DXA analyses was 3.32 years.

3.3.1. Demographics, Metabolic parameters and Bone Mineral Density

We examined the potential link between metabolic control and BMD alteration: Total patient group was divided into two subgroups based on mean Phe levels. Those with mean Phe levels over the upper threshold were grouped as „Suboptimal therapy adherence group" whereas patients with mean Phe levels in the suggested range (120-600 µmol/L) were grouped as „Good therapy adherence group". Basic demographics and metabolic parameters are shown in *Table 11*.

Table 11: Basic demographics and metabolic Parameters of patients. *Tests are two-tailed, using Mann-Whitney test based on the non-normal distribution of variables (results are reported as median (min-max)). (149)*

	All patients	**Good therapy adherence**	**Suboptimal therapy adherence**	**p-value**
Number of patients	59	28	31	-
gender distribution (male/female)	24/35	9\19	15\16	0.18
Age (years)	33 (25-42)	33 (25-40)	34 (25-41)	0.46
Phe (µmol/l)	614.4 (181.8-1222)	506.4 (181.8-598.7)	779 (606.9-1222)	< 0.0001
Tyr (µmol/l)	52.1 (24.4-95.4)	58.3 (29.3 - 99.4)	41.47 (24.4-89.5)	0.01
Phe/Tyr ratio	16.2 (4.5-35.4)	9.2 (4.5-20.6)	20.14 (9.0-35.4)	< 0.0001

Bone fracture was not reported during this time course. Z-score was below -1 SD in 42% of all patients and our examinations revealed low BMD compared to chronological age based on the ISCD guideline criteria (Z-score < - 2.0 SD) in 9 patients. Mean BMD change in the examined interval was +0.0380 (-0.1550-0.7800) g/cm^2 at the femoral neck and +0.0120 (–0.57300–0.3130) g/cm^2 at the lumbar vertebra.

3.3.2. Relationship of BMD change and metabolic control

We found no significant correlation between mean blood amino acid levels (Phe and Tyr) and BMD change in this time frame at any examination sites (*Table 12*). BMD change did not correlate with Phe/Tyr ratio either.

Table 12: Correlation analysis between Phe, Tyr, Phe/Tyr ratio and BMD at femoral neck and lumbar vertebra. *Tests are two-tailed, using Spearman's rank correlation based on the non-normal distribution of variables. Significance: p<0.05. (149)*

FEMORAL NECK:

	r-value	**p-value**
Phe	0.05	0.66
Phe/Tyr	-0.14	0.26
Tyr	0.10	0.42

LUMBAR VERTEBRA:

	r-value	p-value
Phe	-0.05	0.66
Phe/Tyr	-0.08	0.52
Tyr	0.051	0.69

Upon comparing Z-score changes in the two subgroups we did not find significant difference, detailed results are presented in *Table 13* whereas distribution of Z-score changes are shown in *Figure 6.*

Table 13: Change of BMD (Z-score) in the examined time frame in the two groups. *Tests are two-tailed, using Mann-Whitney test based on non-normal distribution of variables (results reported as median (min-max)). Significance: p<0.05. (149)*

	Good adherence	Suboptimal adherence	p-value
Femoral neck	+0.03 ((-0.05) – (+0.78))	+0.04 ((-0.15) – (+0.30)	0.92
Lumbar vertebra	+0.02 ((-0.05) – (+0.31))	+0.002 ((-0.57) – (+0.28))	0.38

