Aus der Kinderklinik und Kinderpoliklinik im Dr. von Haunerschen Kinderspital
der Ludwig-Maximilians-Universität München
Direktor: Professor Dr. med. D. Reinhardt

Comparison of the physical health in adult patients with phenylketonuria (PKU) and healthy age-matched controls

Dissertation
zum Erwerb des Doktorgrades der Humanbiologie
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von
Juliana Thamar Freiin von Berlepsch
aus
Mittweida
2009

Mit Genehmigung der Medizinischen Fakultät
der Universität München

Berichterstatter:	Professor Dr. med. Berthold Koletzko
Mitberichterstatter:	Prof. Dr. Thomas Lang Prof. Dr. Yoon S. Shin-Podskarbi
Mitbetreuung durch den promovierten Mitarbeiter:	Dr. agr. J. Demmelmair
Dekan:	Prof. Dr. med. Dr. h. c. M. Reiser, FACR, FRCR
Tag der mündlichen Prüfung:	25.05.2009

Index of contents **Page**

Index of abbreviations

$A^{1\%}_{1cm}$	extinction coefficient
AA	arachidonic acid
AAS	atomic absorption spectrometry
Apo A1	apolipoprotein A1
Apo B	apolipoprotein B
APS	Working Group on Paedriatric Metabolic Disorders
AVLT	auditory verbal learning test
BH_4	tetrahydrobiopterine
BHT	butylated hydroxytoluene
BMI	body mass index
BMR	basal metabolic rate
CV	coefficient of variation
CWIT	colour word interference test
DHA	docosahexaenoic acid (22:6n-3)
DGE	German Nutrition Society
EDTA	ethylene diamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EPA	eicosapentaenoic acid (20:5n-3)
FAME	fatty acid methyl esters
Fig.	figure
GC	gas chromatography
HDL	high-density lipoprotein
HPA	hyperphenylalaninemia
HPLC	high performance liquid chromatography
IQR	interquartil range (25.- 75. percentiles)
IS	internal standard
LCPUFA	long-chain polyunsaturated fatty acid
LDL	low density lipoprotein
Lp (a)	lipoprotein (a)
MPT	motor performance task
MPKUC	maternal phenylketonuria collaborative study
MUFA	monounsaturated fatty acids
NCT	number combination test
NEFA	non-esterified fatty acids
NIST	national institute of standards and technology
PAL	physical activity level
Phe	phenylalanine
PKU	phenylketonuria
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids
SD	standard deviation
SEM	standard error of the mean
Tab.	table
TC	total cholesterol
TFA	trans fatty acids
TG	triglycerides
TLC	thin layer chromatography
Trp	tryptophan
Tyr	tyrosine
UV	ultraviolet
VEP	visual evoked potential
VLDL	very low-density lipoprotein
vs.	versus
yrs	years

Index of figures **Page**

Index of tables **Page**

1 Introduction

Phenylketonuria (PKU) is an inherited metabolic disorder that appears in about one out of every 10,000 births in Germany. It results from a deficient activity of the liver enzyme phenylalanine hydroxylase (PAH), which leads to elevated levels of the amino acid phenylalanine (Phe) in the blood. When a strict diet is implimentated and maintained children with PKU can expect normal development, whereas dietary noncompliance can result in a decline in mental and behavioral performance.

As a consequence of screening newborns for today there are increasing numbers of patients who have been treated early in life for PKU. Current concepts propose a “diet for life”. Yet studies show that PKU-patients often lack an adequate medical and nutritional supervision in adult life (96;115;159) even after intensive care during childhood. This fact is suggested to additionally influence the long-term physical health of adult PKU-patients (96;159) apart from the early and strict diet in childhood. In contrast to the situation in the USA and Australia, where dedicated centers with professional medical and nutritional care for adult PKU-patients emerge, in Germany health care for this group of patients is not well developed. Therefore adequate medical and nutritional support is not provided for the majority of these patients living in Germany (159).

The dietary compliance of the PKU-patients decreases with age and especially during adolescence since young adults strive for independence and therefore often loosen or even discontinue their diet (96;115). Furthermore, there is a lack of age-adapted dietary guidelines and nutritional education. Since many adult patients are vegetarians, diet might be unbalanced. This can lead to nutrient deficiencies. Studies showed inadequate intakes of long-chain polyunsaturated fatty acids (4;63;130;148;177), minerals (calcium, magnesium, zinc, copper, selenium, iron) (5;22;24;44;67;74;110;122) and vitamins (41;70;144;147;153;158;177) in adult PKU-patients due to a vegetarian diet. Moreover adult PKU-patients tend to have high fat and sugar intakes, which might have adverse effects on their lipoprotein profile (156).

While untreated PKU is characterized by microcephaly, epilepsy, severe mental retardation and progressive supranuclear motor disturbances, patients treated early reveal only minor neurological signs, such as tremors, hypo-, hyperactivity, convulsions, ataxia, loss of coordination and/or brisk deep tendon reflexes (96;107;120;140;173). Although these symptoms seem to be stable over time in the vast majority of patients, progression of neurological symptoms occurred (140;173;180) in a smaller number of patients. Psychological testings reveal lower performance in neuropsychological attention tasks, which is influenced by the current plasma concentration of phenylalanine (186).

A high frequency of psychiatric disorders was detected in PKU-patients, i.e. of the internalizing type, especially behavioral abnormalities, depressions and emotional

impairment (168). However, it is unclear whether this can be attributed to the chronic condition itself or to disease-related impairments in the brain function. While visual evoked potentials (VEP) consistently show prolongation of latencies in 30 % of early-treated adult PKU-patients, somatosensory evoked potentials and peripheral motor nerves sensory tests are inconsistently prolonged in about 10 % of the patients. Early auditory evoked potentials mostly show normal profiles (140;184). A recent multicenter study in the USA found that adult PKU-patients who discontinued their diet suffer more frequently from eczema and bronchial asthma compared to adults who continue their diet (96). There are also indications that PKU-patients show alterations in the immune system (88;146). Karagoz and colleagues (88) found that children with PKU had lower immunoglobulin levels compared to healthy children.

Thus the present work deals with the question in dispute whether and if so, how strictly adult patients with phenylketonuria should be treated and which effects and side effects could then be described as results of a low-Phe diet.

1.1 Definition of phenylketonuria

The autosomal-recessive inherited defect of the liver enzyme phenylalanine hydroxylase (PAH) leads to an increase of Phe in all body tissues by blocking and obstructing of the hydroxylation of the amino acid phenylalanine (Phe) into tyrosine (Tyr). Depending on the existing residual activity of PAH, Phe concentration increases with varying speed and extension in the bodies of patients. Clinical symptoms appear in untreated patients in relation to the amount of blood Phe concentration, mainly in the most extreme case of classical PKU (PAH activity <1 %). In Germany, there is a classification of PAH defects according to the severity code of the disorders:

- *Classical PKU* with blood Phe concentrations above 20 mg/dl (>1205 µmol/dl) without diet and a Phe tolerance below 400 mg/day
- *Mild PKU* with blood Phe concentrations between 10 and 20 mg/dl (602-1205 µmol/dl) and Phe tolerances between 400 and 600 mg/day
- *HPA* with blood Phe concentrations maximal 10 mg/dl (>603 µmol/dl)
- *Tetrabiopterine (BH_4) sensitive PAH defect* with a normally maximal blood Phe concentration of 20 mg/dl (1205 µmol/l)

In the English literature HPA describes all conditions with an increase of blood Phe concentration regardless of the causes.

Eponymous for the disease is the increasing excretion of phenylketones like phenylpyruvate, which is a direct degradation product of Phe (58).

In contrast to PKU and HPA maternal PKU describes an independent clinical picture in newborns caused by high blood Phe-levels of a mother with PKU. High blood Phe concentrations of the mother are dangerous for a developing fetus and can lead to an embryopathy without any relation to the genetic status of the fetus. Therefore women with PKU who are off the special PKU-diet should restart it and have their blood Phe-levels carefully monitored.

1.2 Clinical symptoms of phenylketonuria

If classical PKU remains untreated, developmental delays appear, as well as a special mousy odor and an eczema-like rash can appear at 5-6 months of age or even earlier. In the following early years of life the deficient mental development with microcephaly, mental and psychosocial retardation or fair hair and skin become more explicit (140;161).
In untreated patients with HPA symptoms may appear later or milder than in patients with PKU due to a relatively high residual activity of PAH. Sometimes developmental delays are not even detected until the investigation for school enrolment.

Thus, typical clinical symptoms of untreated, lately detected and/or badly adjusted patients with classical PKU are the following:

- mental retardation (IQ score < 50)
- motor disturbances
- microcephaly
- EEG alterations in 50 % of the cases
- hyperactivity
- increased muscle tone and more active muscle tendon reflexes
- vomiting
- fairer hair and skin than other family members
- irritability and eczema-like rash
- typical mousy odor to the urine (phenylpyruvate)

Less frequently:

- prominent cheek and upper jaw bones
- widely spaced teeth
- poor development of tooth enamel
- cataracts
- decreased body growth (lack of growth hormone)

The higher the residual activity of PAH and the lower the blood Phe-levels the milder are the clinical symptoms. Residual activities below 2 % seem to lead to the classical picture of PKU.

Intelligence deficits do appear, if blood Phe concentrations are permanently above 8 mg/dl (484 µmol/l) during infancy. Children with blood Phe-levels up to 13 mg/dl (792 µmol/l) often show significant disorders related to attentiveness (12).
However, in the literature some symptom-free cases are described in spite of very high Phe-levels. So far, no explanations have been found why these did not develop the typical clinical picture of PKU although lower Phe-levels were found in the brain tissue of such subjects than in patients with the classical PKU phenotype (140;189).
Infants born to mothers with elevated Phe-levels during pregnancy are at risk for mental retardation, microcephaly, low birth weight (< 2500 g), spontaneous abortions, congenital heart diseases and dysmorphic face similar to alcohol embryopathy depending on the Phe-level. Due to an active Phe transport, blood Phe-levels of the fetus are twice as high as in the placenta. The most severe defects were observed in mothers with blood Phe-levels above 20 mg/dl (1210 µmol/l). Furthermore a lack of tyrosine, protein and/or energy and vitamins, as well as too little weight gain can contribute (116).

1.3 Biochemistry of phenylketonuria

There are many investigations into the pathologic mechanisms that lead to the development of the severe mental damage resulting from high Phe-levels, which indicate that it is most likely caused by a multifactor process. A shared, energy-dependant carrier transports the amino acids Phe, Tyr and Trp, which are very important for the brain, but also Val, Ile, Leu, Lys and His. In PKU there is an enormous imbalance of amino acids with very high levels of Phe, as well as normal or even reduced concentrations of other amino acids which lead to an overloading of the carrier with suppression of the other amino acids and reduced intracellular concentrations (43;121). The lack of Trp mainly results in aggregation disorders of the polyribosomes with a reduction in protein synthesis (10;169). It works as a limitating factor of the protein synthesis. Moreover the sulfate synthesis is possibly inhibited by the reduction of cyclic AMP and phosphoadenosin-5-phosphorsulfate, which is needed for sulfate synthesis (81). Furthermore, a reduction of cerebroside synthesis due to reduced cerebroside concentrations is assumed with regard to previous investigations in deceased PKU-patients.
Recent investigations in mice with PKU indicate that elevated Phe-levels can block the cerebral cholesterol synthesis and thus cause alteration of the oligodendrocyte function, as well as a reduction of melanin synthesis (51).
In addition an inhibition of synaptogenesis in neuronal cell cultures was found (76).
It has not become clear so far, whether the inhibition of protein synthesis, energy metabolism, cholesterol, cerebroside or sulfate synthesis or combinations of these parameters might cause alterations in myelinisation and morphologic brain structure.

Moreover the important neurotransmitter dopamine is synthesized from tyrosine and dopa. It has been demonstrated in patients with PKU that their dopamine are reduced as was confirmed by studies of liquor. In addition to that the synthesis of serotonin and Trp is restrained. A lack of these neurotransmitters leads to an inadequate development of the dendrites and thus to alterations of the brain function with a loss of cognitive skills. Some studies have also shown that e.g. the nerve conduction and most cognitive functions depend explicitly on the serotonin and 5-hydroxyindol acetate levels in the liquor, which in turn strongly depends on Phe-levels. So it is obvious that Phe influences brain metabolism in many ways (111).

A further possibly important reason for the reduced synthesis of dopamine is the direct inhibition of tyrosine hydroxylase. Phe concentrations of approximately 15 mg/dl inhibited tyrosine hydroxylase up to 80 % (133) which seems to indicate a lack of neurotransmitters, too. Perhaps the inhibition of amino acid transport into brain is not the considerable cause of structural and functional alterations in the brain. In patients with tyrosinemia type II and histidinemia e.g. similar imbalances of amino acids are observable but do not lead to alterations of neurotransmitters (79).

An overview of the metabolism of Phe is shown in figure 1:

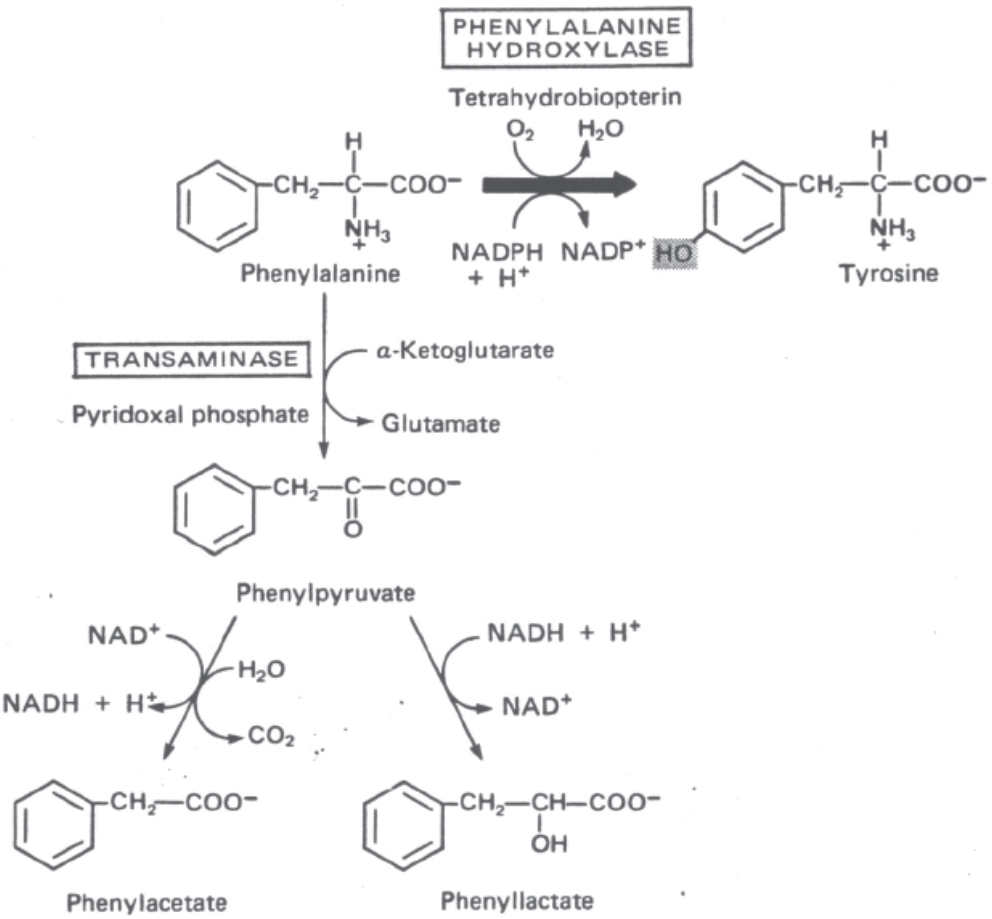


Fig. 1: The major and minor pathways of Phenylalanine (Phe) metabolism

1.4 Diagnosis of phenylketonuria

Newborn screening for elevated blood Phe-levels generally allows for an early diagnosis of the different types of HPA with normal protein intake. The method measures even small changes in Phe-levels. Current methods based on mass spectrometry are much more sensitive and replace the bacteriologic inhibition test pioneered by Robert Guthrie (69). In Germany, today the neonatal screening for inherited metabolism disorders is carried out exclusively by tandem mass spectrometry (1). The optimal time of blood withdrawal is on the 3rd day of life. An early detection within the first 36 hours is definitely possible by measuring Phe, Tyr and their quotient. Phe-levels above 2.4 mg/dl and a ratio of Phe to tyrosine above 2 in the first 36 hours of life are suspicious, but should be confirmed by a 2nd measurement preferable on the 3rd, 4th or 5th day of life, or thereafter.

Biochemical findings

Typical for patients with PKU is a marked elevation of Phe-levels relative to normal levels, and reduced levels of tyrosine (table 1). Table 2 shows different metabolites produced by degradation of Phe and excreted in the urine (128):

Tab. 1: Plasma Phe and Tyr concentrations (39)

Status	Phe (mg/dl)	Tyr (mg/dl)
Normal (birth to 3rd month)	Up to 3	Up to 3.6
Normal, not fasting (3rd month-14 yrs)	0.3-2.2	0.6-1.8
Normal, fasting (3rd month-14 yrs)	0.5-1	0.6-1.4
Classical PKU (older than 72 hrs)	>3	<1
Untreated classical PKU (not later than babyhood)	>15	<1

Tab. 2: Metabolites excreted in the urine in untreated PKU-patients (170)

Metabolite	Normal (mmol/mol creatinine)	Classical PKU (mmol/mol creatinine)
Phenylpyruvate	0-4	300-1,000
Phenyllactate	<2	200-1,000
2-hydroxy phenylacetate	<2	50-2,000

The activity of PAH can be measured directly in liver biopsates. Activities below 1 % lead to the clinical picture of classical PKU and those above 5 % (up to 35 %) lead to HPA. In a few patients with mild PKU direct measurements were carried out *in vivo* by application of deuterized Phe (161).

Genetic findings

The gene encoding PAH is located on chromosome 12q22-q24.2. So far more than 530 mutations have been described (72;72;106;142;161;194). There are three main types of mutations observed in about 50 % of German patients.

While the prevalence of classical PKU in Germany is one in about 11,600, HPA occurs significantly less often (54). However, in other countries PKU is more (1:7,400 in Turkey or 1:4,500 in Ireland) or less common (1:20,000 in Bulgaria and very rarely in Finland) and in Japan HPA is more common than PKU.

The detection of heterozygotes is possible by:

- Measurement of Phe and Tyr levels in blood, as well as Phe metabolites in urine after exposure with protein and Phe (100 mg/kg body weight L-Phe or protein)
- Examination of PAH activity by liver biopsy
- Determination of Phe turnover e.g. by ^{13}C breath test (27)
- Detection of mutations in the PAH gene

1.5 General treatment concepts for phenylketonuria

Since the beginning of the 1950s patients with PKU and HPA have been treated with success (20) if a strict diet was started very early and maintained at least up until puberty (31;140). Cases have been described recently, in which the administration of BH_4 in the scope of the differential diagnosis of the HPA led to an decrease of blood Phe-levels (often within 8-24 hours after administration of BH_4) even without the presence of severe inborn disorders in BH_4 metabolism. The maximal Phe concentrations were mostly below 20 mg/dl (1205 µmol/l). Investigation in the PAH gene showed very different mutations. In some patients the Phe-levels were permanently reduced to normal values by substitution of BH_4 without any further dietetic treatment (17;104).

Principles of dietetic treatment

The treatment normally consists of a low-Phe but Tyr-enriched diet, which should start without delay after an early diagnosis in the neonatal period. After detection of elevated Phe-levels by newborn screening a differential diagnosis must be made to determine if there is an inborn defect in the BH_4 metabolism (BH_4 is a cofactor of PAH). Therefore a BH_4 loading test

is carried out by exposing the patient with 20 mg BH_4/kg body weight followed by an observation time of at least 24 hours. Phe-levels from at least 6 mg/dl (364 µmol/l) at the time of administration are considered to be an optimal precondition (17).

If the cause of Phe elevation is a lack of the cofactor BH_4, e.g. due to a synthesis defect, this results in a rapid decrease of Phe and increase of Tyr levels after oral administration of BH_4. Additionally, different pteridines in the urine before and after administration of BH_4, as well as the activity of dihydropteridine reductase in dried blood samples can be measured to differentiate BH_4 metabolism defects. BH_4-responsive forms of PAH defects often lead to a marked decrease of blood Phe-levels after approx. 24 hours (at all events later than 8 hours). However, the low-Phe diet in patients with Phe-levels above 8 mg/dl (485 µmol/l) is not to be started until a lack of BH_4 is excluded (21).

The main principle of dietetic treatment is a strict restriction of protein supply and a reduction of Phe-intake down to the amount required for protein synthesis. In infancy and during growth spurts such as puberty, the tolerable Phe-intake per kg body weight is higher than during other times of childhood. Since there is a lack of tyrosine, its additional administration is advisable. In practice, the low-Phe diet requires the exclusion of protein rich foods such as meat, fish, eggs, milk, dairy products, nuts, wheat, grains, corn, beans and lentils, whereas fruits, vegetables, rice and potatoes are at least allowed in controlled quantities. Due to the limited intake of natural protein (60-80 % lower than in healthy subjects) administration of an amino acid mixture containing all essential amino acids and tyrosine is necessary to cover the requirements for nitrogen, and this is enriched with vitamins, minerals and trace elements to secure the needs also of these nutrients. Above all these factors, an adequate energy intake is essential to reach normal growth rates and to prevent degradation of body proteins. Basically this diet is achieved with the help of commercial dietetic products low in protein and by replacing foods rich in protein, as well as fat and carbohydrates (178).

The patient should adhere to the diet for their entire lifespan, but beyond the age of 10 years the diet can be gradually loosened (31). Since patients with HPA do have a higher Phe tolerance, a low-protein diet without supplementation of amino acids may be sufficient. HPA patients with plasma Phe-levels <600 µmol/l (<10 mg/dl) do not require any dietetic treatment (188), and in BH_4–responsive patients often an administration of BH_4 alone will suffice.

Thus the aims of the dietetic treatment are:

- Reduction and maintenance of the recommended blood Phe-levels
- Maintenance of normal plasma Tyr concentrations
- Normal equilibrium and mental development
- Normal weight gain up to the end of infancy and weight maintenance in adulthood

- Prevention of catabolic conditions (degradation of protein surpasses synthesis) due to an insufficient energy and protein intake, which leads to elevation of Phe
- Fast termination of catabolic conditions (e.g. fever, vomiting, diarrhea, physical strain, weight loss) by high energy and adequate protein intake

First adjustment in newborns

If no defects in pteridine metabolism have been shown in the BH_4 loading test, a Phe-free diet is started for approximately three days (total withdrawal of natural protein and Phe) to reduce the initially elevated Phe-levels. During these days, breastfeeding mothers have to pump and freeze breast milk to maintain milk production for subsequent breastfeeding. The baby is fed only Phe-free formula made of L-amino acids, lipids, carbohydrates which is enriched with minerals, trace elements and vitamins. The aim of this treatment is to reduce blood Phe-levels below 4 mg/dl (242 µmol/l). Empirically one can assume that Phe-levels decrease approximately by 4 mg/dl (242 µmol/l) per day. Once the Phe-levels are 8 mg/dl (484 µmol/l) or lower some natural protein can be given in addition to the Phe-free formula, e.g. by provision of breast milk. Initially about 30 mg/dl Phe per kg body weight can be administered.