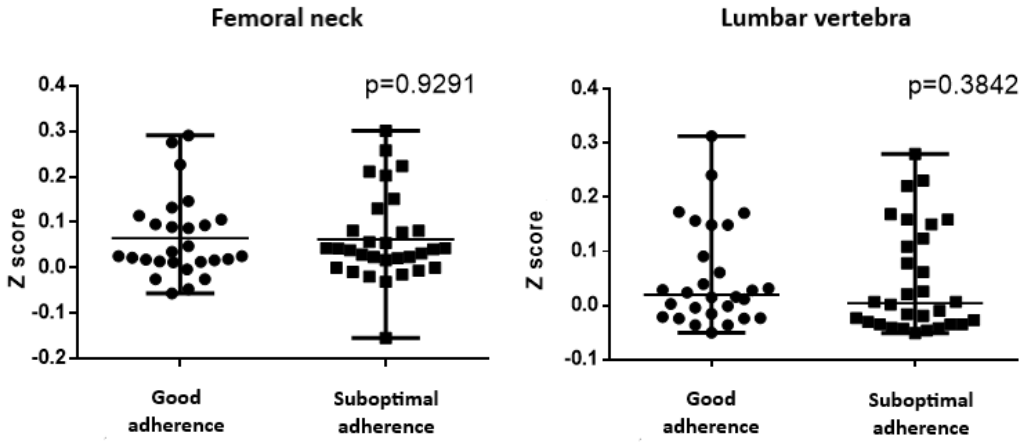

Figure 6: Change of BMD (Z-score) in the two subgroups at the femoral neck and lumbar vertebra. *Tests are two-tailed, using Mann-Whitney test based on non-normal distribution of variables. Significance: p<0.05. (149)*

4. Discussion

4.1. Assessment of Health Related Quality of Life outcomes in early-treated adult PKU patients using the PKU-QoL adult questionnaire

The PKU-QoL disease specific questionnaires provide a novel method to precisely define critical areas of disease management in patients with PKU. Although the complete European guideline on Phenylketonuria specifically recommends using the PKU-QoL questionnaire for all adult PKU patients (6), literature search resulted in only one research using this method at the time of designing our study (150). This can be explained with several reasons: PKU is a rare disease, the questionnaires were developed recently in 2015 and are only available in a few chosen languages. Our study is the first to assess the consequences of suboptimal metabolic control using the PKU-QoL in adult PKU patients.

4.1.1. Results of the questionnaire

For the majority of PKU-QoL domains skewed distributions were found and median scores did not reach severe impact/ frequent symptom (>50). Moreover, most patients rated their general health status as at least good (77%) compared to healthy peers. As several previous studies showed, that quality of life of PKU patients is comparable to the general population, these results are not surprising (110, 112, 114, 115).

In most domains, our results are similar to that of the developers (143). „Anxiety related to high levels of Phe during pregnancy" was the most affected domain altogether. This proves, that PKU patients are well aware of the consequences of uncontrolled pregnancies on their offspring. Patients reported the greatest impact scores related to disease management and to emotional burden (feeling guilty if poor compliance to diet or supplements, emotional impact of PKU) whereas tiredness was the most frequently reported symptom. We found high scores in the taste of Phe-free AAS domain as well, which underlines the need for amino acid supplements with better palatability.

4.1.2. Quality of life scores and metabolic control

Although no strong correlation was observed between therapy adherence and PKU-QoL scores, we report weak to fair correlation in various domains. Trembling hands - which is a well-described long-term consequence of elevated Phe levels – was significantly more burdensome for patients who had higher lifetime or combined-10-years Phe levels (86, 151).

Patients who had higher Phe levels at the time of filling out the questionnaire experienced greater anxiety about their levels than patients with better metabolic control. This finding suggests that patients are well aware of diet-inadherence and this leads to frustration. We observed weak positive correlation between recent, short-term therapy adherence and domain scores of overall and emotional impact of PKU (feeling unfairness having PKU, disease acceptance, self-esteem, worries about children). Similarly, higher lifetime Phe levels correlated with greater burden of emotional impact. Emotional disturbances may occur as consequence of living with a chronic, non-curable disease and not only as a result of having high Phe levels (152).

Several domain scores in the dietary protein restriction module showed weak to fair positive correlation with either long or short-term Phe levels: food temptation, taste of low protein foods, diet adherence and impact scores of dietary protein restriction (both social, emotional and practical). Although living conditions and other socioeconomic circumstances may also influence these results, we speculate that the highly variable tolerance to dietary phenylalanine is the most important factor in determining the burden of keeping low Phe levels (153).

Additionally, we performed a comparison between the classical and non-classical PKU group as well. The only domain in which significant difference was found was the financial impact of PKU domain: this is not surprising as patients with classical PKU need to consume more medical foods which are partially covered by health insurance. Our findings are in line with the results of the developers: Bosch et al. (143).

4.1.3. Subgroup comparison between patients with „Optimal” and „Suboptimal” therapy adherence

Results revealed metabolic control related differences of HRQoL in patients with classical PKU. These patients suffer from significantly impaired Phe tolerance and strict

diet is crucial for maintaining good general health. Ours is the first study, which reports on QoL differences between optimal and suboptimal adherent adult classical PKU patient groups. We report significantly higher impact and symptom scores in the „suboptimally adherent" patient group.