1.6 Therapy of phenylketonuria in adulthood

Adult PKU-patients are a very heterogeneous group:

1) Diagnosed late and untreated
2) Diagnosed late but treated
3) Treated early but early termination of the diet
4) Early treated but badly adhered to diet
5) Early treated with consequently maintained diet
6) Maternal PKU

Diagnosed late and untreated

These patients often live in special institutions for handicapped people, where a dietetic treatment is often poorly administered. However, experience supports to continue treatment or even starting treatment at a late age. Starting this treatment might even reduce the nursing costs and may improve the quality of life.

Diagnosed late but treated

The later the diagnosis, the later treatment is started and therefore the worse the prognosis is for adulthood. Nevertheless, often even treatment started at a later time leads to a better cognitive performance.

Treated early but early termination of the diet

Early termination of the diet results in more marked clinical symptoms in adulthood.

Early treated but badly adhered to diet

Similar observations are also possible for this group when the diet was consequently not maintained during infancy.

Early treated with consequently maintained diet

An early diagnosis, immediately followed by proper treatment and maintenance of said diet leads to adequate physical and mental development.

Dealing with such a heterogeneous group of the PKU-patients, realistic aims of the therapy should be defined and discussed for the individual case in cooperation with the treating expert. Following the recommendations of the Working Group on Pediatric Metabolic Disorders (Arbeitsgemeinschaft Pädiatrische Stoffwechselstörungen, APS), patients at an age of 16 years and older should have serum Phe-levels below 20 mg/dl (1200 µmol/l). If there are neurological symptoms and/or behavioral disturbances detectable, a reduction of the symptoms may be accomplished with a stricter dietetic treatment. In case of a terminated diet, regular cognitive and neuropsychological tests should be performed (11;176). The individual's life situation, their physical and cognitive development as well as their personal aims should be taken into consideration.

Energy supply following the D-A-CH recommendations (47)

The energy requirements of patients with PKU are considered same as in healthy subjects and depend on basal metabolic rate (table 3), working metabolic rate, thermogenesis after feeding, and added requirements such as those for growth, pregnancy and the nursing period. An important part of the energy turnover is the energy requirement for physical activities expressed as the physical activity level (PAL), a factor calculated depending on the intensity of the daily physical activities as shown in table 4.

Tab. 3: Basal metabolic rate (depending on age, gender, body weight)

Age	Body weight		Basal metabolic rate (kcal/day)	
	male	female	male	female
19 < 25 yrs	74	60	1820	1460
25 < 51 yrs	74	59	1740	1340
51 < 65 yrs	72	57	1580	1270

Tab. 4: Examples for physical activity levels

Severity of work and behavior in free-time	PAL*	Examples
Sleeping	0.95	
Exclusively sitting or lying way of life	1.2	Old, frail people
Exclusively sitting activity with little or no strenuous free-time activity	1.4-1.5	Office employee, precision mechanic
Sitting activity, temporary also additional energy demand for walking or standing activities*	1,6-1.7	Laboratory assistant, lorry driver, student, assembly-line worker
Mainly walking and standing work*	1.8-1.9	Housewife, shop assistant, waiter, mechanic, workman
Physically strenuous professional work*	2.0-2.4	Construction worker, farmer, miner, competitive athlete

* For 30-40 min strenuous free-time activities 4-5 times a week 0.3 PAL units can be added daily

Protein supply

The PKU diet is very restricted in protein content, and the amount of natural protein provided depends on the individual's Phe tolerance. Every protein containing food item has a content of approx. 3-6 % Phe, on average about 5 %. The natural protein intake from foods is not sufficient; therefore an Phe-free amino acid mixture should be taken several times per day to compensate for the low protein intake.

There is no reliable data about the biological equivalency and the bioavailability of nitrogen from amino acid mixtures. Therefore protein intake is often calculated following the recommendations of the German Nutrition Society (DGE) from 1985, which lie markedly above the current D-A-CH recommendations (47). This extra intake is supposed to compensate for a lower quality of the nitrogen source and the very fast resorption and metabolisation of the amino acids (59). Further scientific investigations are needed to determine the adequate amino acid intake for PKU-patients.

Phe tolerance

The amount of Phe provided depends on the individual's Phe tolerance and the target value for blood levels. If this is not known it should be determined. The upper limit of the patient's tolerance depends primarily on the residual activity of PAH and the individual's target value. In adults the desired Phe intake values usually are between 150 and 1,000 mg per day (131).

Amino acid mixture

The amino acid mixtures (tab. 5) should be given in at least three portions per day which are dissolved in adequate amounts of liquid (water, juice, lemonade, fruit puree or sugared instant powder) or semi liquid foods (e.g. apple puree) to prevent possible side effects from

high osmolarity such as abdominal disturbances or diarrhea. The amino acid supplement should be taken together with a meal providing natural protein and hence Phe for better utilisation. Studies showed that after the intake of free amino acids the levels of the amino acids initially increased but subsequently decreased faster than normal. The amino acids are quickly reabsorbed and then seem to break down oxidatively especially if insufficient amounts of Phe are available for protein synthesis (59).

Tab. 5: Some amino acid mixtures for adults and for pregnant women available in Germany

P-AM 3	SHS
p-am Anamix (flavored)	SHS
p-am Easiphen or Lophlex (flavored, ready to drink)	SHS
P-AM maternal (during pregnancy)	SHS
PKU 3 advanta	Milupa
PKU 3 shake or active (flavored)	Milupa
PKU 3 tempora (during pregnancy)	Milupa

New generation of amino acid mixtures

The new amino acid mixtures (p-am Anamix, PKU 3 shake or active) are prepacked in portion sizes that can be dissolved directly in an adequate amount of drinking water or even ready to drink (p-am Easiphen or Lophlex). Handling of these portions in everyday life has become a lot easier and at the same time the physiological osmolality supports better tolerance. An increase in the metabolic utilization of these amino acids is supposed to be achieved by the enrichment of these portions with fat, carbohydrates, minerals, vitamins, as well as trace elements. Different flavors are available to cover different tastes.

These amino acid mixtures enable the patient to handle them easily even outside the house, at the work place, at school, at the university or on holiday etc. Combinations with conventional amino acid mixtures are also possible.

Fat intake

Due to a lack of hidden fats, one has to use margarine, butter and oils for meeting the recommendations (tab. 6). The intake of soy, canola or walnut oil is recommended due to their high content of the precursor omega-3 fatty acid.

Tab. 6: D-A-CH recommendations for fat intake (47)

30 % of total energy intake	
Saturated fatty acids	<10 % of total energy intake
Monounsaturated fatty acids	>10 % of total energy intake
Polyunsaturated fatty acids	7-10 % of total energy intake
Linoleic acid (omega-6)	2.0 % of total energy intake
Linolenic acid (omega-3)	0.7 % of total energy intake

Carbohydrate intake

Foods rich in carbohydrates are mainly the special low-protein products and sugar. The assortment of fruits and vegetables eaten depends on the Phe tolerance and can be included in the menu by calculation or estimation. The recommendation of 30 g roughages per day often cannot be met due to a restriction of food assortment.

Patients with loosened dietary restrictions, i.e. only mild restrictions of protein intake and without the intake of an amino acid mixture, have to pay attention to ingest an adequate amount of minerals, vitamins and trace elements. Supplementation might become necessary (e.g. with the product Seravit). Patients with only minor restriction of protein intake may still require supplementation of protein intake by amino acid mixtures in special situations with high rates of protein synthesis, e.g. competitive sports or bodybuilding.

Possible critical nutrients

Iron, selenium, zinc, vitamins B and omega-3 fatty acids are possible critical nutrients in patients with PKU so that the calculation of micro nutrients at larger time intervals is recommended by food records (129).

Therapy monitoring

The following table shows the frequency of laboratory and clinical investigations in adult PKU-patients recommended by the Working Group on Pediatric Metabolic Disorders (11):

Tab. 7: Frequency of laboratory and clinical investigations (176)

Age	Laboratory investigation	Clinical investigation
> 15 yrs	Every 2-3 months	Every 6-12 months

Treatment of an elevated Phe-level

If Phe-levels are elevated the intake of natural protein has to be reduced, while still providing a sufficient energy supply. An increase of the dosage of the amino acid mixture might become necessary. With a reduction of the Phe-intake by 50 % a decrease of Phe-levels of 4 mg/dl can be expected. In such circumstances more frequent testing of lab parameters is often indicated. Additionally, an analysis of the causes of the increase in Phe-levels should be carried out to prevent a further increase of the serum Phe-levels.

Possible causes for an elevated Phe-level include:

- Increased intake of protein
- Disease (influenza, fever, vomiting, diarrhea)
- Insufficient energy intake
- Weight loss (catabolic condition, break down of protein)
- Stop of taking the amino acid mixture (insufficient protein supply)

Special situations

For traveling a sufficient amount of the amino acid mixture and other low-protein products should be packed to supply for the duration of the trip. Furthermore certificates for the amino acid mixture should be taken along to provide to customs officials, depending on the country visited. In many hotels there are breakfast buffets including selections of salads, vegetables, vegetarian dishes and fruits. If a restaurant visit is planned, Phe may be “saved” during the day to enlarge the choices. Often only minor alterations in the choice of side dishes or types of vegetables eaten are sufficient to create a meal with a lower content of Phe. A further possibility for adults is a mild loosening of the diet during holidays but, depending on its extent, the amino acid mixture should be taken regularly.

Further possibilities of treatment

In addition to the diet, there are the following further possibilities of treating patients with phenylketonuria: large neutral amino acids (LNAAs), *phenylalanine ammonia-lyase* (PAL) and somatic gene therapy.

Phe as well as other LNAAs are transported across the blood-brain barrier by means of an L-type amino acid carrier. High Phe-levels reduce the brain uptake of other LNAAs. Some LNAAs such as tyrosine and tryptophan are precursors of neurotransmitters, and it has been suggested that impaired neurotransmitter synthesis would be an additional factor contributing to the cognitive dysfunction observed in PKU. Thus a new therapeutic strategy particularly for adult patients is based on moderate protein restriction combined with supplementation of LNAAs except for Phe. It has not become clear so far, whether supplementation of LNAAs is

effective in patients with classical PKU or not. An elevation of tyrosine levels in the brain was previously demonstrated (9;97;118;123), but further studies are needed.

Another therapeutic resource currently under development for PKU treatment is the oral administration of *phenylalanine ammonia-lyase* (PAL). This enzyme acts by degrading Phe into ammonia and non-toxic trans-cinnamate, preventing the absorption of Phe. Thus Phe can be degraded in the intestinal lumen. The main problems associated with this approach is PAL inactivation by digestive enzymes. Adequate dosages are yet to be determined. So far, PAL has only been tested as a purified enzyme in humans, which has the disadvantage of high production costs. Organisms secreting genetically modified PAL offer better economical viability but have been tested only in animal models. Thus, preliminary results appear to be promising, but further clinical studies are still needed.

The ideal treatment of genetic diseases would consist of taking a normal copy of the defective gene and transferring it into the patient's cells, which should express it. Research based on conventional somatic *gene therapy* has seen some success (38;71;90;138), but the lack of truly appropriate vectors for gene transfer still needs to be overcome.

Maternal PKU

In contrast to determining an individual's target therapy in the heterogeneous group of adults, there are defined target values for the period of preconception and pregnancy. The serum Phe-level should lie between 2-6 mg/dl (120-360 µmol/l) and the tyrosine level between 0.8-1.8 mg/dl (45-100 µmol/l) (174). The amino acid mixture already contains tyrosine but should be prescribed additionally in case of persistent low serum levels. The target level of the therapy should be kept during the entire pregnancy to avoid intrauterine damage to the embryo and fetus due to elevated Phe-levels. Good Phe-levels should already be obtained in the preconception period. Ideally these levels should be stable between the last 2-3 months before conception.

Protein intake

Pregnant women with PKU should get more than 75 g of protein per day (table 8). For overweight pregnant women no recommendations exist.

Tab. 8: Recommended protein intake during pregnancy (174)

I. trimenon	1.1 g / kg body weight*
II.- III. trimenon	1.3-1.4 g / kg body weight*

*natural protein and amino acid mixture

Phe tolerance

The individual's Phe tolerance should be tested. Normally it lies between 200-600 mg/day and increases enormously after the 20th week of pregnancy because of elevated protein requirements due to fetal and uterine growth (approx. 10-5 mg/kg). Furthermore, the activity of PAH increases in the fetal liver (132;174).

Energy intake

In the first instance, an adequate weight gain during the entire pregnancy and especially during the first trimester is important to avoid catabolism with elevation of blood Phe-level (tab. 9). This can be measured by the BMI. Table 10 gives an overview of the recommended weight gain during pregnancy.

Tab. 9: Recommended energy intake (11)

I. trimenon	30-35 kcal / kg body weight
II.- III. trimenon	35-40 kcal / kg body weight

In some cases and especially at the beginning of the pregnancy, additional preparations for enhancing energy intake can become necessary to temporarily obtain an adequate weight gain (e.g. by use of maltodextrine). Due to long-lasting insufficient calorie intake caused by e.g. emesis or disease, a catabolic metabolism may develop and result in degradation of endogenous body proteins, with a resulting elevation of serum Phe-levels, against which an increased energy intake should be employed.

Tab. 10: Protocol of the American MPKUC-Study (117)

Body weight at the beginning of pregnancy (BMI)	< 20 underweight	20-26 normal weight	> 26 overweight
I. trimenon	2.3 kg	1.6 kg	0.9 kg
II.+ III. trimenon	0.5 kg/week	0.5 kg/week	0.3 kg/week
Total duration of pregnancy	12.5-18 kg	11.5-16 kg	7-11.5 kg

Fat intake

The fat intake should ideally be 30-35 % of the total energy intake following the D-A-CH recommendations (47). However, since protein intake is high this is difficult to achieve.

Minerals, vitamins and trace elements

The amino acid mixture is enriched with minerals, vitamins and trace elements so that a substitution of iron, iodine, vitamin B12 and selenium is not necessary but depends on dosage and the kind of supplement.

In patients with HPA, lower quantities of the amino acid mixture are provided, thus supplementation on folic acid and iodine should be provided.

A relative lack of selenium, carnitine and vitamin B12 was observed in adolescents after long-term treatment but no firm recommendations for their supplementation can be given.

Therapy management

The regular monitoring (twice a week) of serum Phe and tyrosine levels is of special importance so that the Phe-intake can be adapted following Phe tolerance. At baseline and in the 12th, 24th, and 36th week of pregnancy there should be a full amino acid profile and a major lab control. A weekly control of the body weight is useful. Continuous outpatient care is required due to regular lab controls and the monitoring and adaptation of the diet. In between, telephone contact usually suffices to give short therapy and practical advices. In the preconception period and during pregnancy an ambulant care of the patients is necessary. If there are extremely high values or patients who are hard to lead, an in-patient treatment can become necessary next to social indications. The treating gynecologist should be provided with general information, information about the treatment and laboratory test results.

1.7 Aims of the study

We aimed to study the quality of life and the nutritional, immune, cardiovascular, metabolic and neurological status of adult PKU-patients without interventions.

Hypotheses to be tested:

- Due to the diet, adult PKU-patients develop nutrient deficiencies (LCPUFA, minerals, trace elements and vitamins).
- An unbalanced diet in PKU-patients has implications for their immune system and cardiovascular risk profile.
- In comparison with healthy subjects of the same age, adult PKU-patients show distinct neurological abnormalities.

2 Materials and Methods

2.1 Subjects and study design

Thirty-three adult PKU-patients attending the Department of Metabolic Diseases of the Dr von Hauner Children's Hospital, University of Munich, as well as 33 healthy subjects of the same gender and age took part in the investigations. The control persons received financial compensation for their participation in the study.
Ethical permission of the study was obtained from the Bavarian Board of Physicians in February 2005. The study was conducted between June 2005 and July 2006.

Eligible were persons who fulfilled the following inclusion criteria:

- PKU-patients had to have a confirmed clinical diagnosis of PKU
- Age ≥ 25 years
- Written informed consent
- Controls had to be omnivores
- Controls should not suffer from metabolic, cardiovascular or psychological disorders
- Controls should not be on drugs influencing the lipometabolism during the last 3 months

2.2 Study procedure

The adult PKU-patients from the local PKU database and the control group were contacted. If they indicated consent, an appointment for the tests was made and informations about the study as well as the three-day dietary protocol and the leaflet with the site plan were sent to them (see 7.3.).
The duration of all investigations totaled approximately 4 hours for each patient, but only 2 hours for the controls since they did not need VEP, MRI, or blood withdrawal.

At the appointed day, the following schedule took place at the Dr von Hauner Children's hospital:

- ❖ Reception of the study subjects
- ❖ Oral study explanation
- ❖ Receiving 3-day dietary protocol and MRI
- ❖ Clinical investigation
- ❖ Standardized interview
- ❖ Atopic disease questionnaire
- ❖ Drawing and preservation of venous blood
- ❖ Visual Evoked Potential (VEP)

- Motor Performance Task (MPT)
- Auditory Verbal Learning Test (AVLT)
- d2 attention test (d2)
- Number Combination Test (NCT)
- Color Word Interference Test (CWIT)
- Handing out of the accelerometer

2.2.1 Clinical investigation

First, anthropometrical measurements were carried out followed by standardized medical procedures. For weight measurement, the subject stood over the center of the scale platform (Seca 702, Vogel & Halke, Hamburg) with the body weight evenly distributed between both feet. Weight was recorded to the nearest 100 g, and 1 kg was subtracted for the light clothes that were worn. Their height was measured using the stadiometer of the same scale. The subject was wearing socks so that positioning of the body could be seen and its weight was distributed evenly on both feet with the heels placed together. The head was in upright position and the moveable headboard was brought onto the most superior point on the head. The height was recorded to the nearest 0.1 cm. The body mass index (BMI) was calculated by dividing the measured body weight in kilograms by the square of body height in meters.

Blood pressure and heart rate were determined using a digital sphygmanometer (Dinamap pro series 100 V2, GE Medical Systems, Milwaukee) wrapping the cuff round the upper right arm, locating the brachial pulse medial to the biceps tendon and the arrow on the cuff over the brachial artery. The subject was sitting comfortably with the right arm at approximately heart level. After resting for five minutes, blood pressure was measured automatically by the digital sphygmanometer.

Skin disturbances and symptoms of atopic disease were investigated by physical examination and filling out a special questionnaire.

Furthermore, subjects were asked for personal data, education, dietetic treatment, contentedness with health and subjective health feelings by using a standardized interview. This applied interview is a revision of a structured questionnaire established at the University Children's Hospital Münster some years ago. The first part consists of questions about personal data, education, health, diet and the family of the patient, whereas the second part is modified to be filled in from control persons comparing both groups.

2.2.2 Neurophysiologic testings

Visual evoked potential (VEP)

Visual evoked potentials are normally used to evaluate optic neuritis, optic disorders, retinal disorders and demyelineating diseases such as multiple sclerosis. VEPs were measured in all patients and controls from the eye to the brain for detection of any differences of the nervous system function between both groups, and furthermore whether any reduction occurs in nerve conduction velocity in patients with PKU.

For measurement, a Medtronic Keypoint workstation (Medtronic Functional Diagnostics A/S, Skovlunde, Denmark) with the manufacture's computer software was used. The VEPs were performed with a 1.5 Hz alternating black-and-white checkerboard pattern (12 x 16 squares, check size 70') displayed on a 17-inch monochrome computer monitor in a darkened, quiet room. The subjects were seated comfortably 100 cm from the screen (fig. 2) wearing optical correction if needed, and the untested eye was patched focusing on a small spot of red light in the center of the computer screen. The recording electrode was placed at the Oz position of the 10-20 International System; the reference electrode was placed at the Fz position and the ground electrode was placed above the right eyebrow. All electrodes were fixed by contact paste and electrode impedence was kept below 5 kΩ. Signals were filtered with a bandwidth of 1 to 100 Hz. VEP recordings were performed twice with at least 50 stimulations each. Latencies P100 were determined and the mean of left and right side was calculated.

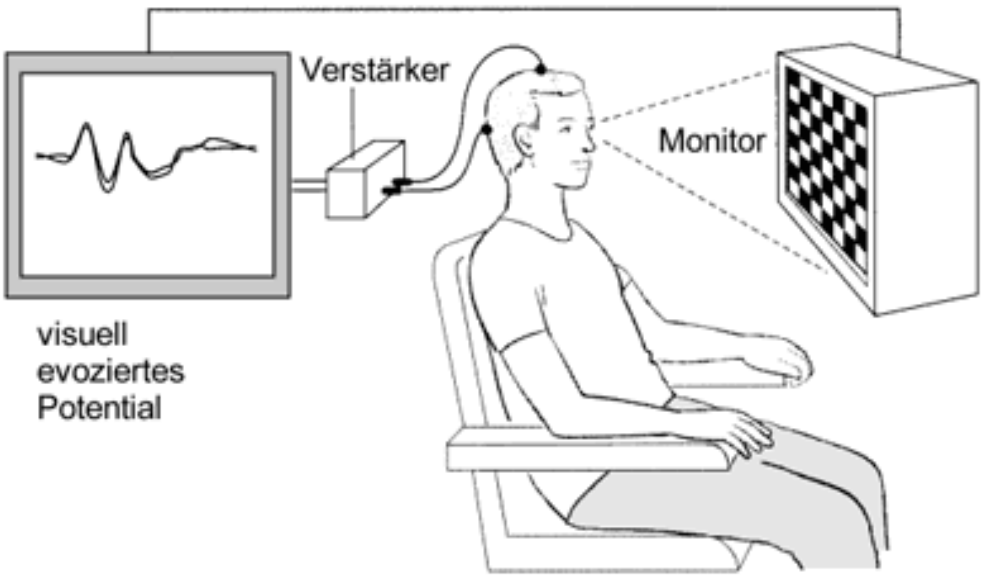


Fig. 2: Test arrangement for measuring VEP (195)

Motor Performance Task (MPT)

The motor performance task was applied to measure fine motor abilities through static and dynamic tasks for finger, hand and arm movement. It is a battery of tests developed by Schoppe based on Fleishman's factor-analytic examinations of fine motor abilities. The MPT analyzes six factors of fine motor abilities: aiming of motion, hand unrest (tremor), precision of arm-hand movements, manual dexterity and finger dexterity, rate of arm and hand movements, as well as wrist-finger speed. The dimensions of the work panel are 300x300x15mm and it is covered with boreholes, grooves and contact surfaces (fig. 3). A pen is attached to each edge (left and right). The right pen is black, the left one red. The following tasks have to be carried out on the work panel: steadiness (holding pen 32 sec vertically into hole without touching sides), line tracking (following line without touching sides), aiming (rapid hitting of all points within a line), inserting pins (rapid inserting of pins into holes of a line) and tapping (tapping 32 sec onto small plate as often as possible). The result table shows speed and/or accuracy measurements calculated for the right and left hand for one-handed and two-handed administrations. The testing time is about 15 to 20 minutes and can be applied to patients sevens of age and older.

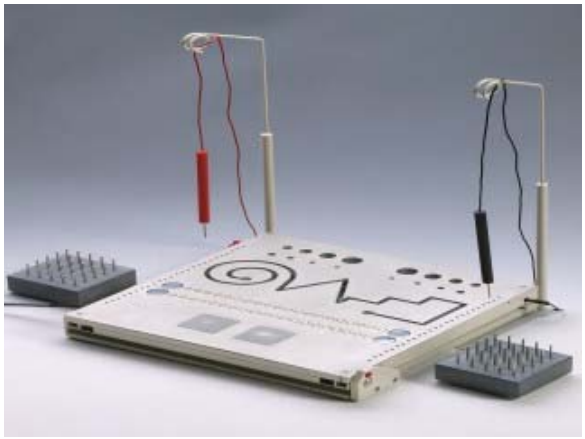


Fig. 3: Test arrangement for measuring fine motor skills (motor performance task)

2.2.3 Neurostructural testing

Magnetic resonance imaging (MRI)

Magnetic resonance imaging of the cranium was performed in PKU-patients due to a standardized protocol (T2 weighted FLAIR sequence using a 1 or 1.5 Tesla MR scanner). As shown in other studies, the aim was to discover deep white matter alterations that correlated to the most recent blood Phe concentrations, as well as to brain Phe concentrations measured by magnetic resonance spectroscopy. The MRIs were performed by practicing radiologists. The generally symmetrical white matter abnormalities were graded from T2-weighted images according to their extension in six anatomical areas (frontal, temporal, parietal and occipital lobes; cerebellum; brainstem). This score was calculated as follows:

1 point for lesions restricted to deep white matter of the cerebral lobes, 2 points for abnormalities extending to subcortical white matter involving the region of the arcuate fibers and 2 additional points for changes in the brainstem or cerebellum. Therefore MRI grades could ranged between zero (normal MRI) and 12 (involving the subcortical white matter in all cerebral lobes as well as the brainstem and cerebellum) (141).

2.2.4 Neuropsychological testings

Auditory Verbal Learning Test (AVLT)

The Auditory Verbal Learning Test (Lezak 1995, Rey 1941) consists of two lists of 15 words each (lists A and B), arranged in a fixed order. The examiner reads list A aloud and immediately thereafter the patients must recall as many words as they can (A1). The examiner then repeats the same procedure four more times (A2 to A5). In a normal performance the patient recalls an increasing number of words throughout the essays, which is usually referred to as the learning curve. List B is read aloud only once, after the fifth recollection of List A (A5) and the patient receives the same previous instructions. List B is actually a distracter to prevent rehearsal of the material held in short-term memory. The examiner then asks the patient to recall List A again without reading it (immediate free recall, A6). After 15 minutes, another free recall is requested (delayed free recall, A7). Recognition, the last stage of the test, consists of a list of 30 words read aloud by the examiner and the patient must indicate which words were on List A.

d2 Attention Test

The d2 attention test established by Brickenkamp in 1994 was used to estimate the neuropsychology of individual's attention and concentration. The test was originally developed to measure driver aptitude and skill. This test has become one of the most widely used measures of attention, particularly visual attention, throughout Europe. Areas of usage include transport and pharmaceutical sectors, as well as sports, educational and clinical psychology. The test consists of a single 'recording blank'. The front page of this is reserved for recording the respondent's personal data and performance results. There is also a practice example so that the respondent can become acquainted with the task. On the reverse side is the standardized test form in a landscape layout of 14 test lines with 47 characters in each line. Each character consists of the letter *d* or *p* marked with one, two, three, or four small dashes (see 7.3.). The respondent is required to scan the lines and cross out all occurrences of the letter *d* with two dashes while ignoring all other characters. Two scoring keys are provided; one for identifying errors of omission (missing characters that should have been crossed out) and one for identifying errors of commission (crossing out

characters that should not have been crossed out). The reliability of the test is high. Extensive norms according to age, sex and education are included. The test-time is about 8 minutes including instructions and it can be applied in patients between 9 and 60 years of age.

Number Combination Test (NCT)

The number combination test (Oswald and Roth, 1997) is a trail-making test mainly used to diagnose brain performance disturbances by measuring the basal and genetically determined cognitive performance velocity, which is related to the so-called perceptual speed. The test is a pencil and paper test consisting of the numbers 1 to 90, which subjects have to join chronologically as fast as possible (see 7.3.). The time taken to complete this task is measured by a stopwatch and used for data analysis. Test scores indicate speed of information processing and furthermore the IQ. The test can be done in 5-10 minutes and can be applied in patients between 8 and 60 years of age.

Color-Word Interference Test (CWIT)

The so-called Stroop test established in 1935 examines the mental vitality and flexibility and the color-word interference respectively. It is based on the assumption that reading speed of a color-word is slower, if the word is written in a differently colored font. There is always a delay in naming the color of this word, if color and color-word do not match. It is a fair and highly reliable assessment of the ability to inhibit overlearned answers to simple tasks used to detect impairments of the reading speed or color recognition due to interfering information. There are two experimental conditions without interfering influences: determination of the reading speed of a colored word alone and determination of the color naming speed. This initial performance can be used as a baseline and can be related to the two so-called interference conditions. The reading speed of the color word decreases if the word is written in a different color and naming the color is made more difficult since the color-word and the color in which it was written do not match. Therefore, the task is to first read three columns of words written in black and then the color-words (red, green, yellow, blue), then say the color of 3 columns with color-strokes, and finally read 3 columns with color-words written in different color than the word says. This task has to be done three-times. The main variables are reading interference (the difference of the reaction time medians of naming interference condition and naming baseline). Additionally, the following variables are issued for each individual test part: median reaction time and number of incorrect answers. The test protocol shows each single reaction of the respondent with reaction time of the respondent and evaluation of the reaction. The testing time is about 15 minutes.

2.2.5 Nutritional, cardiovascular and metabolic status

The nutritional status of the PKU-patients was determined by recording of dietary intakes using self-reported three-day estimated dietary protocols (see 7.3.). Additionally, analyses of blood samples were performed (see 2.2.5). Thus, the content of those nutrients as minerals and vitamins were measured (see 3.2), which often are low in patients following a low-protein diet. Values from the food record were compared with the D-A-CH recommendations (47) and blood level ranges which were given from the laboratory. To determine cardiovascular risk, homocysteine and the lipoprotein profiles (TC, LDL, HDL, VLDL, TG, Apo A, Apo B, Lp (a)) were measured in blood. Furthermore, all patients and controls received accelerometers recording of an array of physiological data.

3-day dietary protocol

As dietary protocols, we used a modified form of the “Freiburger Ernährungsprotokoll” adding special low-protein products often consumed by PKU-patients. The protocol consists of different food groups with typical foods. The patients estimated the quantities of consumed foods and beverages using common household measures according to provided instructions (slice, piece, teaspoon, tablespoon, cup, mug etc.). They were asked to make precise descriptions e.g. the content of fat. Their food consumption was recorded over three consecutive days covering two weekdays and one day at the weekend. For calculation of the nutrient intake, recorded dietary intakes were entered into the software PRODI, version 4.5 LE 2003 (NutriScience GmbH, Freiburg, Germany) based on the German Nutrient Data Base BLS, version II.3 (BgVV, Berlin, Germany). The results were compared with recommended ranges of the German Nutrition Society (47).

Sense wear

All patients and controls were asked to wear for 3 days a SenseWear armband (SenseWear Pro 2, BodyMedia, Pittsburgh, USA) acting as a wearable metabolic monitor collecting and analyzing physical activity level and lifestyle information in a free-living context. The Sense Wear armband continuously records an array of physiological data, which is analyzed by special software (InnerView Professional, version 5.0, BodyMedia, Pittsburgh, USA). The resulting information can be organized into graphs and reports that clearly display energy expenditure, duration and level of physical activity level, number of steps taken, sleep/wake states and further parameters.

2.2.6 Analysis of blood samples

Venous blood samples (18 ml) were collected from an antecubital vein of the forearm into serum tubes or EDTA containing tubes (Sarstedt, Nümbrecht, Germany) in sitting or lying position of the subject. Tubes were centrifuged at 4 °C (1300 g for 7 minutes) immediately after blood withdrawal and the obtained serum and plasma were aliquoted into plastic storage vials. Plasma samples for vitamin A and E, as well as for fatty acid analysis were stored at -80°C until analysis, whereas the aliquots for all other analyses were sent to the laboratories by using cooling elements immediately after centrifugation.
The venous blood samples were analyzed for:

- Homocysteine and lipoprotein profile (TC, LDL, HDL, VLDL, TG, Apo A, Apo B, Lp (a))
- Calcium, magnesium, zinc, copper, selenium and ferritin
- Vitamin A and E, vitamin B12, 25(OH) vitamin D_3 and folic acid
- Fatty acid composition of plasma phospholipids
- IgG, IgA, IgM and total IgE
- Specific IgE, if total IgE was elevated

Vitamin A and E, fatty acids and selenium, as well as specific IgE were analyzed during up to 12 months of storage whereas all other biochemical parameters were analyzed at the day of blood drawing. Selenium was analyzed in the laboratories of the National Research Centre for Environment and Health (GSF). All other biochemical parameters were determined using established routine methods in the clinical chemistry laboratories of our hospital at Munich.
Phe was examined from dried blood spots on filter paper, which all patients three times (every four weeks) sent to the laboratory of Becker, Olgemöller & Colleagues in Munich.
All used methods, as well as the analytical equipment are shown in the following tables:

Tab. 11: Analyses of biochemical parameters

Parameter	Test kit / method	Analytical equipment
Phenylalanine	Fingerhut et al. (55)	API 365 ESI-MS/MS (PE-SCIEX GmbH, Darmstadt)
Total cholesterol	CHOD-PAP-method (Greiner Diagnostic GmbH, Bahlingen)	Hitachi 911 (Roche Diagnostics GmbH, Mannheim)
Triglycerides	GPO-PAP-method (Greiner Diagnostic GmbH, Bahlingen)	Hitachi 911 (Roche Diagnostics GmbH, Mannheim)
LDL cholesterol	Enzymatic test LDL-C (Wako Chemicals GmbH, Neuss)	Hitachi 911 (Roche Diagnostics GmbH, Mannheim)
HDL cholesterol	Immune inhibitation method HDL-C (Wako Chemicals GmbH, Neuss)	Hitachi 911 (Roche Diagnostics GmbH, Mannheim)

VLDL cholesterol	Calculated	Hitachi 911 (Roche Diagnostics GmbH, Mannheim)
Apo A1	APO A-I ver.2 (Roche Diagnostics GmbH, Mannheim)	Hitachi 911 (Roche Diagnostics GmbH, Mannheim)
Apo B	APO B vers.2 (Roche Diagnostics GmbH, Mannheim)	Hitachi 911 (Roche Diagnostics GmbH, Mannheim)
Lp(a)	Lp (a)-HA (Wako Chemicals GmbH, Neuss)	Hitachi 911 (Roche Diagnostics GmbH, Mannheim)
Homocysteine	Advia Centaur Assay (Bayer HealthCare, Leverkusen)	Advia Centaur Reagents (Bayer HealthCare, Leverkusen)
Calcium	Photometric Colour Test (Olympus, Hamburg)	AU 2700 (Olympus, Hamburg)
Magnesium	Photometric Colour Test (Olympus, Hamburg)	AU 2700 (Olympus, Hamburg)
Iron	Photometric Colour Test (Olympus, Hamburg)	AU 2700 (Olympus, Hamburg)
Ferritin	Turbidimetric End Point Method (Olympus, Hamburg)	AU 2700 (Olympus, Hamburg)
Copper	Atomic Absorption Spectrometry (PerkinElmer, Darmstadt)	AAS 3110 (PerkinElmer, Darmstadt)
Coeruloplasmin	N Antisera Ceruloplasmin (Dade Behring Marburg)	BN Prosper (Dade Behring, Marburg)
Zinc	Atomic Absorption Spectrometry (PerkinElmer, Darmstadt)	AAS 3110 (PerkinElmer, Darmstadt)
Selenium	graphite-furnace atomic absorption spectrometry	GFAAS (PerkinElmer, Darmstadt)
Folate	Cobas Vitamin B 12 (Roche Diagnostics GmbH, Mannheim)	Elecsys2010 (Roche Diagnostics GmbH, Mannheim)
Vitamin B12	Cobas Folate II (Roche Diagnostics GmbH, Mannheim)	Elecsys2010 (Roche Diagnostics GmbH, Mannheim)
25(OH) vit. D_3	RIA	Berthold LB 2111
IgG	Turbidimetric End Point Method (Olympus, Hamburg)	AU 2700 (Olympus, Hamburg)
IgA	Turbidimetric End Point Method (Olympus, Hamburg)	AU 2700 (Olympus, Hamburg)
IgM	Turbidimetric End Point Method (Olympus, Hamburg)	AU 2700 (Olympus, Hamburg)
IgE	N Latex IgE mono (Dade Behring, Marburg)	(Dade Behring, Marburg)
Cytokines	Immunoassay Elisa (R&D Systems Inc., Minneapolis)	Elisa reader & components (R&D Systems Inc., Minneapolis)
Elisa tests		
Phleum pratense	Immunoassay Elisa (Euroimmun AG, Lübeck)	Allercoat 6 Microtiter (Euroimmun AG, Lübeck)
Secale cereale	Immunoassay Elisa (Euroimmun AG, Lübeck)	Allercoat 6 Microtiter (Euroimmun AG, Lübeck)
Quercus alba	Immunoassay Elisa (Euroimmun AG, Lübeck)	Allercoat 6 Microtiter (Euroimmun AG, Lübeck)
Artemisia vulgaris	Immunoassay Elisa	Allercoat 6 Microtiter

	(Euroimmun AG, Lübeck)	(Euroimmun AG, Lübeck)
Cat dander	Immunoassay Elisa (Euroimmun AG, Lübeck)	Allercoat 6 Microtiter (Euroimmun AG, Lübeck)
Dog dander	Immunoassay Elisa (Euroimmun AG, Lübeck)	Allercoat 6 Microtiter (Euroimmun AG, Lübeck)
Cladosporium herbarum	Immunoassay Elisa (Euroimmun AG, Lübeck)	Allercoat 6 Microtiter (Euroimmun AG, Lübeck)
Dermatophagoides pteronyssinus	Immunoassay Elisa (Euroimmun AG, Lübeck)	Allercoat 6 Microtiter (Euroimmun AG, Lübeck)

For analysis of the fatty acid profile in plasma phospholipids, as well as vitamin A and E, in our laboratory the following chemicals, consumables and equipment were used:

Tab. 12: List of used chemicals for fatty acid analysis

Chemicals	Source	Quality
2.7 Dichlorfluorescein	Merck, Darmstadt	for analysis
2-Propanol	Merck, Darmstadt	for analysis
Acetic acid	Merck, Darmstadt	for analysis
Butylated hydroxytoluene (BHT)	Fluka, Neu-Ulm	≥ 99% GC
Chloroform	Merck, Darmstadt	extra pure
Diisopropyl ether	Merck, Darmstadt	for analysis
Ethanol	Baker, Deventer, Holland	absolute
Methanol	Merck, Darmstadt	for analysis
Methanolic HCl (3N)	Supelco, Bellefonte, USA	for analysis
n-Heptane	Merck, Darmstadt	for analysis
n-Hexane	Merck, Darmstadt	for analysis
Sodium carbonate	Merck, Darmstadt	anhydrous for analysis
Sodium hydrogen carbonate	Merck, Darmstadt	for analysis
Sodium sulfate	Merck, Darmstadt	for analysis

Tab. 13: List of used chemicals for vitamin A and E analysis

Chemicals	Source	Quality
Acetonitrile	Merck, Darmstadt	LiChrosolv
Ammonium acetate	Merck, Darmstadt	Fractopur
Butylated hydroxytoluene (BHT)	Fluka, Neu-Ulm	≥ 99% GC
Chloroform	Merck, Darmstadt	LiChrosolv
Distilled water	Braun, Melsungen	ad iniectabilia
Ethanol	Baker, Deventer, Holland	absolute
Methanol	Merck, Darmstadt	LiChrosolv
n-Hexane	Merck, Darmstadt	LiChrosolv
Tetrahydrofuran	Merck, Darmstadt	LiChrosolv

Tab. 14: List of consumables for fatty acid analysis

Consumables	Source
Brown glass bottle R1, G4	CS-Chromatographie Service, Langerwehe
Crimp cap R11-1.0	CS-Chromatographie Service, Langerwehe
Micro inlay G30/6	CS-Chromatographie Service, Langerwehe
Pipette tip CP100	Gilson, Villiers-le-Bel, France
Pipette tip 50-1000 µl, 500-2500 µl	Eppendorf, Hamburg
Pipette tip 10-100 µl, 100-1000 µl	Greiner bio-one, Frickenhausen
Screw cap G13	CS-Chromatographie Service, Langerwehe
Sealing disc G13	CS-Chromatographie Service, Langerwehe
TLC plate, silica gel 60	Macherey-Nagel, Düren

Tab. 15: List of consumables for vitamin A and E analysis

Consumables	Source
Brown glass bottle G1, G4	CS-Chromatographie Service, Langerwehe
Micro inlay G30/5	CS-Chromatographie Service, Langerwehe
Pipette tip CP100, CP250, CP1000	Gilson, Villiers-le-Bel, France
Pipette tip 50-1000 µl	Eppendorf, Hamburg
Pipette tip 10-100 µl, 100-1000 µl	Greiner bio-one, Frickenhausen
Screw cap G8-L, G13	CS-Chromatographie Service, Langerwehe
Sealing disc G13	CS-Chromatographie Service, Langerwehe
Silicone-PTFE septum	Merck, Darmstadt
Silicone-PTFE septum, slitted	Merck, Darmstadt

Tab. 16: List of equipment for fatty acid analysis

Equipment	Source
Analytical balance type 1801	Sartorius, Göttingen
Centrifuge Beckman GPR	Beckman, Bucks, UK
Centrifuge Hettich Universal 1200	Hettich, Tuttlingen
Centrifuge Universal 30 F	Hettich, Tuttlingen
Centrifuge glass tube, conical angle 30° (12 ml)	Schott Duran, Mainz
Funnel D35m	Duran Schott, Mainz
Metal block thermostat type 2102	Bachofer, Reutlingen
Nitrogen evaporation system type 5000 6101	Bachofer, Reutlingen
Pipette 10-100 µl, 100-1000 µl (Transferpette)	Brand, Wertheim
Pipette 50-250 µl, 500-2500 µl	Eppendorf, Hamburg
Pipette Microman M100	Gilson, Villiers-le-Bel, France
Solvent chamber for thin layer chromatography	Desaga, Heidelberg
Ultraviolet lamp	Benda, Wiesloch

Vortexer VF2	IKA-Labortechnik, Heitersheim
Gas chromatography	
Autosampler A200S	Carlo Erba Instruments, Milan, Italy
Capillary column, BPX-70 (60 m x 0.32 mm)	SGE, Weiterstadt
Controller HP 7673	Hewlett Packard, Böblingen
Gas chromatograph HP 5890 Series II	Hewlett Packard, Böblingen
Integrator HP 3396 Series II	Hewlett Packard, Böblingen
Software EZChromEliteV2.61	Scientific Software, Pleasanton, USA

Tab. 17: List of equipment for vitamin A and E analysis

Equipment	Source
Analytical balance type 1801	Sartorius, Göttingen
Brown glass bottle (25 ml)	Schott Duran, Mainz
Centrifuge Universal 30 F	Hettich, Tuttlingen
Nitrogen evaporation system type 5000 6101	Bachofer, Reutlingen
Pipette 10-100 µl, 100-1000µl (Transferpette)	Brand, Wertheim
Pipette 50-250µl	Eppendorf, Hamburg
Pipette Microman M100, M250, M1000	Gilson, Villiers-le-Bel, France
Quartz cuvette SUPRASIL	Hellma, Müllheim
Ultrasonic bath Sonorex Super RK 102 H	Bandelein, Berlin
UV/Vis Spectrophotometer Cary 1E	Varian, Darmstadt
Volumetric flask 10 ml, 25 ml	Schott Duran, Mainz
Vortexer VF2	IKA-Labortechnik, Heitersheim
HPLC chromatography	
Autosampler AS-2000	Merck Hitachi, Darmstadt
Column, LiChroCART 250-3, LiChrospher 100, RP18 (5 µm)	SGE, Weiterstadt
Column oven STH 585	Gynkotek, Germering
Pump L-6200 Intelligent Pump	Merck Hitachi, Darmstadt
D-2500 Chromoato-Integrator	Merck Hitachi, Darmstadt
UV/Vis detector L-4250	Merck Hitachi, Darmstadt

Tab. 18: List of used standard substances for fatty acid analysis

Standard substance	Source
Internal standard	
Cholesteryl pentadecanoic acid	Sigma, Deisenhofen
Dipentadecanoyl phosphatidylcholine	Sigma, Deisenhofen
Pentadecanoic acid	Sigma, Deisenhofen
Tripentadecanoin	Sigma, Deisenhofen

Standards for peak identification	
GLC-85 (reference standards)	Nu-Chek, Elysian, MN, USA
14:1t	Sigma, Deisenhofen
16:1t	Sigma, Deisenhofen
18:1n-7	Sigma, Deisenhofen
18:2tt	Sigma, Deisenhofen
18:4n-3	Sigma, Deisenhofen
20:3n-9	Sigma, Deisenhofen
20:5n-3	Sigma, Deisenhofen
22:1t	Sigma, Deisenhofen
22:4n-6	Sigma, Deisenhofen
22:5n-3	Sigma, Deisenhofen
22:5n-6	OmegaTech, CO, USA
24:0	Sigma, Deisenhofen

Tab. 19: List of used standards and control sera for vitamin A and E analysis

Standards/Control sera	Source	Quality
Internal standard		
Tocol	Eisai, Tokyo, Japan	for HPLC
Standards for peak identification		
α-tocopherol	Supelco, Bellefonte, PA, USA	Purity 99.3%
Retinol	Merck, Darmstadt	Purity >98%
Control serum		
NIST Standard Reference Material 968c	Gaithersburg, USA	

2.2.6.1 Analysis of fatty acid profiles in plasma phospholipids

Fatty acids in plasma lipid fractions were quantified using defined concentrations of cholesteryl pentadecanoic acid, dipentadecanoyl phosphatidylcholine, pentadecanoic acid and tripentadecanoin (equalled 10 µg 15:0 per 100 µl for each lipid fraction).