Although mean Phe level of the suboptimally adherent group was 753±137 μmol/l in the last 10 years, it is important to stress that these patients attended regular metabolic care and were compliant to therapy at least partially. We presume, that disease impact and symptom module score differences would have been more unanimous if comparison between patients totally off-diet and patients with good adherence would have been possible.

Classical PKU patients who had suboptimal therapy compliance continuously (both during the preceding 10 years and at the time of filling out the questionnaire) suffered from higher anxiety scores about their Phe levels. Those, who had only a short-term fallout at the time of participating in the questionnaire reported higher overall and practical impact of supplements (lack of spontaneity, embarrassment taking supplements), food temptation and practical impact of dietary protein restriction (burden of estimating quantity of protein, difficulty eating out). Bik-Multanowski et al. reported similar results earlier: they concluded that a subset of adult patients who are on a relaxed diet suffer from severe psychological burden and therapy resumption may improve HRQoL by them (117).

Although patients with short-term therapy fallout reported an increased burden of slow-thinking, this was not seen in patients with long-term inadherence. Unawareness of minor cognitive changes is a possible explanation to our findings. A recent study, which involved both PKU patients, observers and clinicians supports this explanation. Burton et al found that lack of self-awareness can aggravate care – especially by patients with suboptimal metabolic control. Some patients lack insights on mood swings, temper tantrums, irritability and forgetfulness (154).

No significant difference was found between the patient groups with good and suboptimal metabolic control in short term regarding subjectively rated health status compared to peers. However, those with a 10 year history of good metabolic control rated their health-status as significantly better than patients with suboptimal compliance.

We conclude, that optimal dietary therapy adherence in adult classical PKU patients is associated with significantly better HRQoL. Mutze et al (141) reached a similar conclusion earlier: they stated that patients on diet have higher life satisfaction than patients with partial diet adherence. One may either hypothesize that good compliance leads to favorable changes in life satisfaction or the other way around: a positive perception of subjective health status helps to adhere better. Future research is needed to clarify the cause-effect relationship.

4.1.4. Limitations

Ceiling effect is a frequently occurring distorting factor in QoL questionnaires. We found that the majority of PKU-QoL scores approached the upper limit of the scale, hence comparison of these results with alternative methods would have been useful. Such methods include reporting family members, using structured interviews led by experts, using patient generated indexes of QoL or organizing focus group meetings (155-157). We enrolled a large patient population compared to previous HRQoL related studies in PKU, but involving more patients would have allowed us to detect even minor differences of Qol between subgroups with different therapy adherence. Finally, as the PKU-QoL questionnaire for adult PKU patients addresses problems which are only relevant in this patient group it was not possible to compare results with a healthy control group (143).

4.2. Body composition assessment of early-treated, adult Phenylketonuria patients using MF-BIA

Although severe complications of PKU are rarely reported in the ETPKU population, subtle impairment of several organs (e.g. higher risk for cardiovascular consequences, neuropsychological alterations) are present in a subset of adult patients (80, 138). Providing appropriate patient care in the long-term with regular follow-ups is essential to maintain good health. Studies, which focus on the impact of lifelong therapy with intake of AAS are crucial in revealing relevant alterations. The main goal of this investigation was to explore alterations of body composition in adult ETPKU patients who adhere to therapy at least partially. Multifrequency bioimpedance analysis was used for in-depth

analysis of body composition and results were compared with an age and gender matched healthy control group.

4.2.1. Body composition analysis

We report higher tendency for being overweight in female patients in contrast to males. Both the conventional BMI and body fat percentage exceeded normal range in most women. When comparing female PKU patients to healthy peers, significantly higher percent body fat values were found in the patient group, although difference of BMI was statistically non-significant. These findings confirm previous investigations which concluded that female ETPKU patients are more prone to excess weight gain than male ETPKU patients (132-135).

We report higher waist-to-hip ratio in patients compared with controls too. This is a relevant finding as waist circumference is a well-known predictor of abdominal obesity and the latter leads to increased risk for cardiovascular and metabolic diseases (158, 159). On the other hand, female patients had relatively decreased muscle mass, mineral content and protein levels based on the MF-BIA examination. These alterations were not present when comparing male patients to controls. Our results are in contrast to a recently conducted study which also used MF-BIA and involved children: the authors reported increased body fat percentage in boys compared to healthy controls (131).