Tab. 20: Composition of the internal standard for fatty acid analysis

	Molecular weight (g/mol)	Correction factor for non-15:0 residue	Weighted sample (g/200 ml methanol/chloroform)
Cholesteryl pentadecanoic acid	611.0	2.52	50.47
Dipentadecanoyl phosphatidylcholine	706.0	1.46	29.22
Pentadecanoic acid	242.4	1.00	20.05
Tripentadecanoin	765.3	1.05	21.06

The different amounts shown in table 20 were dissolved in 200 ml mixture of methanol and chloroform (35+15 by vol.) with added 2 g/l BHT, aliquoted in 4 ml brown glass bottles and stored at -80 °C until usage.

For the extraction of plasma lipids according to a modified method of Kolarovic & Fourier (98), 100 µl internal standard and 2 ml mixture of hexane and isopropanol (3+2 by vol.) were added to 250 µl plasma. After 30 seconds of vortexing and 7 minutes of centrifugation (1000 g), the hexane phase was transferred into a 4 ml brown glass vial. Thereafter, two extractions with 2 ml hexane were performed and the combined extracts were taken to dryness under a gentle stream of nitrogen.

The lipid residue was then dissolved in a 400 µl mixture of chloroform and methanol (1+1 by vol.) and deposited on a thin layer chromatography plate (TLC). Phospholipids, free cholesterol, non-esterified fatty acids (NEFA), triglycerides and cholesterol esters were separated using a mixture of heptane, diisopropylether and acetic acid (60+40+3 by vol.) as mobile phase (36). The plate was dried and sprayed with 2.7-dichlorofluorescein (1% in ethanol). Bands were visualised by UV-light using an ultraviolet lamp. The fluorescending band with the phospholipid fraction was scraped from the TLC plate (fig. 4) and transferred into a 4 ml brown glass vial.

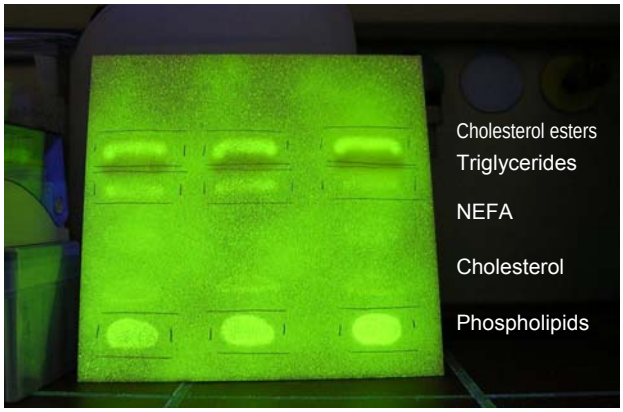


Fig. 4: Thin layer chromatography of fatty acids in plasma

Methyl esters of fatty acids (FAME) were obtained by reaction with 3M methanolic hydrochloric acid at 85 °C for 45 min. After neutralization by mixture of sodium carbonate, sodium hydrogen carbonate and sodium sulphate buffer (1+2+2 by weight), 1 ml hexane was added. After centrifugation at 1000 g for 4 min, the hexane layer was transferred into a 2 ml brown glass vial. The hexane extraction was repeated and the combined extracts were taken to dryness under a gentle stream of nitrogen. For storage until GC analysis at -80 °C, the samples were dissolved in 50 µl hexane with 2g/l BHT added.

FAME were separated by injection of 3 µl sample into the gas chromatograph (GC). The applied program for capillary gas chromatography is shown in table 21:

Tab. 21: Conditions of the GC for fatty acid analysis

Oven temperature	
Initial temperature	*130°C*
Initial time	0.50 min
Rate	3.0°C / min
Final temperature	*150°C*
Final time	0.00 min
Rate A	1.5°C / min
Final temperature	*180°C*
Final time	0.00 min
Rate B	3.0°C / min
Final temperature	*210°C*
Final time	23.00 min
Pressure	
Initial pressure	1.10 bar
Rate	0.025 bar/min
Final time	40.00 min
Injector/Detector	
Injector temperature	250°C
Detector temperature	300°C

Flame ionization detector signals were evaluated using special scientific software (EZ-Chrom Elite version 2.6, Scientific Software, Pleasanton, USA) and identified by comparison with the retention times of a standard mixture run previously. The instrument was calibrated regularly by using a quantitative standards mixture (GLC-85, Nu-Chek, Elysian, MN, USA).

Absolute fatty acid concentrations (mg/l) of all identified fatty acids with 14–24 carbon atoms were determined via comparison with peak area of the internal standard (15:0) and correction by accordant response factors. In plasma phospholipids, the area of the internal standard equaled 10 µg 15:0 / 250 µl plasma = 40 mg 15:0/l plasma.

The fatty acid weight percentages (wt %) were calculated following this formula:

$$\text{Individual fatty acid (wt\%)} = \frac{\text{Individual fatty acid (mg/l)} \times 100}{\text{Sum of all identified C14 – C24 fatty acids (mg/l)}}$$

Intra-assay reproducibility was assessed by analyzing eight pool samples in the same analytical run, whereas inter-assay reproducibilities of the used methods were assessed by analyzing five pool samples during 5 weeks.

2.2.6.2 Analysis of vitamin A and E in plasma

The analysis of vitamin A and E was performed according to a modified method of Schaffer (151) and Göbel et al. (65) using an isocratic mobile phase consisting of acetonitril, tetrahydrofuran, methanol and 1 % ammonium acetate (684+220+68+28 by vol.) (73). For avoiding the formation of explosive peroxides from tetrahydrofuran, the mobile phase was prepared prior to use and degassed ultrasonically.

Preparation of the internal standard

For consideration of losses during the extraction process, as well as unsteady injection amounts into the HPLC system, tocol was added as internal standard to plasma and standard samples at the beginning of the analysis. To prepare the internal standard, the concentrate consisting of 250 mg tocol dissolved in 100 ml ethanol/BHT (0.0625 %) was diluted 1:1000 to a concentration of 2.5 mg/l to get an adequate peak height in the chromatogram. The internal standard was frozen at -80 °C until usage.

Prearrangements for calibration curves

For preparation of standard concentrates, the amounts of retinol and α-tocopherol (tab. 22) were dissolved in 10 ml ethanol each. From these standard concentrates, dilutions were prepared as described in table 22. Standard concentrates and dilutions were stored at -80°C until usage.

Tab. 22: Preparation of standard concentrates and dilutions

Substance	Standard concentrate	Standard dilution
Retinol	10 mg/10 ml ethanol	200 µl concentrate/10 ml ethanol (=0.02 g/l)
α-tocopherol	50 mg/10 ml ethanol	1000 µl concentrate/10 ml ethanol (=0.5 g/l)

Preparation of standard curves for vitamin A and E

For preparation of new standard curves, the standard dilutions were defrosted and their absorbance was measured on a spectrophotometer at 325 nm for retinol and at 292 nm for α-tocopherol (tab. 23). Lambert-Beer Law was used to determine the exact concentration (c) from absorbance (A) ($A^{1\%}_{1cm}$, extinction coefficient of a compound at a certain wave length; d, thickness of cuvette).

Used formula:	A = A1%1cm * c (g/dl) * d (cm)
Calculation of the concentration:	c (g/dl) = A / [A1%1cm * d (cm)]

Tab. 23: Extinction coefficients for retinol and α-tocopherol

Substance	Molecular weight (g/mol)	Wave length (nm)	Extinction coefficient ($A^{1\%}_{1cm}$)	Source
Retinol	286.5	325	1780	(151)
α-tocopherol	430.7	292	75.8	(151)

Based on the photometrically determined concentrations, separate stock standards (which equal the highest concentrated standard of the standard curve) were obtained for each vitamin from standard dilutions. According to plasma levels in healthy adults, the following concentrations were chosen as the highest concentrated standards.

Retinol	~1.0 mg/l
α-tocopherol	~20.0 mg/l

Exact concentrations of stock standards for retinol and alpha-tocopherol were determined photometrically as described above. For preparation of the 7-point calibration curve, the following volumes of the internal standard and each stock standard were pipetted into brown bottles:

Tab. 24: Preparation of the seven standard dilutions for the calibration curves

	Std.7	Std.6	Std.5	Std.4	Std.3	Std.2	Std.1
Tocol (ISTD)	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl
Retinol	250 µl	210 µl	170 µl	130 µl	90 µl	50 µl	10 µl
α-tocopherol	250 µl	210 µl	170 µl	130 µl	90 µl	50 µl	10 µl

The concentrations of standards 1-7 were calculated according to their dilution factors as shown in the example for the seven standard dilutions for retinol and α-tocopherol calibration curves (mg/l):

Tab. 25: Calculation of the concentrations of the seven standard dilutions

	Std. 7	Std. 6	Std. 5	Std. 4	Std. 3	Std. 2	Std.1
Dilution factor	x1	x0.84	x0.68	x0.52	x0.36	x0.2	x0.04
Retinol	1.00*	0.84	0.68	0.52	0.36	0.20	0.04
α-tocopherol	20.0*	16.8	13.6	10.4	7.2	4.0	0.8

* determined photometrically (Lambert-Beer-Law), equals stock standard concentration

These seven standards were dried under a gentle stream of nitrogen. The dried extract was redissolved in 100 µl mobile phase, shaken mechanically for 10 min and transferred into microvials. For UV-Vis detection, 20 µl were injected into the HPLC system with an isocratic flow rate of 0.65 ml/min.

A 7-point standard curve was constructed for retinol and α-tocopherol using the software Microsoft Excel 97 SR-2 (Microsoft GmbH, Unterschleißheim) by plotting vitamin concentrations (tab. 24) against the peak-area ratios (vitamin/ IS, x-axis) (fig. A1). Subsequently, the equation of the regression line and the coefficient of determination (r^2) were calculated.

Tab. 26: Program of the UV-Vis detector

Time (min)	Wave length (nm)	Detection of
0	325	Retinol
4	292	α-tocopherol

Extraction of plasma samples

For protein precipitation, 250 µl plasma, 250 µl internal standard and 500 µl ethanol/BHT (62.5 mg/dl) were pipetted into a 4 ml brown glass vial and vortexed for 15 seconds. Then, two extraction steps followed using 1 ml Hexan/BHT (5 mg/dl) and one further extraction step using 1 ml Hexan. After every extraction, the samples were vortexed for 30 sec each and centrifuged for 5 min (2.300 U/min). Thereafter, vials were placed on ice for 5 min. The upper phases were transferred into a 1.5 ml brown glass bottle and taken to dryness under a gentle stream of nitrogen. The dried extract was redissolved in 100 µl mobile phase, shaken mechanically for 10 min and transferred into microvials. For UV-Vis detection, 20 µl were injected into the HPLC system with an isocratic flow rate of 0.65 ml/min. The program of the UV-Vis detector is shown in table 26. Detector signals were evaluated with an automatic integrator (Chromato-Integrator D-2500, Merck-Hitachi, Darmstadt) and identified by comparison with the retention times of a standard mixture run previously (fig. 5).

For calculation of the vitamin concentrations, peak-area ratios (vitamin/ IS) were computed for retinol and α-tocopherol. The appropriate concentrations were calculated using the equations of the standard curves (see 7.2, fig. A1).

Intra-assay reproducibility was assessed by analyzing six pool samples in the same analytical run, whereas inter-assay reproducibilities of the used methods were assessed by analyzing nine pool samples during one week.

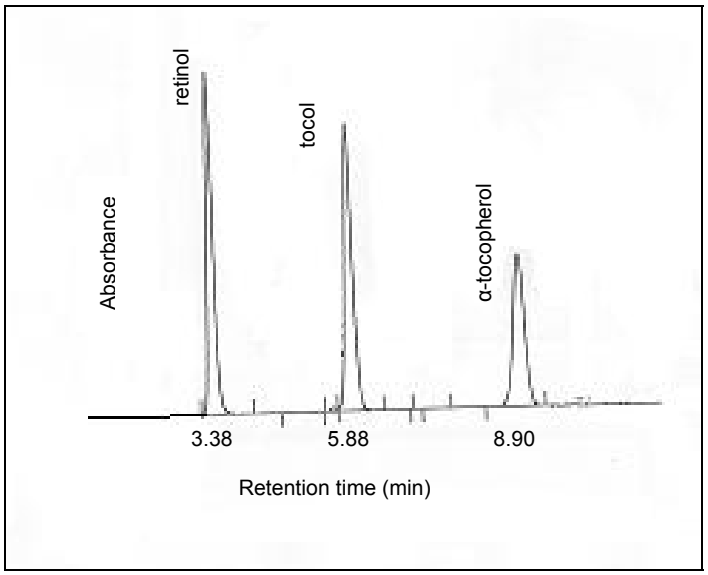


Fig. 5: Chromatogram of a standard mixture using UV-Vis detection*

2.3 Data management and statistical analyses

The information collected from each enrolled subject was stored in paper form and electronically for statistical analysis in consultation with a statistician using the software Statistical Package for Social Sciences (Version 12.0, SPSS Inc., Chicago, USA).

For all data, mean values and corresponding standard deviations were calculated. Whether the data were normally distributed was tested by visual inspection, as well as by Kolmogorov-Smirnov test. Parametric tests were used for normally distributed variables and nonparametric tests for variables that were not normally distributed. For all nonparametric tests, exact significances were calculated.

If normal distribution was probable, Student's unpaired t-test was used for between group comparisons; otherwise, tests according to Mann-Whitney were applied.

Correlations between parameters were estimated by computing Pearson's correlation coefficient in case of normally distributed values and the Spearman-Rho correlation coefficient in case of other distributions respectively. For bivariate tabular analysis, exact calculation by chi-square test was used, whereas in case of expected values smaller than five, the fisher exact test was used.

In either case, p-values ≤ 0.05 were considered as statistically significant and $p \leq 0.01$ as highly significant.

2.4 Ethical aspects and insurance

Ethical permission of the study was obtained from the Bavarian Board of Physicians after reviewing the study protocol. During our study, physical health was determined by clinical investigation, the questionnaire of atopic disease, three-day dietary food records, accelerometer data, biochemical measurements, neurophysiological and neuropsychological testings. Since no adverse effects were observed, all investigations seemed to be justified.

The liability insurance of the patients of the clinical trial would have been affected according to the requirements, but was not necessary in any case.

The study subjects received written and oral information about the nature of the study and associated procedures to clarify all arising questions before giving written consent.

3 Results

3.1 Subjects

The present study is based on 33 adult PKU-patients aged 26 to 59 years (12 men, 21 women) out of 53 adult patients with PKU from the database of the Dr von Hauner Children's Hospital. Five out of the 53 patients did not want to take part in the investigations, further five subjects were too handicapped and 10 PKU-patients failed to have left their current addresses or telephone numbers. For comparison of the study results, thirty-three healthy, age- and gender-matched subjects (11 men, 22 women) at an age between 23 and 49 years were recruited as controls by intranet advertisement on the website of the Ludwig Maximilian University hospitals.

The mean age of the patients (34.5 ± 6.3 yrs) was not significantly different from the mean age of the controls (34.6 ± 7.6 yrs), whereas the height was significantly lower and the body weight significantly higher in PKU-patients compared to the controls. The BMI, as well as diastolic and systolic blood pressure were revealed to be highly significantly larger in adults with PKU. The education level was different between both groups (p=0.054) because the control group was chosen according to the education level of the population reported by the Statistical Yearbook (2).

Tab. 27: Baseline characteristics of the subjects

	Mean ± SD or N as appropriate		PKU vs. controls p-value*
	Patients (n=33)	Controls (n=33)	
Gender ♀ / ♂	21 / 12	22 / 11	0.796
Age (yrs)	34.5 ± 6.3	34.6 ± 7.6	0.949
Education[1]	3 / 14 / 9 / 5	0 / 9 / 11 / 13	0.054
Height (cm)	168.5 ± 9.2	172.8 ± 8.6	0.036
Weight (kg)	75.6 ± 16.6	67.5 ± 12.2	0.027
BMI (kg/m^2)	26.5 ± 4.5	22.5 ± 3.1	<0.001
Blood pressure (mm Hg)			
Systolic	140.6 ± 22.0	126.3 ± 16.2	0.006
Diastolic	87.6 ± 13.7	75.4 ± 10.9	<0.001
Heart rate (beats/min)	79.9 ± 13.7	73.0 ± 12.5	0.080

* derived from Student's unpaired t-test, Mann-Whitney-U-test, Chi-square-test or Fisher's exact test as appropriate
[1] German schools: Sonderschule / Hauptschule / Realschule / Gymnasium; 2 patients have no graduation

3.2 Nutritional status of the patients

3.2.1 Measurement of fatty acids in plasma phospholipids

The applied gas chromatography method (2.2.5.1.) allowed the separation of the following fatty acids identified by comparison with the peaks of a standard mixture (fig. 6):

- *Saturated fatty acids:*
  14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0
- *Trans fatty acids:*
  14:1t, 16:1t, 18:1t, 18:2tt, 22:1t
- *Monounsaturated fatty acids:*
  14:1n-5, 15:1n-5, 16:1n-7, 17:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, 22:1n-9, 24:1n-9
- *Polyunsaturated fatty acids:*
  18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6 (including small amounts of 22:3n-3), 22:5n-6, 22:5n-3, 22:6n-3

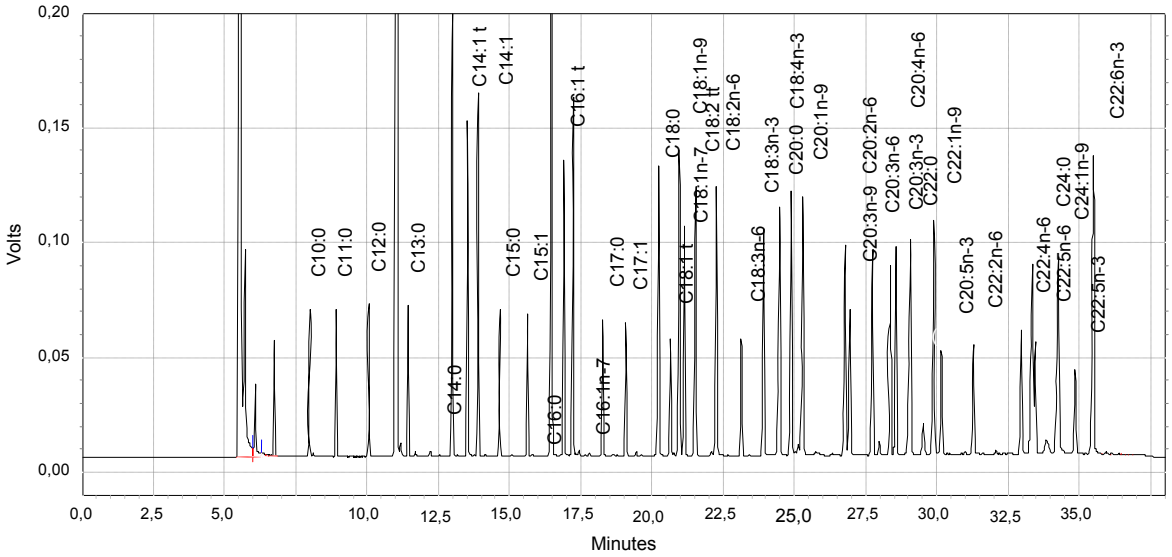


Fig. 6: Chromatogram of a fatty acid standard mixture

Reproducibility

For most of the fatty acids in plasma phospholipids, intra- and inter-assay variation tests proved a good reproducibility. Intra-assay coefficients of variation (CV) of the described n-3 and n-6 fatty acids (absolute and relative concentration) remained below 5 % as expected and mostly even below three, whereas inter-assay CV remained mostly below 6 %. The

following two tables show the determined coefficients of variation for selected n-6 and n-3 fatty acids, for complete intra-assay and inter-assay CV see the attached table A3.

Tab. 28: Intra-assay CV of fatty acid analyses in plasma phospholipids

		CV (%) of fatty acid concentrations (n=8)	
		Absolute (mg/l)	Relative (wt%)
n-6 fatty acids	18:2n-6	1.75	0.19
	18:3n-6	3.13	2.90
	20:3n-6	1.80	0.41
	20:4n-6	1.79	0.36
	22:4n-6	2.47	1.00
	22:5n-6	2.31	1.00
n-3 fatty acids	18:3n-3	3.21	1.42
	20:5n-3	2.07	0.71
	22:5n-3	2.05	0.67
	22:6n-3	2.11	1.28

Tab. 29: Inter-assay CV of fatty acid analyses in plasma phospholipids

		CV (%) of fatty acid concentrations (n=5)	
		Absolute (mg/l)	Relative (wt%)
n-6 fatty acids	18:2n-6	3.28	0.52
	18:3n-6	1.54	3.85
	20:3n-6	5.20	2.48
	20:4n-6	4.91	2.40
	22:4n-6	4.55	1.93
	22:5n-6	5.31	3.59
n-3 fatty acids	18:3n-3	5.40	2.62
	20:5n-3	5.19	3.46
	22:5n-3	6.85	4.36
	22:6n-3	7.11	5.20

3.2.2 Vitamin A and E in plasma

Linearity of calibration curves

The calculated coefficients of determination (r^2) obtained by the described method (2.2.5.2) using seven vitamin concentration levels within the stated concentration range are the following ones: 0.040 – 1.005 mg/l (r^2 = 0.9989) for retinol and 0.812 – 20.299 mg/l (r^2 = 0.9986) for α-tocopherol (fig. A1).

Reproducibility

The performed variation tests proved very good reproducibilities of the used method. Table 30 shows the determined coefficients of variation for retinol and α-tocopherol, which remained below 1 % in the intra-assay and below 3 % in the inter-assay for both substances. More details show the attached tables A4 and A5.

Tab. 30: Reproducibility of vitamin A and E analysis in plasma

	Intra-assay (n = 6)		Inter-assay (n = 9)	
	Mean (mg/l)	CV (%)	Mean (mg/l)	CV (%)
Retinol	0.40	0.94	0.40	2.97
α-tocopherol	8.96	0.68	8.95	2.91

Accuracy of the measurements

The results for the α-tocopherol level proved to be in good correspondence with the approved values of the NIST standard reference material (bias < 5 %), whereas the retinol level exceeded the certified values about 9.7 %. However, the coefficients of variation for accuracy were excellent (1.53 % for retinol, 0.45 % for α-tocopherol).