Altered body composition in ETPKU patients is a result of a combination of several causative factors. One of these factors is the PKU specific carbohydrate and fat rich diet from birth. It is well described in non-PKU patients, that altered nutritional habits during early ages contribute to higher risk of metabolic alterations later (160). Previous studies found that daily calory intake for classical PKU patients is significantly higher than that of patients with mild PKU and of patients with HPA (approximately 100 and 200 kilocalories respectively) (130). Appetite stimulative effects of amino acid supplements is another potential contributing factor. These medical products absorb rapidly and lead to ghrelin-induced appetite stimulation (125, 161, 162). As a consequence, the intake of medical and Phe-poor foods high in fats and sugars increases (163). Partially contradicting to this statement, male patients with stricter dietary and better supplement adherence did not suffer from higher prevalence of obesity.

Studies, which examine physical activity among PKU patients are gravely sought for as sedentary lifestyle significantly increases risk for obesity. A decrease in physical activity is proven in healthy children from age 5, but it is still not known whether this statement is true for PKU patients (164). Results regarding adult patients are scarce and contradictory. A recent investigation concluded that adolescents with PKU are inactive physically and that activity levels further decline during transition to adulthood. However, this finding did not go hand in hand with a correlation between fat mass and physical activity in this age group (165). On the other hand, Jani et al found, that intense physical activity did correlate with lower fat content in both adult and pediatric PKU population (166).

4.2.2. Nutritional parameters

Patients who have optimal Phe-free diet adherence but do not consume the calculated amount of AAS are at risk to protein insufficiency (167, 168). Serum albumin, prealbumin and total protein concentrations are widely used markers for assessing protein malnutrition (168). We report normal levels of prealbumin, albumin and protein by all participants but a relative decrease in prealbumin and weaker therapy adherence was found by female patients compared to male counterparts.

Not only obesity was more prevalent in female patients, we also found significantly higher total blood cholesterol compared to male patients whereas LDL also showed a tendency to be higher. LDL was used as an important surrogate marker for cardiovascular risk earlier but recently more precise lipoprotein ratios were introduced. LDL/HDL cholesterol ratio is proven to be effective in showing the risk caused by the unfavourably modulated balance in atherogenic and protective lipoproteins (169). We cannot conclude that female PKU patients have a greater risk for cardiovascular diseases as consequence of altered lipoproteins as LDL/HDL ratio was not significantly different between males and females.

4.2.3. Correlation analysis between body composition and therapy adherence

No significant correlation was found between long-term (10 years) therapy adherence and MF-BIA parameters in this adult PKU patient group. Although there are conflicting previous results, we assume based on our results that medium to long term dietary

compliance and AAS consumption does not lead to altered body composition in adult patients. Doulgeraki et al. investigated adolescents with PKU and reported correlation between unfavourable body composition changes and suboptimal diet adherence (170).

Similarly, Evans et al reported that higher natural protein consumption in children with PKU is associated with healthier body composition (125). In contrast to these findings and in line with our results, authors of a recent publication (which also used MF-BIA) concluded that neither natural protein intake nor metabolic control influence body composition in adolescent patients (165). A recently published meta-analysis investigating the relationship of dietary adherence and obesity in PKU patients reached the conclusion, that dietary Phenylalanine restriction itself is not a risk factor for obesity (171).

We report better therapy compliance (both better dietary adherence and AAS intake) in adult male patients compared with females. This is unusual as women of childbearing age are supposed to follow diet stricter to avoid potential MPKUS (172).

4.2.4. Limitations

Despite the fact that several published articles use BIA methodology in PKU patients, validation in this population is insufficient. Although a specific multifrequency-BIA machine was proven to be precise in patients with PKU (173), in this study we used the same methodology but a machine of a different manufacturer. Mean age of female PKU patients was higher – although not statistically significantly - than that of males. It can not be ruled out, that this contributed to differences found in the two sexes when assessing dietary compliance and body composition. Future studies are needed to further prove or refute these findings.

4.3. Bone mineral density in early-treated, adult PKU patients using DXA

The long-term significance of decreased BMD found in some patients is not entirely discovered yet. We performed the first study in Hungary to assess bone mineral density in adult ETPKU patients and to investigate potential correlation with dietary adherence.