Tab. 31: Accuracy of vitamin A and E analysis in plasma

	Mean ± SD in mg/l or % as appropriate		
	Certified value[1]	Measured value (n=4)	Bias[2]
α-tocopherol	7.47 ± 0.47	7.66 ± 0.03	+ 2.68
Retinol	0.484 ± 0.012	0.53 ± 0.01	+ 9.69

[1] true concentration expected with 95 % confidence to be in the interval (certified value ± expanded uncertainty)
[2] bias= mean of samples - certified value * 100% / certified value

3.2.3 Dietary intake and nutritional supply

During the study, sixteen of the patients (48.5 %) were still or again adhering to the low-protein diet supplemented with special amino acid mixtures, three of these had begun again due to health problems (n=1) or pregnancy (n=2). The daily dietary intake of Phe calculated from the applied food records amounted to 2362 mg. Eleven (34.4 %) patients exceeded the recommendations of the APS (11) with a blood Phe-level greater than 20 mg/dl. The mean blood Phe-level during the study amounted to 15.9 mg/dl and the mean blood Phe-level throughout life (mean of all yearly medians of Phe-levels since start of treatment collected in patient files) amounted to 13.6 mg/dl (tab. 32).

Tab. 32: Daily dietary Phe-intake and blood Phe-levels of the PKU-patients

	PKU-patients (n=33)		
	Mean	SD	Median
Phe-intake per day (mg)	2362	1755	1904
Blood Phe-level during study[#] (mg/dl)	15.9	8.1	14.5
Blood Phe-level throughout life* (mg/dl)	13.6	3.9	12.8

[#] including very small levels in subjects with strict diet due to pregnancy or desire to have a child (n=5)
* Phe-level throughout life= mean of all yearly medians of Phe-levels since start of treatment (collected in patient files); excluding very low values of pregnant patients with a lack of Phe-levels during life (n=6)

The following two figures show the highly significant correlation between the blood Phe-level during the study (excluding very low values, n=6) and daily mean Phe-intake calculated from the applied food records (fig. 7) or the blood Phe-level throughout life respectively (fig. 8).

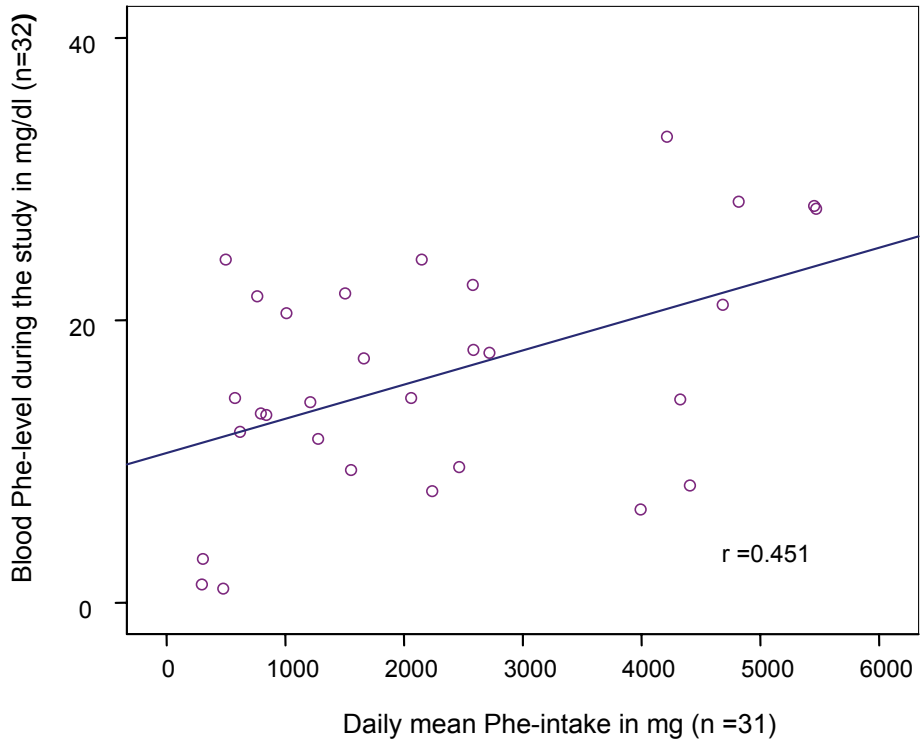


Fig. 7: Spearman-Rho correlation between blood Phe-level during study and daily Phe-intake

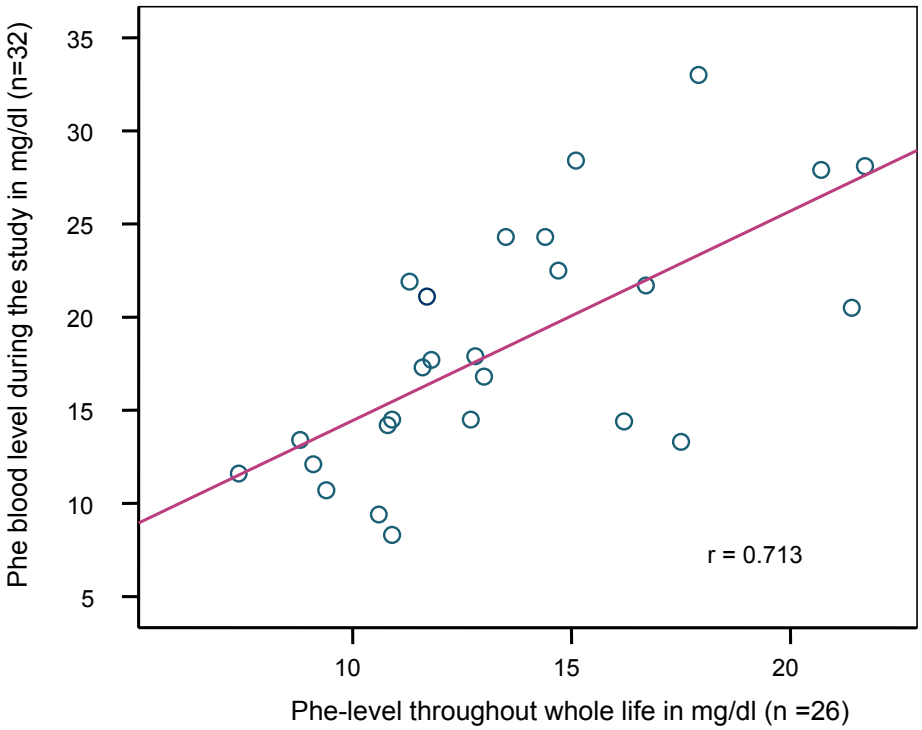


Fig. 8: Spearman-Rho correlation between the blood Phe-level during study and blood Phe-level throughout life

Each patient filled in estimated dietary protocols for three days. The evaluation of the applied food records shows intakes of energy, protein, fat, carbohydrates, as well as of vitamins, minerals and trace elements. Table 33 gives an overview of the dietary intake of energy, macronutrients and some critical nutrients; further results are shown in the attached table A1. The mean intakes of carbohydrates, folic acid, vitamin D, EPA and DHA, as well as of fiber, cholesterol, iodine and fluoride were found to be lower than recommended. The mean intakes of saturated fatty acids, as well as of the vitamin A, B-group vitamins, vitamin C, α-tocopherol and of minerals like sodium, potassium, magnesium, iron, zinc, copper, chloride and manganese exceeded the recommendations. All other parameters met more or less the reference values.

Tab. 33: Daily dietary intake of the PKU-patients (including amino acid mixtures)

	PKU-patients (n=31; 7 ♂, 24 ♀)				
	Mean ♀ / ♂	SD ♀ / ♂	Median ♀ / ♂	Reference value* ♀ / ♂	Mean in % of reference value
Energy (kcal)	2283 / 2772	646 / 565	2180 / 2843	2300 / 2900	99 / 96
Protein (g)	74 / 90	34 / 31	72 / 93	68.5 / 86.3	108 / 104
Total fat (g)	82 / 92	41 / 39	72 / 96	74.2 / 93.5	111 / 98
Carbohydrates (g)	259 / 356	79 / 84	297 / 354	314.9 / 397	82 / 90
PUFA (g)	12.5 / 13.4	6.7 / 6.0	10.8 / 13.9	≤ 24.7 / 31.2	51 / 43
MUFA (g)	26.6 / 29.6	18.0 / 17.4	20.1 / 26.3	≥ 24.7 / 31.2	108 / 95
SFA (g)	30.5 / 36.3	19.1 / 19.7	25.8 / 33.1	≤ 24.7 / 31.2	123 / 116
Retinol (mg)	1.4 / 1.5	0.8 / 1.1	1.3 / 1.1	0.8 / 1.0	175 / 150
Vitamin B12 (µg)	5.2	3.4	4.7	3	170
Folic acid (µg)	332	182	288	400	83
Vitamin D (µg)	4.6	4.8	2.7	5	92
Vitamin E (mg)	18.6 / 11.9	15.5 / 2.5	15.4 / 11.5	12 / 14	155
Calcium (mg)	1084	427	993	1000	108
Magnesium (mg)	412 / 472	129 / 127	440 / 421	300 / 350	137 / 135
Iron (mg)	19 / 18	9.1 / 5.6	17.6 / 18.3	15 / 10	193 / 121
Zinc (mg)	15.1 / 15.0	7.5 / 5.4	14.1 / 16.4	7 / 10	216
Copper (µg)	2604	1083	2453	1250	208
Linoleic acid (g)	10.4 / 10.5	5.6 / 4.4	8.7 / 10.3	6.2 / 7.8	168 / 135
α-Linolenic acid (g)	1.3 / 1.5	1.0 / 0.8	1.0 / 1.7	1.2 / 1.6	108 / 94
EPA (g)	0.002	0.01	0.0	> 0.23 / 0.29	1.1 / 1.1
DHA (g)	0.042	0.01	0.0	> 0.23 / 0.29	19.7 / 19.5

* reference values derive from D-A-CH recommendation (47); for EPA and DHA from ISSFAL (86)

The proportion of macronutrients (means) in comparison with recommended ranges or values of the German Nutrition Society (47) shows figure 9. According to that, PKU-patients tend to have higher intakes of fat and protein (or protein equivalent), but lower intakes of carbohydrates compared with German recommendations.

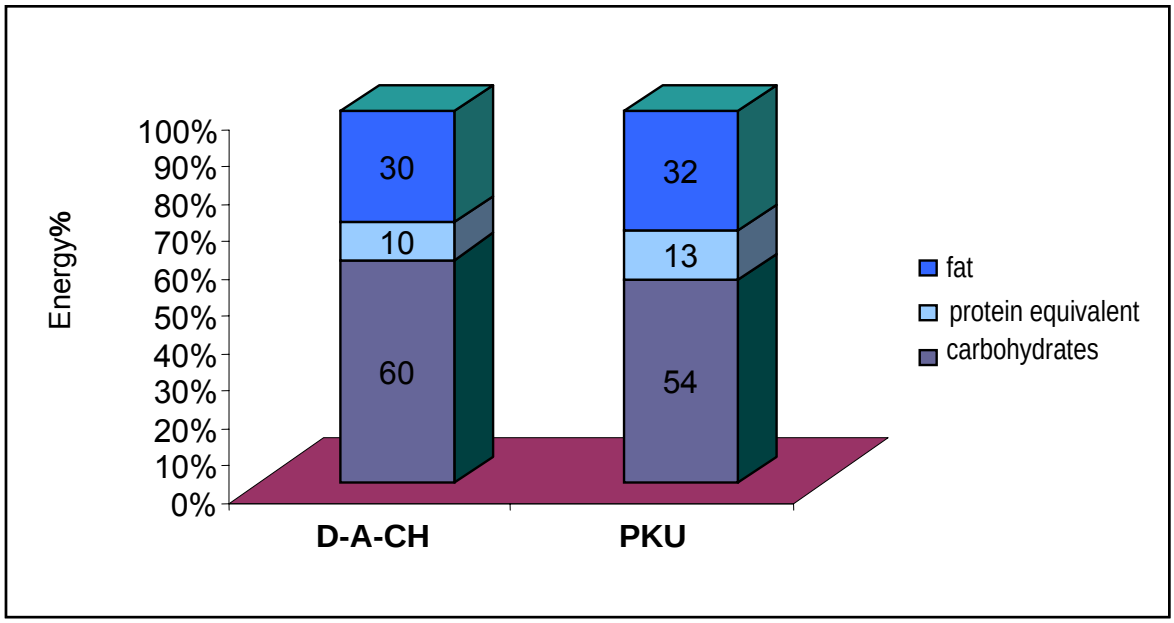


Fig. 9: Macronuttrient intake of PKU-patients and controls (% of energy intake)

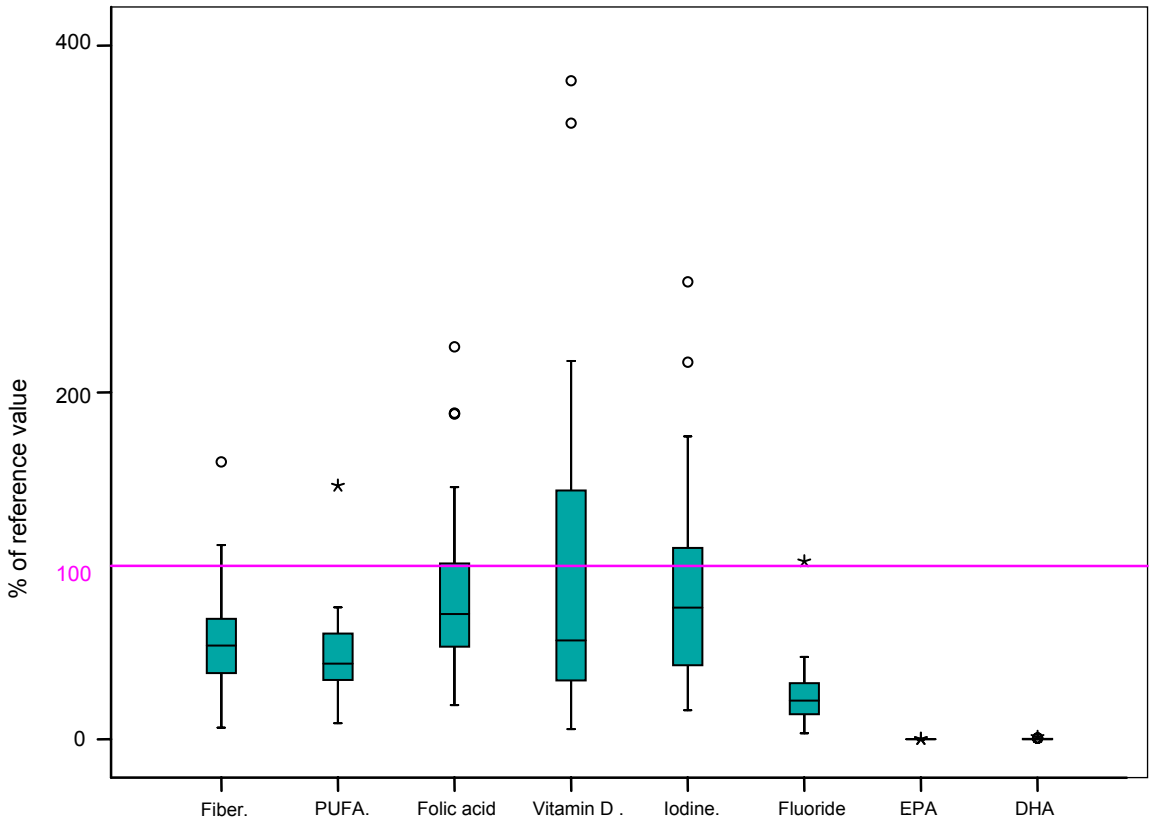


Fig. 10: Intake of nutrients considered critical in PKU-patients (expressed as % of reference values; (47))

Figure 10 gives an overview of nutrients whose median intakes are lower than the reference values. Bottom and top edges of the box indicate 25th and 75th percentiles, the horizontal line in the box indicates the median value of the data, the whiskers extend to a maximum of 1.5 times the interquartil range and the points outside the ends of the whiskers are outliers or suspected outliers. It is shown that the intakes of fiber, fluoride, PUFA, EPA and DHA are less than reference intakes in more than 75 % of the PKU-patients. Table 34 gives an overview of the nutritional supply of the patients with some important nutrients. The listed nutrients might prove to be too low in patients on a diet without foods rich in protein. For detection of deficiencies, measured blood concentrations were compared with reference ranges given from the laboratory.

The iron or zinc concentrations in the blood of almost 38 % of the patients were found below the laboratory's reference range. Furthermore, two PKU-patients had low contents of ferritin. In 56 % of the subjects, reduced blood levels of selenium were found. One subject had a retinol concentration below the reference value and three patients had reduced levels of α-tocopherol.

In some patients, higher concentrations compared with reference ranges were revealed. Thus, two patients had higher calcium, one patient higher ferritin, three patients higher selenium, seven patients higher copper, two patients higher retinol, five patients higher folic acid and one patient higher cobalamin levels than regarded as normal. Blood levels of selenium were significantly correlated with blood Phe-levels during life (Spearman-Rho, $r=0.451$, $p=0.024$).

Figure 11 shows the proportions of selected plasma fatty acids of the PKU-patients in comparison to relative fatty acid levels in 31 healthy adult omnivores of a comparable age and gender ratio (Kraft et al., unpublished data). PKU-patients had lower mean levels of linoleic acid and DHA, no difference was observed for α-linolenic acid levels.

In addition to these reported values the median arachidonic acid proportion in plasma phospholipids of adults with PKU proved to be higher and the median DHA level was lower compared to healthy omnivores (fig. 12) which could explain the alterations in the immune status. Comparing both groups p-values showed significant differences regarding Linoleic acid ($p<0.001$) and DHA ($p=0.013$).

However, compared with reference values of our own laboratory (derived from healthy controls), most of the PKU-patients were within normal ranges.

Tab. 34: Nutritient status of the PKU-patients (blood levels)

	Patients (n=33)				
	Mean	SD	Median	Reference range	Below /in /above reference range*
Calcium (mmol/l)	2.4	0.14	2.4	2.05-2.65	0 / 31 / 2
Magnesium (mmol/l)	0.83	0.07	0.80	0.65-1.20	0 / 32 / 0
Iron (µg/dl)	99	34	101	80-180	9 / 24 / 0
Ferritin (µg/l) ♀ / ♂	45 / 219	31 / 131	35 / 220	15-160 / 30-300	2 / 30 / 1
Selenium (µg/l)	78	21	80	80-100	18 / 11 / 3
Zinc (µg/dl)	82	18	81	75-140	9 / 24 / 0
Copper (µg/dl)	112	38	98	75-130	0 / 24 / 7
Retinol (mg/l)	0.61	0.13	0.57	0.33-0.85	1 / 29 / 2
Folic acid (ng/ml)	12.6	4.3	12.5	2.0-17.5	0 / 26 / 5
Vitamin B12 (pg/ml)	509	205	466	185-1100	0 / 32 / 1
Vitamin D (ng/ml)	29	9	28	10-120	0 / 30 / 0
Vitamin E (mg/l)	7.6	1.7	7.6	5.7-13.3	3 / 29 / 0

* one patient could not come into the hospital, therefore only some parameters were measured by her family doctor; and in few cases parameters could not be measured due to too less material

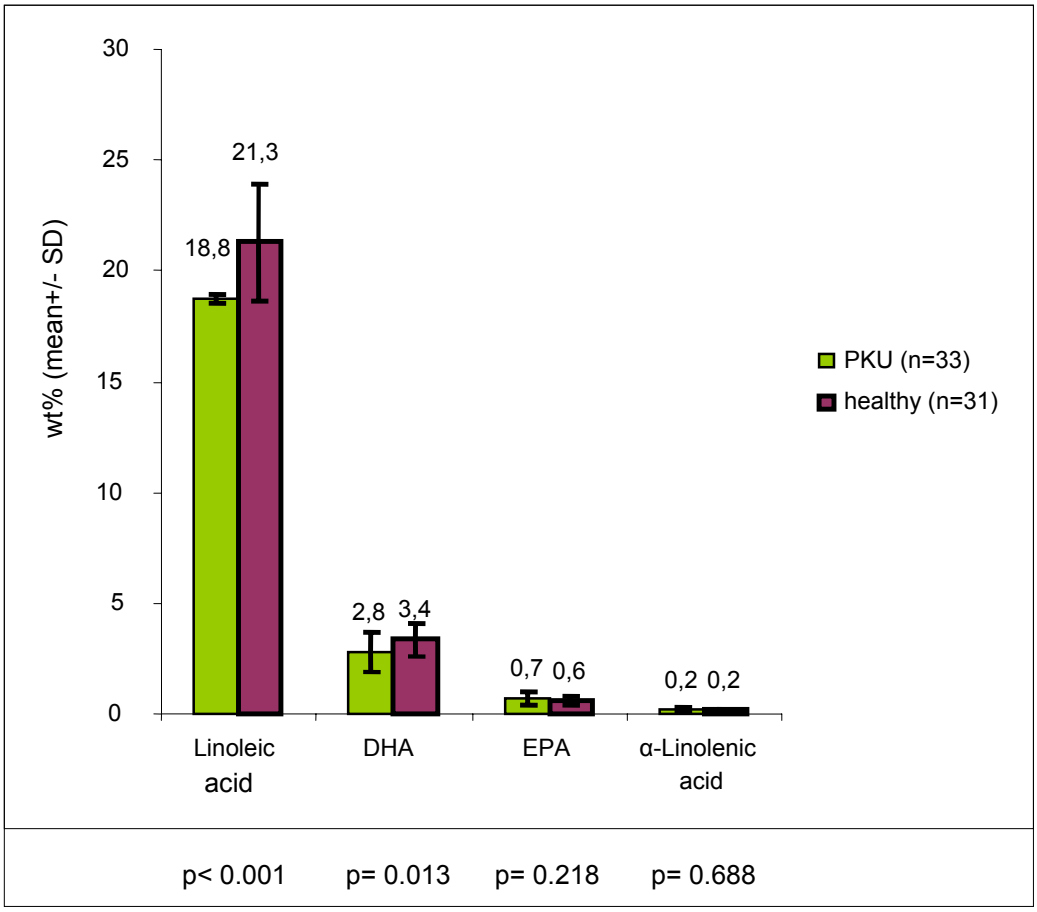


Fig. 11: Comparison of essential fatty acids, EPA and DHA (wt%) in plasma phospholipids between PKU-patients and healthy omnivores (p-value, Mann-Whitney-U-Test)