4.3.1. Interpretation of Bone Mineral Density results

DXA is the golden standard to assess BMD. Two derived values are used to define the deviation from the population mean of an individuals bone density. As T-scores can be misleading before substantial bone loss has already occurred, Z-scores are used in children and young adults: Z-score reflects an ethnicity adjusted value normalized to age and sex matched controls. According to the ISCD guideline a Z-score below -2 represents BMD „below the expected range for age" but osteoporosis should not be diagnosed unless it is associated with significant fracture anamnesis (174). The prevalence of BMD „below the expected range for age" is estimated to be around 10% in young adult patients with PKU (93). We found slightly more patients with a Z-score under -2 (13%). We also report, that 42% of young, adult ETPKU patients had Z-scores below -1 SD whereas international literature suggests a prevalence of 33-60% (93, 175, 176). Frank osteoporosis was not diagnosed as no patient had significant fracture history. Our results suggest that the distribution of bone mineral density in Hungarian patients is similar to that reported worldwide. Although BMD was within the normal range in the majority of these patients, reduced bone density in a minority of patients may increase their risk of developing osteoporosis later in life. Osteoporosis may evolve silently but it leads to significant morbidity as pathological fractures occur (177, 178). Hence there is no specific osteoporosis prevention and treatment strategy for PKU, general prevention can be applied to these patients as well (179). Continuous monitoring and longitudinal studies with repeated assessments are much needed to achieve proper patient care and to prepare for further bone loss.

4.3.2. The relationship between metabolic parameters and BMD

The change of BMD did not show significant correlation with Phe, Tyr or the Phe/Tyr ratio. It seems, that suboptimal metabolic control in adulthood does not lead to significant decrease in bone density in the medium term. This is further supported by the fact that we found no difference in bone mineral density change between the therapy adherent group and the group on a relaxed diet. Recent studies in children and in young adults with PKU have not been able to confirm the previous belief that high blood Phe levels are strongly associated with reduced bone density (176, 180). Our results fit into this trend. Although the average blood Phe concentration in our study population of young adult PKU patients treated from birth was only slightly above the upper target

treshold (614 μmol/L), we found that more than half of these patients (53%) only partially adhere to therapy, having supraoptimal Phe values regularly. Short-term adverse effects of diet inadherence on bone density is not supported by this study, but its adverse neurocognitive effects are already known (181).

4.3.3. Limitations and strengths

The study has several limitations that need to be taken into account when evaluating the results. The metabolism and forming of bone tissue is regulated by a complex system. Several factors which may have an important role were not investigated in this study. A more accurate picture could have been obtained by measuring markers of bone metabolism, lifetime blood Phe levels, or by analysing microstructural alterations of bone biopsies. Furthermore, we determined diet adherence by blood Phe levels, although keeping and analysing a food diary would have given a more accurate picture. It is also known that physical activity influences bone metabolism and bone density, which this study did not investigate.

On the other hand, we were able to recruit a large patient group compared to the rarity of PKU and to previous investigations in this field. We were the first to study adult, early-treated PKU patients in Hungary for bone mineral density.

5. Conclusion

5.1. Assessment of Health Related Quality of Life outcomes in early-treated adult PKU patients using the PKU-QoL adult questionnaire

1. Suboptimal metabolic control is negatively associated with HRQoL. Similarly, therapy adherent patients with classical PKU reached better scores in most domains compared to partially-adherent peers.

2. Most patients rated their general health status as at least good compared to healthy peers and they rarely reported severe complications of living with PKU. We conclude, that disease management and PKU related emotional burden causes the most difficulties in this patient group. We recommend using the newly developed disease specific PKU-QoL in adults as we see it as an useful tool to estimate patient well-being and to improve long-term care.

5.2. Body composition assessment of early-treated, adult Phenylketonuria patients using MF-BIA

1. Although lifelong natural protein restricted diet with amino acid supplements is supposed to alter body composition, we found no significant correlation between body composition parameters and therapy adherence in a 10-year interval among early-treated, adult PKU patients.

2. We conclude, that overweight and obesity is highly prevalent in young adult PKU patients, especially in women. It is important to aim for better weight management in PKU care to achieve healthier body composition and therefore minimise the risk for secondary consequences (e.g. metabolic syndrome, cardiovascular diseases).