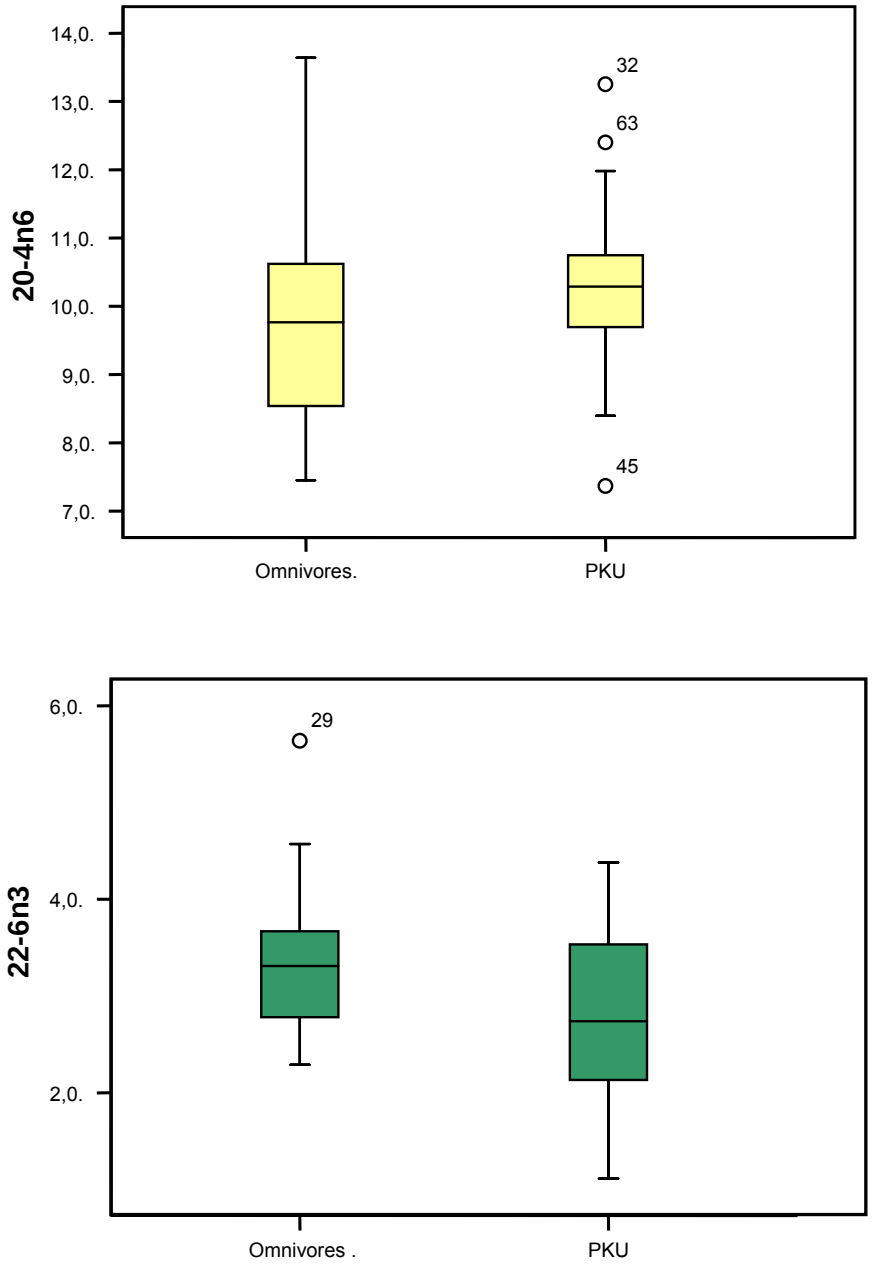


Fig. 12: Comparison of arachidonic acid and DHA levels in plasma phospholipids (wt%) between PKU-patients (n=33) and healthy omnivores (n=31)

3.2.4 Comparison of the nutritient status data with data from patients followed at Münster

Besides the present study based on 33 adult PKU-patients aged 26 to 59 years (12 men, 21 women), blood samples and estimated dietary protocols from further 52 adult PKU-patients out of the database of the University Hospital of Muenster were evaluated in the Dr von Hauner Children's Hospital in Munich. The mean age of the PKU-patients from Münster (32.3 ± 6.3 yrs; 28 men, 53 women) was not significantly different from the mean age of the patient group of Munich (34.5 ± 6.3 yrs).

Table 35 gives an overview of the dietary intake of energy, macronutrients and some critical nutrients. Further results are shown in the attached table A6. As previously reported in the patient group of Munich the mean intakes of carbohydrates, folic acid, vitamin D, EPA and DHA, as well as of fiber, cholesterol, iodine, fluoride, PUFA, linoleic and α-linolenic acid were lower than reference intake values. In contrast to the results of PKU-patients from Munich (tab. 33), the intake of energy was found to be slightly lower than recommended evaluating both patient groups together. Furthermore, the intake of EPA was remarkably lower (1.1 vs. 8 or 10 %) and DHA was approximately 10 % lower. The mean intakes of saturated fatty acids, as well as of the vitamin A, B-group vitamins, vitamin C, α-tocopherol and of minerals like sodium, potassium, magnesium, iron, zinc, copper, chloride and manganese exceeded the recommendations in the PKU-patients from Munich as well as in the PKU-patients from Münster. All other parameters met more or less the reference values.

Figure 13 shows the proportion of macronutrients (means) in comparison with reference ranges or values of the German Nutrition Society (47). PKU-patients tend to have higher intakes of fat and protein, but lower intakes of carbohydrates compared to German reference values. Evaluating data from Munich and Münster together showed even more unpropitious results. The intake of fat and protein in the combined group was higher than in the Munich patient group alone (33 vs. 32 % and 14 vs. 13 %) and the intake of carbohydrates was lower in the combined patient group (52 vs. 54 %).

Tab. 35: Dietary intake of all PKU-patients (including amino acid mixtures)

	PKU-patients (n=73, 24 ♂, 59 ♀)				
	Mean ♀ / ♂	SD ♀ / ♂	Median ♀ / ♂	Reference value* ♀ / ♂	Mean in % of reference value
Energy (kcal)	2015 / 2436	628 / 671	2009 / 2076	2300 / 2900	89 / 83
Protein equivalent (g)	66 / 79	31 / 27	59 / 74	68.5 / 86.3	99 / 98
Total fat (g)	74 / 91	35 / 39	69 / 78	74.2 / 93.5	100 / 97
Carbohydrates (g)	259 / 299	86 / 96	255 / 275	314.9 / 397	84 / 71
PUFA (g)	11.7 / 13.3	6.0 / 6.8	10.1 / 12.4	≤ 24.7 / 31.2	47 / 44
MUFA (g)	24.8 / 30.4	14.8 / 16.0	20.8 / 25.2	≥ 24.7 / 31.2	101 / 96
SFA (g)	26.9 / 34.1	15.8 / 16.6	24.2 / 32.5	≤ 24.7 / 31.2	108 / 111
Retinol (mg)	1.8 / 1.5	1.7 / 1.1	1.3 / 1.0	0.8 / 1.0	215 / 149
Vitamin B12 (µg)	4.4 / 5.8	3.3 / 3.6	3.4 / 4.9	3	162 / 185
Folic acid (µg)	256 / 281	111 / 116	232 / 260	400	72 / 77
Vitamin D (µg)	4.8 / 4.2	5.6 / 3.8	2.3 / 2.2	5	98 / 88
Vitamin E (mg)	13.2 / 12.0	7.2 / 3.8	11.3 / 11.3	12 / 14	131 / 90
Calcium (mg)	981 / 1006	475 / 363	879 / 986	1000	102 / 107
Magnesium (mg)	391 / 458	143 / 142	393 / 437	300 / 350	134 / 135
Iron (mg)	16.1 / 17.0	7.6 / 7.4	14.5 / 14.8	15 / 10	150 / 132
Zinc (mg)	12.2 / 13.4	6.1 / 5.7	10.6 / 14.8	7 / 10	185 / 139
Copper (µg)	2234 / 2558	895 / 896	2188 / 2166	1250	188 / 214
Linoleic acid (g)	9.9 / 10.5	5.2 / 5.1	8.6 / 10.1	6.2 / 7.8	158 / 142
α-Linolenic acid (g)	1.2 / 1.5	0.8 / 0.7	0.9 / 1.3	1.2 / 1.6	96 / 94
EPA (g)	0.03 / 0.005	0.15 / 0.01	0.00 / 0.00	> 0.23 / 0.29	10 / 8
DHA (g)	0.06 / 0.08	0.16 / 0.10	0.00 / 0.03	> 0.23 / 0.29	27 / 35

* reference values derive from D-A-CH recommendation (47); for EPA and DHA from ISSFAL (86)

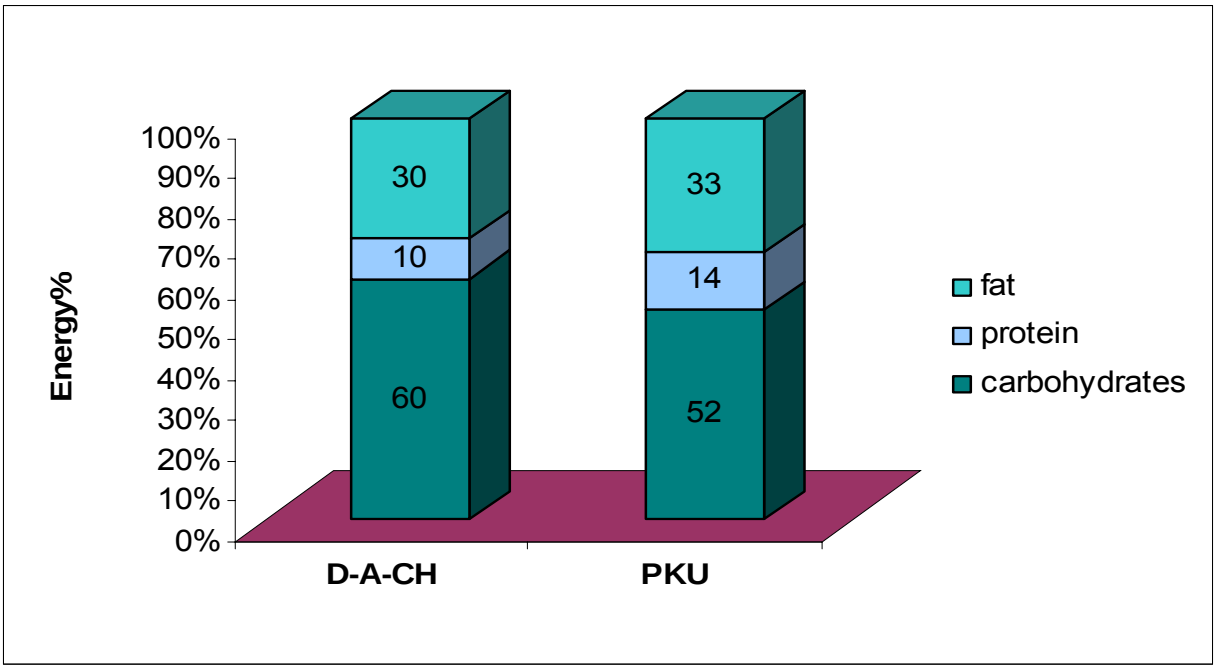


Fig. 13: Macronutrient intake (expressed as % of energy intake) of all PKU-patients relative to reference values (47)

In PKU-diet, the vast amount of protein is consumed as amino acid mixtures for which protein-equivalents are calculated by multiplying the percentage of nitrogen from amino acids in a diet with the factor 6.25.

In comparison to table 34, table 36 includes data from the PKU-patients of Münster showing the supply of some important nutrients. The listed nutrients might prove to be too low in patients on a diet without foods rich in protein.

In the group of patients from Munich and Münster, zinc blood levels of 40 % of the patients were found below the laboratory's reference range. In almost 42 % of all PKU-patients low selenium levels and in approximately 10 % low copper blood levels were detected. In 14 % of the subjects, reduced blood levels of Vitamin E were found. Nine subjects had a Vitamin D concentration below the reference value and six patients had reduced levels of ferritin. Furthermore, three PKU-patients were found to have lower retinol concentrations than recommended and two patients showed a lack of vitamin B12.

In two patients higher calcium blood levels were revealed, two patients had higher ferritin, 23 patients higher selenium, 27 patients higher copper, six patients higher retinol, eight patients higher folic acid, two patients higher cobalamin and one patient higher vitamin E levels than regarded as normal.

However, compared with reference values of the Children's hospital laboratories in Munich (derived from healthy controls), most of the PKU-patients were within normal ranges.

The combining data from Munich and Münster shows even greater nutritional deficiencies as data from Munich alone.

Tab. 36: Nutritional supply of all PKU-patients (blood levels)

	Patients (n=113)				
	Mean	SD	Median	Reference range	Below /in /above reference range* (N)
Calcium (mmol/l)	2.44	0.11	2.44	2.05-2.65	0 / 105 / 2
Magnesium (mmol/l)	0.85	0.06	0.85	0.65-1.20	0 / 106 / 0
Iron# (µg/dl)	99	34	101	80-180	9 / 24 / 0
Ferritin (µg/l) ♀ / ♂	56 / 156	44 / 97	47 / 141	15-160 / 30-300	6 / 99 / 2
Selenium (µg/l)	88	24	86	80-100	39 / 31 / 23
Zinc (µg/dl)	77	17	77	75-140	42 / 63 / 0
Copper (µg/dl)	112	41	97	75-130	10 / 66 / 27
Retinol (mg/l)	0.60	0.15	0.59	0.33-0.85	3 / 97 / 6
Folic acid (ng/ml)	9.6	4.6	8.3	2.0-17.5	0 / 97 / 8
Vitamin B12 (pg/ml)	463	207	431	185-1100	2 / 103 / 2
Vitamin D (ng/ml)	22	10	20	10-120	9 / 94 / 0
Vitamin E (mg/l)	7.3	2.0	6.6	5.7-13.3	15 / 90 / 1

*one patient could not come into the hospital, therefore only some parameters were measured by her family doctor; and in few cases parameters could not be measured due to too less material

iron was only measured in PKU-patients from Munich

3.3 Immune status of the patients

For detection of alterations in the immune status due to the PKU-diet, a questionnaire about atopic disease was used, immunoglobulins were measured and in case of elevated total IgE, an immunoassay for special IgE was carried out.

Regarding the concentrations of immunoglobulins there were no great abnormal results detectable for IgG, IgA and IgM. IgG was normal in all patients, elevated IgA was found in one and two PKU-patients had greater IgM values compared with normal ranges. The most significant result was that about 30 % (n=10) of all patients had elevated IgE levels in blood. In these patients, an Elisa immunoassay was performed for measurement of special IgE (tab. 39). There were significant positive correlations detectable between IgE and blood Phe-level in the study (p=0.029) and furthermore significantly negative correlations between PUFA blood level and IgA (p=0.036), as well as IgE (p=0.012) as shown in table 38.

Tab. 37: Immunoglobulins of the PKU-patients

	PKU-patients (n=33)					
	Mean ± SD	Median	Min	Max	Reference ranges	Below /in /above reference range
IgG (mg/dl)	997 ± 169	982	732	1394	700-1800	0 / 33 / 0
IgA (mg/dl)	196 ± 97	169	44	551	70-450	1 / 31 / 1
IgM (mg/dl)	162 ± 197	128	59	1222	40-250	0 / 31 / 2
IgE (U/ml)	1839 ± 9358	70	0.5	53930	<200	0 / 23 / 10

Tab. 38: Spearman-Rho correlations between the blood Phe-level, blood PUFA content and cardiovascular risk factors in PKU-patients

	Phe-level during study (n=32)		Phe-level throughout life (n=26)		Sum of PUFA[#] in wt% (n=32)	
	r	p	r	p	r	p
IgG (mg/dl)	-0.265	0.143	-0.066	0.749	-0.252	0.164
IgA (mg/dl)	0.089	0.628	0.001	0.997	-0.371*	0.036
IgM (mg/dl)	0.157	0.392	0.131	0.524	-0.065	0.723
IgE (U/ml)	0.387*	0.029	0.388	0.050	-0.440*	0.012

*significant, **highly significant, [#]sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3

As shown in table 39, patients had positive results in different allergens of the Elisa immunoassay. Most of the 10 patients with elevated IgE showed allergic reactions with phleum pratense (n=6), secale cereale (n=5), quercus alba (n=4), as well as dermatophagoides pteronyssinus (n=4).

Using a questionnaire, atopic disease was diagnosed if the subject had or ever had had at least one of the diseases listed in table 40. Almost 55 % of all PKU-patients were suffering from atopic disease compared with only 21 % of the age-matched controls, which means that adults with PKU suffered highly significantly more often from atopic disease than the controls (tab. 40). As shown by the p-values, adults with PKU had significantly more often hay fever, allergic coryza or allergic conjunctivitis. A trend towards a higher prevalence of asthma in PKU-patients compared to healthy controls was observed (p=0.053). A running nose and swollen eyes as typical allergic symptoms, as well as neurodermitis, urticaria or food allergy were not significantly but still more often observable in patients compared with controls.

Searching for correlations there was no significant correlation between the frequency of allergic diseases and blood Phe, or blood PUFA level observable in PKU-patients (tab. 41).

Tab. 39: Elisa test results of 10 PKU-patients with elevated IgE

Allergen	N out of 10 patients					
	Negative	Marginal positive	Slightly positive	Positive	Strongly positive	Sum of positives
Phleum pratense	4	0	1	2	3	6
Secale cereale	5	0	1	4	0	5
Quercus alba	6	0	1	2	1	4
Artemisia vulgaris	9	0	1	0	0	1
Cat dander	7	1	1	0	1	3
Dog dander	9	0	0	1	0	1
Cladosporium herbarum	10	0	0	0	0	0
Dermatophagoides pteronyssinus	6	0	0	2	2	4

* Elisa was carried out in 10 PKU-patients with elevated IgE, # phleum pratense= timothy grass, secale cereale= ray grass, quercus alba= white oak, artemisia vulgaris= mugwort, cladosporium herbarum = common species of mould, dermatophagoides pteronyssinus= house dust mite

Tab. 40: Frequency of allergic diseases in all subjects

	Frequency (N)			PKU vs. controls p-value*
	PKU-patients (n=33)	Controls (n=33)	Total (n=66)	
Atopic disease#	18	7	25	0.005
Asthma	5	0	5	0.053
Hay fever, allergic coryza / conjunctivitis	14	6	20	0.032
Running nose, swollen eyes	11	6	17	0.159
Neurodermitis	5	2	7	0.230
Urticaria	2	1	3	0.555
Food allergy	3	2	5	0.642

* derived from Chi-square-test or Fisher's exact test as appropriate
# positive diagnosis if subject has or ever had one of the listed diseases

Tab. 41: Spearman-Rho correlations between the blood Phe-level, blood PUFA content and frequency of allergic diseases in PKU-patients

	Phe-level during study (n=32)		Phe-level throughout life* (n=26)		Sum of PUFA# in wt% (n=32)	
	r	p	r	p	r	p
Atopic disease#	-0.014	0.941	0.165	0.422	-0.125	0.494
Asthma	-0.014	0.939	-0.195	0.339	-0.308	0.092
Hay fever, allergic coryza / conjunctivitis	0.024	0.896	0.279	0.167	-0.059	0.750
Running nose, swollen eyes	-0.245	0.177	0.012	0.955	-0.103	0.574
Neurodermitis	-0.051	0.780	0.000	1.000	-0.200	0.271
Urticaria	0.056	0.761	0.077	0.709	0.168	0.359
Food allergy	-0.296	0.100	-0.019	0.926	0.180	0.324

* excluding extreme values, # sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3

3.4 Cardiovascular risk markers of the patients

For evaluating the cardiovascular risk of the PKU-patients, BMI, consumption of cigarettes, blood pressure, hypertension, heart rate and blood parameters like homocysteine and the lipoprotein profile (TC, LDL, HDL, VLDL, TG, Apo A1, Apo B, Lp (a)) were determined.
Table 42 shows highly significantly higher values for BMI, blood pressure and heart rate in PKU-patients compared with controls. Subjects with PKU were significantly more often obese. Eleven patients in contrast to only three of the controls had BMI values of 25 - <30, obesity of the grade I (BMI 30 - <35) was revealed in 4 of the patients but in only 2 of the controls and BMI values of 35 and higher were found solely in two of the adults with PKU. Subjects from the control group were not significantly but more often smokers. Hypertension was not revealed to be significantly different comparing in both groups. Six of the patients but eight of the controls had mild, two patients but six controls moderate and only one subject of the control group suffered from heavy hypertension.

Tab. 42: Cardiovascular risk factors in PKU-patients compared to healthy controls

	Mean ± SD			PKU vs. controls p-value*
	PKU-patients (n=33)	Controls (n=33)	Total (n=66)	
BMI (kg/m^2)	26.5 ± 4.5	22.5 ± 3.1	24.5 ± 4.3	<0.001
Obesity[1] (n)	11 / 4 / 2	3 / 2 / 0	14 / 6 / 2	0.011
Smoker (n)	3	7	10	0.170
Blood pressure (mmHg)				
Systolic	140.6 ± 22.0	126.3 ± 16.2	133.4 ± 20.5	0.004
Diastolic	87.6 ± 13.7	75.4 ± 10.9	81.5 ± 13.7	<0.001
Hypertension[2] (n)	6 / 2 / 0	8 / 6 / 1	14 / 8 / 1	0.175
Heart rate (beats/min)	79.9 ± 13.7	73.0 ± 12.5	76.5 ± 13.5	0.037

* derived from Student's unpaired t-test, Mann-Whitney-U-test, Chi-square-test or Fisher's exact test as appropriate
[1] BMI between 25-<30 / 30-<35 / 35-<40, no subject had BMI>40
[2] mild < 130/85 / moderate 130-139/85-89 / heavy ≥140/90 (85)

Regarding the blood lipid status and blood homocysteine levels of the PKU-patients, elevated levels (compared to the laboratory's reference range) were often observed: 3 % up to 19 % had higher concentrations of blood parameters that are considered as risk factors for cardiovascular disease (CVD).

Tab. 43: Spearman-Rho correlations between the blood Phe-level, blood PUFA content and cardiovascular risk factors in PKU-patients

	Phe-level during study (n=32)		Phe-level throughout life* (n=26)		Sum of PUFA# in wt% (n=32)	
	r	p	r	p	r	p
BMI (kg/m^2)	0.437*	0.012	0.181	0.376	0.242	0.182
Obesity (n)	0.423*	0.016	0.194	0.342	0.161	0.378
Smoker (n)	0.308	0.087	0.217	0.288	0.064	0.728
Blood pressure (mmHg)						
Systolic	0.306	0.088	0.405*	0.040	0.112	0.542
Diastolic	0.266	0.141	0.399*	0.044	0.210	0.250
Hypertension (n)	0.275	0.128	0.473*	0.015	0.104	0.571
Heart rate (beats/min)	0.273	0.130	0.505*	0.008	0.109	0.552

* significant, ** highly significant, # sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3; Obesity = BMI $\geq$25

Thus, five patients (15 %) had higher total cholesterol and six (18 %) higher triglyceride levels. Regarding the different cholesterol types there were again higher VLDL concentrations detectable in six adults with PKU (19 %), in one patient, the LDL level exceeded the reference range and two subjects had LDL/HDL quotients above the laboratory's reference range. Furthermore, Lp (a) was elevated in six patients (18 %), Apo A1 also in six (19 %) and Apo B was too high in one of the subjects with PKU. Homocysteine was found to be beyond the reference range in 16 % of the patients.

Searching for significant correlations between cardiovascular risk factors and blood Phe, or blood PUFA level, significantly positive differences between the concurrent blood Phe-levels and BMI (p=0.012), or obesity (p=0.016) respectively. Additionally, the blood Phe-level throughout life and the blood pressure, as well as hypertension and heart rate showed significant positive correlations (tab. 43). Additionally, blood PUFA content in wt% was significantly positive related to body weight (r=0.396, p=0.025).

Tab. 44: Blood lipid status and homocysteine levels of the PKU-patients

	PKU-patients (n=33)				
	Mean	SD	Median	Reference ranges	Below /in /above reference range
Total cholesterol (mg/dl)	203.8	41.2	204.0	120-240	0 / 28 / 5
Triglycerides (mg/dl)	125.0	68.0	111.0	50-200	2 / 25 / 6
LDL (mg/dl)	125.8	34.7	123.0	60-190	0 / 32 / 1
HDL (mg/dl)*	56.4	15.3	55.0	35-75	2 / 28 / 2
LDL/HDL	2.4	0.9	2.4	1-4	1 / 30 / 2
VLDL (mg/dl)*	21.4	10.3	22.0	5-30	1 / 25 / 6
Lp (a) (mg/dl)	22.4	24.8	10.0	<30	0 / 27 / 6
Apo A1 (mg/dl)*	150.2	29.1	140.0	100-180	0 / 26 / 6
Apo B (mg/dl)*	91.8	25.6	88.0	45-150	0 / 31 / 1
Homocysteine* (mol/l)	11.5	3.1	11.2	<13.9	0 / 27 / 5

* one patient's value is missing

Tab. 45: Spearman-Rho correlations between the blood Phe-level, blood PUFA content and blood lipid status, as well as homocysteine levels in PKU-patients

	Phe-level during study (n=32)		Phe-level throughout life (n=26)		Sum of PUFA# in wt% (n=32)	
	r	p	r	p	r	p
Total cholesterol (mg/dl)	0.418*	0.017	0.099	0.630	-0.004	0.981
Triglycerides (mg/dl)	0.349*	0.050	0.134	514	-0.125	0.497
LDL (mg/dl)	0.286	0.113	-0.010	0.962	-0.115	0.530
HDL (mg/dl)*	0.086	0.640	0.084	0.684	0.075	0.684
LDL/HDL	0.174	0.342	-0.013	0.948	-0.199	0.275
VLDL (mg/dl)*	0.355*	0.033	0.149	0.476	-0.021	0.907
Lp (a) (mg/dl)	0.093	0.614	0.250	0.218	-0.141	0.440
Apo A1 (mg/dl)*	0.265	0.149	0.144	0.491	0.209	0.260
Apo B (mg/dl)*	0.284	0.128	-0.001	0.995	-0.034	0.857
Homocysteine* (mol/l)	0.258	0.162	-0.005	0.980	0.070	0.709

* significant, ** highly significant, # sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3

Testing correlations between cardiovascular risk factors and blood Phe, or blood PUFA level significantly positive differences were detected between the blood Phe-level during the study and total cholesterol (p=0.017), as well as TG (p=0.050) and VLDL (p=0.033) shown in table

45. Additionally, blood DHA content in wt% was significantly positive related to HDL (r=0.466, p=0.007), as well as to LDL/HDL (r=0.371, p=0.036) and ApoA1 (r=0.514, p=0.003).

3.5 Metabolic status of the patients

For determination of differences in metabolic parameters, all patients and controls were asked to wear an accelerometer for three days. Thus, the total and the active energy, the duration of physical activity level, the number of steps taken, as well as the lying and sleeping duration could be compared between patients and controls.

The total energy expenditure, the number of steps taken, as well as the sleeping duration showed no significant differences between the two groups (tab. 46). The lying duration tends to be different with 8.6 hours for patients and only 8.1 hours per day for the controls (n.s.). Significantly higher values for the daily active energy expenditure were found in the control group, as well as highly significant greater physical activity levels per day.

Tab. 46: SenseWear results in the PKU-patients and healthy controls

Per day	Mean ± SD		PKU vs. controls p-value*
	PKU-patients (n=32)	Controls (n=31)	
Total energy expenditure (kcal/day)	2791 ± 578	2896 ± 799	0.801
Active energy expenditure (kcal/day)	595 ± 513	977 ± 659	0.013
Physical activity (min/day)	115 ± 99	180 ± 89	0.009
Number of steps (per day)	10930 ± 4201	11170 ± 3733	0.667
Lying duration (hrs/day)	8.6 ± 1.5	8.1 ± 2.3	0.051
Sleeping duration (hrs/day)	6.6 ± 1.4	6.2 ± 1.4	0.203

* derived from Student's unpaired t-test or Mann-Whitney-U-test as appropriate

The significant correlations found between SenseWear results and activity parameters are emphasized. Significant positive correlations were found between active energy expenditure, as well as physical activity level and the duration of doing sports per week, but significantly negative correlations between the duration of watching TV per day and activity expenditure, as well as physical activity level per day. Furthermore, a significant positive correlation was found between the active energy expenditure and obesity, as well as the body weight, but a negative relation between the physical activity level per day and obesity, as well as the body weight. In addition, the number of steps taken per day and body weight were significantly positively correlated (tab. 47).

Tab. 47: Spearman-Rho correlations between accelerometer results and activity dependent parameters in all subjects

		Doing sports min/week	Watching TV min/day	PC work min/day	BMI kg/m^2	Obesity n	Weight kg
Total energy expenditure (kcal/day)	r	0.072	-0.220	0.156	0.049	0.135	0.141
	p	0.573	0.083	0.223	0.705	0.293	0.269
Active energy expenditure (kcal/day)	r	0.349**	-0.355**	-0.045	-0.473**	0.357**	0.414**
	p	0.005	0.004	0.727	<0.001	0.004	0.001
Physical activity (min/day)	r	0.299*	-0.346**	-0.128	-0.583**	-0.455**	-0.559**
	p	0.017	0.006	0.317	<0.001	<0.001	<0.001
Number of steps (per day)	r	0.082	-0.182	-0.198	-0.154	-0.143	0.335**
	p	0.525	0.153	0.120	0.229	0.263	0.007
Lying duration (hrs/day)	r	0.033	0.128	-0.117	0.129	0.064	0.103
	p	0.798	0.315	0.356	0.309	0.614	0.417
Sleeping duration (hrs/day)	r	-0.024	0.189	0.034	0.105	0.076	0.201
	p	0.850	0.134	0.788	0.407	0.550	0.112

Obesity = BMI $\geq$25

Testing correlations between accelerometer data and the blood Phe-level, or blood PUFA level there was a significant positive correlation determined between the blood PUFA content and the physical activity level (r=0.371, p=0.040), as well as the number of steps taken (r=0.406, p=0.023) shown in table 48.

Tab. 48: Spearman-Rho correlations between the blood Phe-level, blood PUFA content and accelerometer data in PKU-patients

	Phe-level during study (n=32)		Phe-level throughout life (n=26)		Sum of PUFA# in wt% (n=32)	
	r	p	r	p	r	p
Total energy expenditure (kcal/day)	0.060	0.746	0.212	0.308	0.071	0.704
Active energy expenditure (kcal/day)	0.104	0.576	0.139	0.507	0.336	0.064
Physical activity (min/day)	0.062	0.740	0.069	0.744	0.371*	0.040
Number of steps (per day)	0.067	0.722	-0.018	0.933	0.406*	0.023
Lying duration (hrs/day)	0.092	0.618	0.087	0.673	-0.029	0.877
Sleeping duration (hrs/day)	0.081	0.659	0.206	0.314	-0.158	0.388

* significant, ** highly significant, # sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3

Moreover, asking for free-time activities showed that healthy adults do highly significantly more frequently sports but watch highly significantly less frequently television than adults with PKU. The duration of using the computer during free time was not significantly but different between patients and controls (tab. 49).

Tab. 49: Activity parameters of the PKU-patients and healthy controls

	Mean ± SD		PKU vs. controls p-value*
	PKU-patients (n=33)	Controls (n=33)	
Doing sports (min/week)	96 ± 119	223 ± 197	0.002
Watching TV (min/day)	117 ± 73	78 ± 52	0.016
PC work (min/day)	26 ± 54	44 ± 57	0.205

* derived from Student's unpaired t-test or Mann-Whitney-U-test as appropriate

Searching for significant correlations between activity parameters and the blood Phe-level, or the blood PUFA level there were no significant differences observable (tab. 50).

Tab. 50: Spearman-Rho correlations between the blood Phe-level, blood PUFA content and activity parameters in PKU-patients

	Phe-level during study (n=32)		Phe-level throughout life* (n=26)		Sum of PUFA# in wt% (n=32)	
	r	p	r	p	r	p
Doing sports (min/week)	-0.133	0.467	-0.281	0.165	0.158	0.387
Watching TV (min/day)	-0.149	0.419	-0.186	0.363	0.229	0.207
PC work (min/day)	0.004	0.981	0.271	0.181	0.068	0.710

* excluding extreme values, # sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3

3.6 Neurological status of the patients

Neurophysiological testings and neuropsychological tests were performed to explore potential dietary effects on neurological outcomes.

3.6.1 Neurophysiological testings

Motor performance task (MPT)

For detection of alterations of fine motor skills, all patients and controls were asked to do the motor performance task (MPT). The number of failures and the duration of the different tasks, as well as fine motor factors like the aiming of motion, the hand unrest (tremor), the arm-hand precision, the arm-hand and the wrist-finger speed were compared between PKU-patients and controls.

Table 51 shows that adults with PKU committed about three times more failures in the steadiness task, compared to the controls, with almost one third more in line tracking (statistically significant) and approximately twice as many failures in aiming. Regarding the measured duration of failures, significant differences were found for the steadiness and differences for the line tracking. The total duration of line tracking and aiming were proven to be highly significant for inserting pins. Controls had a higher rate of hits in tapping and almost as many hits as the PKU-patients in aiming.

Tab. 51: Motor performance task results of the PKU-patients and healthy controls (measured with the right hand)

		Mean ± SD of duration (sec) or N as appropriate		PKU vs. controls p-value*
		PKU-patients (n=32)	Controls (n=28)	
Steadiness	NF	6.1 ± 8.0	2.4 ± 3.2	0.051
	DF	2.1 ± 6.3	0.22 ± 0.38	0.049
Line tracking	NF	26.0 ± 12.6	18.9 ± 7.0	0.015
	DF	3.0 ± 3.1	1.6 ± 0.8	0.004
	TD	30.2 ± 13.2	28.6 ± 11.2	0.584
Aiming	NF	1.6 ± 1.9	1.0 ± 1.7	0.162
	DF	0.12 ± 0.16	0.39 ± 0.06	0.164
	TD	9.3 ± 2.9	7.4 ± 1.5	0.006
	NH	19.7 ± 1.3	19.9 ± 0.6	0.513
Inserting pins	TD	48.9 ± 9.2	43.3 ± 6.3	0.003
Tapping	RH	186.7 ± 35.5	203.3 ± 20.3	0.057

* derived from Student's unpaired t-test, or Mann-Whitney-U-test as appropriate
NF=number of failures, DF=duration of failures, TD=total duration, HN=number of hits, RH=rate of hits
For explanations of the different tasks please have a look on pages 20/21.

Tab. 52: Spearman-Rho correlations between the blood Phe-level, blood PUFA content and the Motor performance task results in PKU-patients

		Phe-level during study (n=32)		Phe-level throughout life* (n=26)		Sum of PUFA# in wt% (n=32)	
		r	p	r	p	r	p
Steadiness	NF	0.370*	0.031	0.090	0.667	0.103	0.573
	DF	0.241	0.191	0.262	0.206	-0.038	0.838
Line tracking	NF	0.289	0.114	0.167	0.424	0.051	0.782
	DF	0.262	0.154	0.182	0.384	0.190	0.297
	TD	0.030	0.872	0.033	0.874	-0.089	0.628
Aiming	NF	0.398*	0.026	-0.012	0.956	0.058	0.751
	DF	0.469**	0.008	0.110	0.599	-0.002	0.991
	TD	0.389*	0.031	0.090	0.667	-0.216	0.234
	NH	-0.020	0.916	0.175	0.404	-0.216	0.234
Inserting pins	TD	-0.327	0.073	-0.318	0.121	-0.361*	0.042
Tapping	RH	0.241	0.191	0.262	0.206	-0.012	0.948

MPT=motor performance task, *significant, **highly significant, #sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3

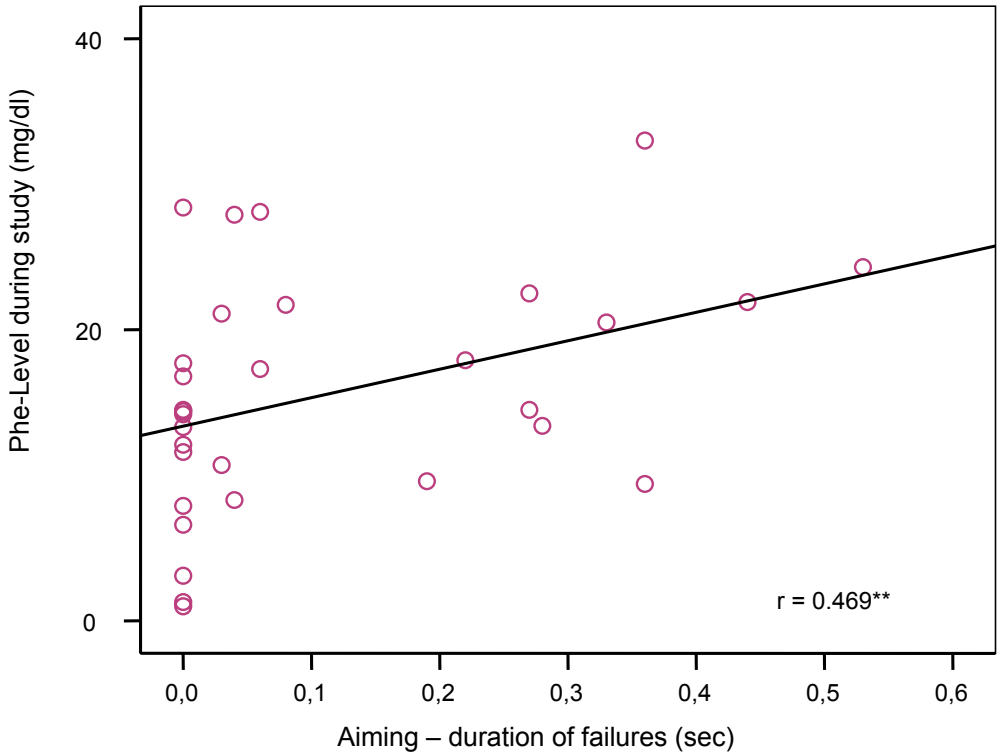


Fig. 14: Correlation between Phe-level during study and duration of failures in PKU-patients (Aiming) (n=32)

Table 52 shows a significant positive correlation between the blood Phe-level in the study and the number of failures in steadiness (r=0.370, p=0.031) and aiming (r=0.398, p=0.026), the duration of failures in aiming (r=0.469, p=0.008), as well as the total duration of aiming (r=0.389, p=0.031). Additionally, a significantly negative correlation was found between the blood PUFA content and the total duration of inserting pins (r=-0.361, p=0.042) (tab. 52).

As shown in figure 14, the results of the PKU-patients were significantly correlated with the blood Phe-level in the study (Spearman-Rho).

The raw values obtained were transformed into t-values by using tables with normative values. Conceptually, t-values represent the mean number of standard units varying from the average of 50 within a scale between 0 and 100 points. The higher t-values are, the better. Differences in motor performance became observable by comparison of fine motor factors obtained by the motor performance task. The PKU-patients showed worse t-values for every factor compared with controls. The differences in the aiming of motion, the arm-hand precision, as well as in the arm-hand speed were significant. Non significant trends were found for the hand unrest and the wrist-finger speed.

Investigating potential correlations between fine motor factors and blood Phe or blood PUFA levels, we found a significant negative correlation between the current blood Phe-level and the aiming of motion, as well as the arm-hand speed (tab. 54).

Tab. 53: Fine motor factors of the of the PKU-patients and healthy controls (t-values)

	Mean		PKU vs. controls p-value*
	PKU-patients (n=32)	Controls (n=28)	
Aiming of motion	60.16	69.86	0.019
Hand unrest (tremor)	56.00	63.86	0.067
Arm-hand precision	24.69	37.14	0.005
Arm-hand speed	25.05	36.73	0.009
Wrist-finger speed	26.47	35.11	0.056

* derived from Mann-Whitney-U-test or Student's unpaired t-test as appropriate

Tab. 54: Spearman-Rho correlations between the blood Phe-level, blood PUFA content and fine motor factors in PKU-patients

	Phe-level during study (n=32)		Phe-level throughout life (n=26)		Sum of PUFA# in wt% (n=32)	
	r	p	r	p	r	p
Aiming of motion	-0.430*	0.016	0.005	0.980	-0.055	0.765
Hand unrest (tremor)	-0.307	0.093	-0.303	0.141	-0.012	0.947
Arm-hand precision	-0.277	0.131	-0.179	0.391	-0.136	0.459
Arm-hand speed	-0.387*	0.031	-0.104	0.619	0.289	0.109
Wrist-finger speed	-0.328	0.072	-0.131	0.127	-0.020	0.915

AVLT= auditory verbal learning test, *significant, **highly significant, #sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3

Visual evoked potentials (VEP)

VEPs were measured for P100 latencies. Pathological VEPs were observed in more than 50 % of the PKU-patients (tab. 55). From evaluable VEP results (n=23) a mean latency of 110.5 ms was calculated for PKU-patients as shown in table 56. In comparison, mean VEP (70') latency in 30 healthy omnivores of a comparable age and gender ratio amounted to 100.8 ms (100). P100 latencies above 115 ms were classified as prolonged, based on clinical experience (Prof. W. Müller-Felber, Munich). In eight (34.8 %) of all 23 evaluable VEPs prolonged P100 latencies above 115 ms were determined, whereas in none of the 30 omnivores prolonged latencies were found.

Tab. 55: VEPs findings in PKU-patients

	N (%)	
	Pathologically altered	Normal
VEPs (n=30)*	15 (51.7)	14 (48.3)

*some patients did not want to take part in these neurological tests

Tab. 56: VEP (70') P100 latencies in PKU-patients compared with healthy omnivores

	Mean ± SEM[$]	
	PKU-patients* (n=23)	DHAVEG-Study[#] (n=30)
Latency P100 (ms)	110.5 ± 3.2	100.82 ± 1.08

* derived from 23 patients, others were not evaluable due to pathological alterations
[#] Kraft et al., unpublished data; * SEM = standard error of the mean

3.6.2 Neurostructural testing

Magnetic Resonance Imagings (MRI)

MRIs of the PKU-patients were evaluated to explore neurological abnormalities. All measured MRIs turned out to be abnormal. Pathological alterations were found in all of the 16 performed MRIs. Figure 15 shows the MR image of a 30-year-old male patient. The MRI made during the study shows an atrophy of the brain as well as focal high signal intensity in the periventricular white matter.

The severity of these findings ranged from one up to 6 points according to their extension. These generally symmetrical white matter abnormalities were graded as follows: 1 point for lesions restricted to deep white matter of the cerebral lobes (six areas), 2 points for subcortical alterations and 2 additional points for changes in the brainstem or cerebellum. MRI grade could therefore total between zero (normal MRI) and 12 (subcortical white matter

in all cerebral lobes, brainstem and cerebellum) (141). Table 55 shows the number of pathological altered MRIs in PKU-patients, whereas table 57 shows the severity of the detected alterations.

Searching for significant correlations between severity of deep white matter alterations and blood Phe, or blood PUFA level no significant correlation was detected (tab. 58).

Tab. 57: Severity of deep white matter alterations in PKU-patients

	Number N (%) of points given for alterations							
	0	1	2	3	4	5	6	7-12
Pathological MRIs (n=16)	0	2 (13)	3 (19)	3 (19)	5 (31)	2 (13)	1 (6)	0

Tab. 58: Spearman-Rho correlations between the blood Phe-level, blood PUFA content and severity of deep white matter alterations in PKU-patients

	Phe-level during study (n=32)		Phe-level throughout life* (n=26)		Sum of PUFA# in wt% (n=32)	
	r	p	r	p	r	p
Severity of deep white matter alterations in PKU-patients	0.328	0.215	-088	0.764	0.190	0.482

*excluding extreme values, #sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3

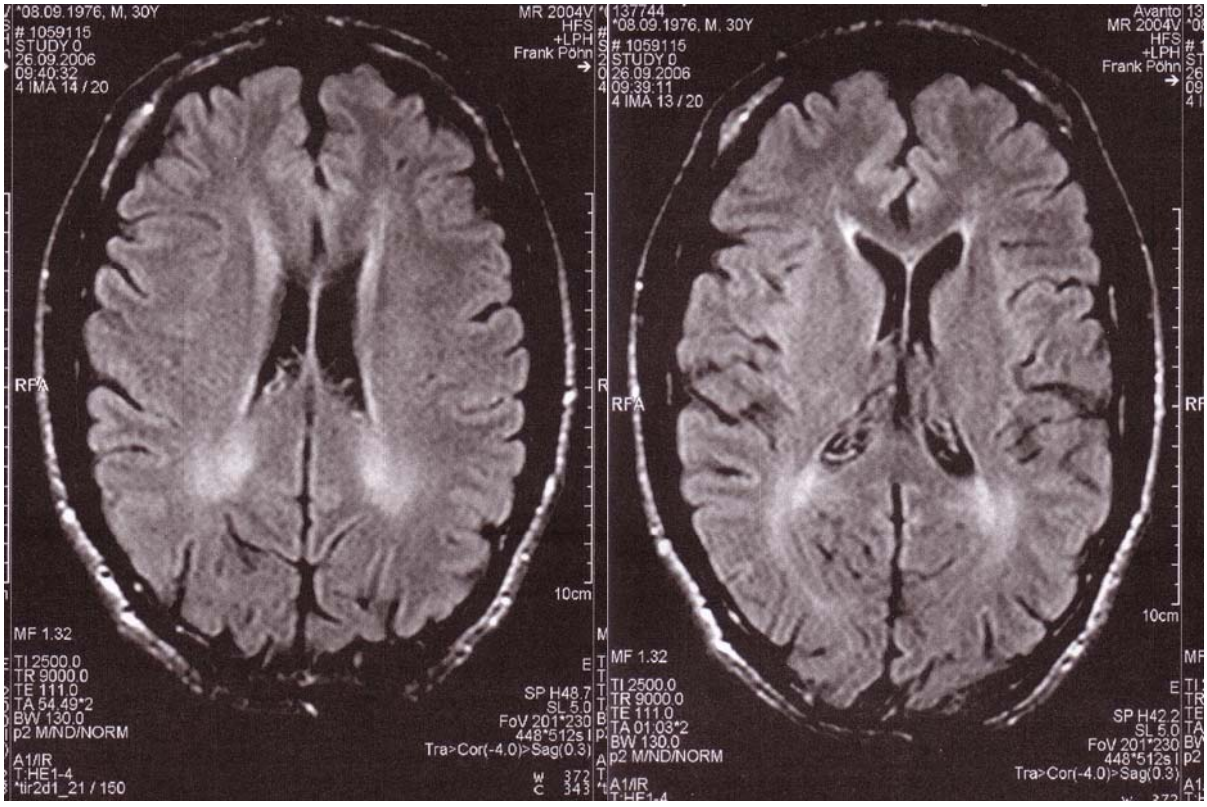


Fig. 15: Typical periventricular deep white matter alterations (♂, 30 yrs)

3.6.3 Neuropsychological tests

d2 attention test

From the variety of neuropsychological tests, the d2 attention test, the color-word interference task, the auditory verbal learning test and the number combination test were chosen for the PKU-patients and the healthy controls.