5.3. Bone mineral density in early-treated, adult PKU patients using DXA

1. We were the first to assess Bone Mineral Density among early-treated, adult PKU patients in Hungary. We report a similar prevalence of BMD below the expected range of age compared with previous reports in the international literature. We stress the importance of regular monitoring for patients with already decreased BMD as a further decrease is expected with advancing age.

2. We found no significant association between BMD and metabolic control during this investigated few year interval. This finding suggests that suboptimal diet adherence may not contribute substantially to further decrease in BMD in adults. We stress the importance of conducting further studies to reveal the exact pathomechanism of bone loss in PKU.

6. Summary

Although the prognosis of PKU has greatly improved in the last decades, the pathomechanism is still insufficiently understood and life-long adherence to therapy involves many sacrifices. Severe symptoms are seldom reported in this patient group but both the disease and its treatment may contribute to long-term complications. The main aims of my doctoral research were to assess and contribute to the timely prediction of these long-term complications and to investigate the relationship between these abnormalities and treatment adherence.

We assessed HRQoL in Hungarian adult patients using the newly developed PKU-QoL questionnaire and investigated the relationship between therapy adherence and subjectively perceived QoL outcomes. We conclude, that suboptimal metabolic control is negatively associated with patients HRQoL. Furthermore, we found that classical PKU patients with good therapy adherence reached better scores in most domains compared to peers with partial adherence. We stress the importance of maintaining good therapy adherence in adulthood and of regular monitoring HRQoL to improve outcome.

Life-long natural protein restricted diet with amino acid supplements may lead to altered body composition. We used MF-BIA for detailed assessment of body composition and found no distinct correlation between body composition parameters and adherence to diet in a 10-year interval. We conclude, that obesity is an important comorbidity in young adult PKU patients, especially in women. Weight management should be an additional goal of PKU care to achieve lower rates of obesity and therefore to minimise the risk for metabolic syndrome.

We were the first to study bone mineral density and to compare BMD change with adherence to therapy in adult PKU patients in our country. Similarly, to what is seen in international literature, we found BMD below the expected range of age in a proportion of patients. Low bone mineral density observed in young adults is relevant because of potentially increased risk of osteoporosis later in life. Our results suggest that keeping a relaxed diet does not contribute significantly to decrease in bone mineral density in adulthood. We presume, that peak BMD is already decreased in affected patients, but further studies are much needed to prove or reject this hypothesis.

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8. Publications under my authorship

8.1. Publications directly related to the thesis

1. **Barta AG**, Sumanszki Cs, Turgonyi Zs, Kiss E, Simon E, Serfozo Cs, Reismann P, (2020). Health Related Quality of Life assessment among early-treated Hungarian adult PKU patients using the PKU-QOL adult questionnaire. Molecular Genetics and Metabolism Reports 23: 100589.
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2. **Barta AG**, Sumanszki Cs, Reismann P, (2017). Csontanyagcsere felnőtt phenylketonuriás pácienseknél – hazai adatok [Bone metabolism in adults with phenylketonuria - Hungarian data]. Orvosi Hetilap 158: 1868-1872.
   Impact factor 2017: 0.322

3. **Barta A, G,** Becsei D, Kiss E, Sumánszki C, Simonová E, Reismann P: The Impact of Phenylketonuria on Body Composition in Adults. Ann Nutr Metab 2021. doi: 10.1159/000520047
   Impact factor 2021: 5.923

8.2. Publications not directly related to the thesis

4. Sumánszki Cs, **Barta AG**, Reismann P, (2017). Phenylketonuria felnőttkorban [Adult phenylketonuria]. Orvosi Hetilap 158 : 1857-1863.
   Impact factor 2017: 0.322

5. Serfozo Cs, **Barta AG**, Horvath E, Sumanszki Cs, Csakany B, Resch M, Nagy ZZ, Reismann P (2020). Altered visual functions, macular ganglion cell and papillary retinal nerve fiber layer thickness in early-treated adult PKU patients. Molecular Genetics and Metabolism Reports 25: 100649.
   Impact factor 2020: 2.797

6. Serfozo Cs, **Barta AG**, Horvath E, Sumanszki Cs, Csakany B, Resch M, Nagy ZZ, Reismann P. Reduced macular thickness and macular vessel density in early-treated adult patients with PKU. Mol Genet Metab Rep. 2021 May 5;27:100767. doi: 10.1016/j.ymgmr.2021.100767. PMID: 34026550; PMCID: PMC8121983.
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