Regarding the d2 attention test (tab. 59), subjects from the control group could complete a significantly higher number of items in the whole test, they achieved a higher number of items completed subtracted by failures, as well as a higher concentration performance (number of crossed out characters minus failures) compared to the PKU-patients. The number and percentage of failures, as well as the spread of the number of items completed (between the lines) did not differ significantly comparing the PKU-patients and the controls.

Tab. 59: d2 attention test results of the PKU-patients and healthy controls

	Mean ± SD		PKU vs. controls p-value*
	PKU-patients (n=30)	Controls (n=32)	
Number of items completed (n)	336.2 ± 89.0	465.5 ± 99.3	<0.001
Number of failures (n)	23.1 ± 23.4	32.0 ± 29.4	0.194
Failures (%)	7.6 ± 8.3	10.0 ± 13.7	0.410
Number of items completed minus failures (n)	309.8 ± 95.0	447.1 ± 79.5	<0.001
Concentration performance # (n)	120.0 ± 46.1	188.9 ± 79.2	<0.001
Spread between lines (n)	13.5 ± 7.2	12.6 ± 5.0	0.591

*derived from Mann-Whitney-U-test or Student's unpaired t-test as appropriate
#Concentration performance = number of crossed out characters minus number of failures

Tab. 60: Spearman-Rho correlations between the blood Phe-level, blood PUFA content and results of the d2 attention test in PKU-patients

	Phe-level during study (n=32)		Phe-level throughout life (n=26)		Sum of PUFA# in wt% (n=32)	
	r	p	r	p	r	p
Number of items completed (n)	-0.435*	0.018	-0.201	0.357	-0.072	0.706
Number of failures (n)	0.521**	0.004	0.422*	0.045	0.016	0.931
Failures (%)	0.566**	0.001	0.456*	0.029	0.033	0.862
Number of items completed minus failures (n)	-0.512**	0.005	-0.265	0.222	0.020	0.917
Concentration performance# (n)	-0.607**	<0.001	-0.428*	0.041	0.008	0.966
Spread between lines (n)	0.286	0.133	0.089	0.686	-0.037	0.847

*significant, **highly significant, #sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3

In PKU-patients (n=30), the number and the percentage of failures were significantly correlated with the blood Phe-level in the study and throughout life, whereas the number of items completed, the number of items completed minus failures, as well as the concentration performance were significantly negative correlated with the blood Phe-level in the study and throughout life. In addition to that, the concentration performance was significantly negative correlated with the blood Phe-level throughout life (tab. 60).

Color-word interference test

Differences in the color-word interference test (CWIT) comparing results of the PKU-patients and of the controls are shown in table 61. Significant differences were detected in the reading duration of the color-words, the color-strokes, as well as of the interference list comparing the PKU-patients and the controls. The raw values obtained were transformed into t-values by using tables with normative values (196). The subjects from the control group had higher t-values in the reading duration of all three tasks. No differences were observed between both groups for results of the nomination and the selectivity skills. Significant negative correlations were found between the reading duration of all three different tasks and blood Phe-levels in the study (tab. 62).

Tab. 61: Color-word interference test results of the PKU-patients and controls (t-values)

	Mean ± SD		PKU vs. controls p-value*
	PKU-patients (n=30)	Controls (n=32)	
Reading duration of color-words	47.7 ± 9.3	54.9 ± 8.2	0.002
Reading duration color-strokes	45.1 ± 11.7	54.1 ± 10.2	0.003
Reading duration of interference task	49.1 ± 9.4	56.1 ± 7.4	0.001
Nomination	48.9 ± 14.4	49.1 ± 10.5	0.928
Selectivity	57.8 ± 10.2	57.7 ± 8.3	0.994

CWIT= color-word interference test, *derived from Mann-Whitney-U-test or Student's unpaired t-test

Tab. 62: Spearman-Rho correlations between the blood Phe-level, the blood PUFA content and results of the Color-word interference test in PKU-patients

	Phe-level during study (n=32)		Phe-level throughout life (n=26)		Sum of PUFA[#] in wt% (n=32)	
	R	p	r	p	r	p
Reading duration of color-words	-0.593**	0.001	-0.392	0.064	-0.176	0.353
Reading duration color-strokes	-0.517**	0.004	-0.519*	0.011	0.121	0.523
Reading duration of interference task	-0.454*	0.013	-0.608**	0.002	0.117	0.538
Nomination	0.086	0.658	0.043	0.845	0.131	0.491
Selectivity	0.265	0.164	0.102	0.644	-0.017	0.928

CWIT= color-word interference test, *significant, **highly significant, [#]sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3

Auditory verbal learning test

Table 63 shows results of the auditory verbal learning test (AVLT) used in the present study. Significant differences between PKU-patients and controls were detected in the number of immediately recalled words (of 15 presented words) and in the number of recalled words after the fifth recollection, whereas there were no significant differences for the learning and the recalling performance, for loss after distraction, and for the total number of recognized words.

Tab. 63: Auditory verbal learning test results of the PKU-patients and healthy controls

	Mean ± SD		PKU vs. controls p-value*
	PKU-patients (n=32)	Controls (n=32)	
Immediately recalled words (n)	5.4 ± 1.6	6.4 ± 2.2	0.047
Recalled words after 5th recollection (n)	10.9 ± 3.1	12.9 ± 2.1	0.007
Learning performance[#] (n)	5.5 ± 2.5	6.5 ± 2.3	0.129
Recalling performance[$] (n)	11.4 ± 2.9	12.8 ± 2.1	0.073
Loss after distraction (n)	-0.29 ± 1.13	0.13 ± 1.19	0.160
Recognized words (n)	13.8 ± 1.8	14.5 ± 1.1	0.102

AVLT= auditory verbal learning test
* derived from Mann-Whitney-U-test or Student's unpaired t-test as appropriate
[#] number of recalled words after 5th recollection minus number of words of the first recalling
[$] number of recalled words after reading the 5th recollection and after recalling of another distracting list of words

There were significant negative correlations between the blood Phe-levels throughout life and the number of recalled words after the fifth recollection, the learning performance, as well as the recalling performance in PKU-patients. The blood Phe-level in the study showed a significan negative correlation with the recalling performance and a positive one with the loss after distraction as (tab. 64).

Tab. 64: Spearman-Rho correlations between the blood Phe-level, blood PUFA content and results of the Auditory verbal learning test in PKU-patients

	Phe-level during study (n=32)		Phe-level throughout life (n=26)		Sum of PUFA[#] in wt% (n=32)	
	r	p	r	p	r	p
Recalled words after the fifth recollection (n)	-0.347	0.056	-0.520**	0.008	0.218	0.232
Learning performance [#] (n)	-0.270	0.142	-0.531**	0.006	0.310	0.084
Recalling performance [$] (n)	-0.482**	0.007	-0.544**	0.006	0.146	0.432
Loss after distraction (n)	0.489**	0.006	0.073	0.733	0.101	0.587

* significant, ** highly significant, [#] sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3

Number combination test

Significant differences regarding the number combination test (NCT) results were found between the PKU-patients and the healthy controls. The PKU-patients needed more time to complete the whole test (tab. 65). Furthermore, values of the IQ equivalent were found to be much higher in the controls (mean IQ 104) compared with the PKU-patients (mean IQ 85).

Tab. 65: Number combination test results of the PKU-patients and healthy controls

	Mean ± SD or N as appropriate		PKU vs. controls p-value*
	PKU-patients (n=29)	Controls (n=32)	
Total duration (sec)	418.4 ± 170.8	286.7 ± 81.0	0.001
IQ equivalent	84.7 ± 18.5	104.1 ± 16.8	<0.001

NCT, number combination test
* derived from Mann-Whitney-U-test or Student's unpaired t-test as appropriate

Current and lifetime blood Phe levels were significantly correlated with total duration of the NCT, as well as the IQ equivalent the Phe-level, whereas PUFA content showed no correlation (table 66).

Tab. 66: Spearman-Rho correlations between the blood Phe-level, blood PUFA content and results of the Number combination test in PKU-patients

	Phe-level during study (n=32)		Phe-level throughout life (n=26)		Sum of PUFA[#] in wt% (n=32)	
	r	p	r	p	r	p
Total duration (sec)	0.689**	<0.001	0.591**	0.004	-0.074	0.703
IQ equivalent	0.673**	<0.001	0.564**	0.006	0.065	0.739

NCT, number combination test, * significant, ** highly significant, [#] sum of following PUFA: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3

3.7 Subjects' own assessment of their health

For verification of the hypothesis that adults with PKU are less healthy than age-matched controls all subjects were asked for their health status, as well as how healthy they feel.
Although there was no significant group difference detected, the following two tables show an indication that PKU-patients might be less content with their health compared to controls. The same number of patients, as well as of the controls felt content, but about 39 % of the controls in contrast to only 8 % of the PKU-patients felt very content with his health status (table 67). In comparison with the controls more PKU-patients feel less content (12 % compared with 0 %) or even not at all content (6 % compared with 3 %).

Tab. 67: Measure of contentedness with health in the PKU-patients and healthy controls

	N (%)				PKU vs. controls p-value*
	Not at all content	Less content	Content	Very content	
PKU-patients (n=33)	2 (6.1)	4 (12.1)	19 (57.6)	8 (24.2)	0.130
Controls (n=33)	1 (3.0)	0 (0.0)	19 (57.6)	13 (39.4)	
Total (n=66)	3 (4.5)	4 (6.1)	38 (57.6)	21 (31.8)	

* derived from Chi-square test

Asking for the feelings about their health in more detail, there are again some trends detectable. As shown in table 68, more than 50 % of the PKU-patients, as well as of the controls feel healthy, but the proportion of the controls feeling very healthy exceeds that one in the group of the PKU-patients (39 % vs. 27 %). Five PKU-patients felt less healthy in contrast to only one of the controls and one PKU-patient compared to none of the controls.

Tab. 68: Feelings of the PKU-patients and healthy controls about their health status

	N (%)				PKU vs. controls p-value*
	Not at all healthy	Less healthy	Healthy	Very healthy	
PKU-patients (n=33)	1 (3.0)	5 (15.2)	18 (54.5)	9 (27.3)	0.198
Controls (n=33)	0 (0.0)	1 (3.0)	19 (57.6)	13 (39.4)	
Total (n=66)	1 (1.5)	6 (9.1)	37 (56.1)	22 (33.3)	

* derived from Chi-square test

4 Discussion

4.1 Subjects

The present study is based on 33 adult PKU-patients aged 26 to 59 years (12 men, 21 women) out of a total of 53 adult patients aged ≥25 years with PKU from the metabolic outpatient clinic at the Dr von Hauner Children's Hospital, Univ. of Munich. Five out of the 53 patients did not want to take part in the investigations, further five subjects were too handicapped and for 10 PKU-patients current addresses or telephone numbers could not be determined. As a reference goup, thirty-three healthy, age- and gender-matched subjects (11 men, 22 women) at an age between 23 and 49 years were recruited. The number of patients was comparable to other PKU studies in which the number of cases was sufficient to obtain significant test results for the comparison of the PKU-patients and the control group. For example, Moseley et al. (130) determined a significant decline in serum fatty acids in 27 adult PKU-patients, and Weglage et al. (185) showed significant alterations in the motor performance, as well as a positive correlation with the elevated Phe-levels in 20 PKU-patients.

The educational level tended to be different comparing both of our groups (p=0.054) because the control group was chosen according to the educational level of the current German population reported by the Statistical Yearbook (2) for adults of the same age like the patients. Thus, two patients did not have any graduation at all; three patients had graduated from special school (Sonderschule) compared to none of the controls; and 13 controls were qualified for University entrance (Gymnasium) compared to only 5 of the patients (tab. 27). The mean age of the patients (34.5 ± 6.3 yrs) matched with the one of the control group (34.6 ± 7.6 yrs).

There was no difference between the groups with respect to gender, age and heart rate; whereas PKU-patients were significantly shorter than the control group. Fisch et al. (57) and Verkerk et al. (179) reported a significantly smaller longitudinal growth in untreated patients with PKU, even after adjustment of the Phe-levels <3 mg/dl (78). One possible explanation for that might be a lower supply of Phe and other amino acids, vitamins, minerals and trace elements (162) e.g. malnutrition of zinc and other trace elements (149). Weight and BMI were highly significantly greater in adults with PKU. Avoidance of sport club activities due to reported social isolation and anxiety (167) of the PKU-patients might explain the higher weight and BMI. Besides, three of the patients live in homes with sportive activities only once a week. Additionally, Acosta et al. (6), as well as Mc Burnie et al. (119) found a Phe-associated tendency to overweight and Fisberg et al. (56) revealed lower height/weight ratios in PKU-children compared with healthy children. In the present study, blood pressure was

highly significantly greater, as well as the heart rate was found to be higher in the group of PKU-patients compared with the control group. This might be a result of the higher body weight and lower height, as well as of less sportive activity and nutritional influences which will be discussed in a different chapter.

4.2 Nutritional status

Phe-levels of the patients

As index for the quality of dietary control the blood Phe-level throughout life (mean of all yearly medians of Phe-levels since start of treatment collected in patient files) was used. The advantage of this method is reflected in the lower rates of extreme values compared to current blood Phe-levels (56). During the study, sixteen patients (48.5 %) were still or again adhering to low-protein diet supplemented with special amino acid mixtures. Three of them had restarted due to health problems (n=1) or pregnancy (n=2). These proportions are higher than data presented about phenylketonuric adults in the collaborative study of PKU in adulthood (96) in which 12.3 % never discontinued and 87.7 % never resumed low-protein diet although they received some additional treatment.

Daily dietary intake of Phe calculated from food records was about 2,362 mg. In adults the target value usually lies between 150 and 1,000 mg per day (131). Seven PKU-patients (including 3 pregnant women on strict diet) had Phe-intakes less than 10 mg per kg body weight and about 58.1 % of the PKU-patients (n=18) had Phe-intakes greater than 20 mg per kg weight. The average Phe-tolerance normally determined in adult PKU-patients amounts to 10-20 mg/kg. Only about every fifth PKU-patient had a Phe-intake within these ranges (n=6). Most of the patients seem to have higher Phe-intakes than considered advisable.

Regarding the blood Phe-levels, eleven (34.4 %) patients exceeded the recommendations of the APS (11) with a blood Phe-level greater than 20 mg/dl. The mean blood Phe-level in the study amounted to 15.9 mg/dl and the mean blood Phe-level throughout life amounted to 13.6 mg/dl. There was a highly significant positive correlation between the blood Phe-level in the study (excluding very low values, n=6) and the daily mean Phe-intake calculated from food records, or the blood Phe-level throughout life respectively. In the collaborative study of PKU in adulthood (96) the majority of the 73 PKU-patients had blood Phe concentrations above 19-20 mg/dl except the group of PKU-patients who always continued diet (n=9, mean Phe-level=15.4 mg/dl). Both facts demonstrate the importance of a strict low-Phe diet and adequate treatment which should be implemented early and continued for lifetime (96).

Method for dietary evaluation

Data of nutritional supply of the PKU-patients was collected by using dietary protocols. A modified form of the “Freiburger Ernährungsprotokoll” was used adding special low-protein products often consumed by PKU-patients for reminding patients of food like sweets usually eaten between the dishes. The dietary protocol consists of different food groups with typical food items. The patients estimated the quantities of consumed food items and beverages using common household measurements according to provided instructions (slice, piece, teaspoon, mug etc.). PKU-patients were asked to make precise descriptions, e.g. the content of fat to minimize failures in calculation of fat and calorie intake. Their food consumption was recorded over three consecutive days covering 2 weekdays and one weekend day for obtaining results which are representative for the intake of the whole week. PRODI, version 4.5 LE 2003 (NutriScience GmbH, Freiburg, Germany) commonly used for dietary treatment in Germany was used to record dietary intake based on the German Nutrient Data Base BLS, version II.3 (BgVV, Berlin, Germany). The results were compared to recommended ranges of the German Nutrition Society (47). To minimize failures in calculation of nutritional supply using PRODI we proved results for similar nutrients. “Vitamin E” concentrations were used because “α-tocopherol” values were lower or missing. In case of vitamin A, the “retinol equivalent” was used for calculation because “vitamin A” was not measured in amino acid mixtures. For folic acid there were three possibilities by using PRODI. Values were available for “free folic acid”, “free folic acid equivalent” and “folate”. The concentrations of folate exceeded those of free folic acid equivalent and free folic acid, therefore “folate” was used to calculate folic acid supply. Above choosing the right method for calculation, eating a variety of food products enriched with vitamins, or supplementation with vitamins and minerals might bias the results. For that reason blood vitamin levels might be higher than expected by calculated dietary intake derived from dietary protocols. In contrast to that, underreporting might result in lower calculated nutrient levels than the real intake.

Dietary intake and nutrient supply in PKU

Many studies reported untoward effects of the semi-synthetic diet on the intake or status of critical nutrients (6;22;24;35;44;62;70;108-110;144;146;148;172). In the present study *energy* intake was about 96 % of D-A-CH reference values (47) in women and 99 % in men, compared to 101 % (men) and 122 % (women) determined in the German population (46). The energy requirements of patients with PKU are the same as in healthy subjects (11;176;193) and result from basal metabolic rate, working metabolic rate and thermo genesis after dietary intake, as well as the requirements for growth, pregnancy and nursing period. An important part of the energy turnover is based on the energy requirement for

physical activities. The daily energy requirement results from the duration of activities. As PKU-patients seem to do rarely sports, they might have lower energy requirements. In contrast to our results meeting the recommendations, Fisberg et al. (56) found lower energy intakes in PKU-children compared with healthy children which might be a result of the strict diet excluding many high caloric food items e.g. chocolate. However, the participation in a nutritional study might have caused larger reductions in dietary intakes or underreporting of consumed foods than before the investigation at the days of the dietary record. Another explanation might be an appetite lowering effect of the amino acid mixtures.

The *protein* intake (calculated from total nitrogen intake) of the PKU-patients in the present study was about 108 % (women) and 104 % (men) of D-A-CH reference values, compared to 138 % or 169 % referring to the Nutrition Report 2004 (46). The protein intake measured in % of energy taken in exceeded the D-A-CH recommendations (14 % vs. 10 %). In contrast to results in adults, Fisberg et al. (56) found normal protein intakes in PKU-children which shows again the importance of diet in adulthood. The PKU-diet is very restricted in protein content and the amount of natural protein depends on the individual Phe-tolerance. Studies showed that patients with PKU have the same capacity of protein turnover as healthy subjects and requirements of Phe are the same as in healthy subjects regarding the compensation of tissue losses, specific functions of Phe, as well as need for growth (3;6;26;101;127). Therefore the protein intake is often calculated following the recommendations of the German Nutrition Society (DGE) from 1985, which are the same for adult women. The recommendation for adult men was about 0.9 g protein per kg body weight whereas current D-A-CH recommendations (47) propose 0.8 g per kg. As determined in other studies PKU-patients tend to have protein intakes higher than recommended (128). Patients with little restriction of protein intake may require supplementation of protein by amino acid mixtures in special situations e.g. in competitions or bodybuilding. However, further scientific investigations are needed to allow a final comment about a sufficient protein intake.

The *fat* intake of the PKU-patients was about 111 % in women and 98 % in men compared with D-A-CH recommendations (47). The fat intake determined in % of energy was about 107 %. These facts show that adult PKU-patients tend to have fat intakes higher than recommended (128). Results from the Nutrition Report 2004 (46) revealed a different fatty acid profile among the German population reporting higher intakes of fat (142 % ♀, 117 % ♂), SFA (176 % ♀, 136 % ♂) and MUFA (119 % ♀, 102 % ♂) but a lower intake of PUFA (88 % ♀, 84 % ♂). The PUFA intake in our PKU-patients was also lower than recommended but much lower compared with results of the Nutrition Report (PKU: 51 % ♀, 43 % ♂). The MUFA intake was similar to the recommendations (108 % ♀, 95 % ♂) and SFA intake (123 % ♀, 116 % ♂) exceeded the recommendations by D-A-CH (47). The intake of

linoleic acid was about 10.4 g in women and 10.5 g in men compared with higher results of 12.8 g and 16.3 g reported in the German population (46). The intake of α-linolenic acid amounted to 1.3 g in women and 1.5 g in men (168/135 % of D-A-CH) compared to 2.0 g and 2.2 g from the Nutrition Report (46). Referring to D-A-CH (47) the intake was higher than reference intakes (168/135 % ♀/♂). Regarding the fatty acid profil of plasma phospholipids PKU-patients had lower mean blood levels (wt%) of linoleic acid and DHA compared with healthy omnivores of a same age and gender (61). No difference was observed for α-linolenic acid levels, whereas the median arachidonic acid proportion was higher compared to healthy omnivores. However, some of the critical nutrients in adults with PKU are the same as in the German population e.g. PUFA which might be a result of the diet containing small amounts of fatty acids. Furthermore, the dietary intake of EPA (2 mg/d) and DHA (42 mg/d) was very low in PKU-patients possibly due to abdiction of fish and fish products. In comparison to our results, higher intakes of both EPA (53 mg ♀, 98 mg ♂) and DHA (87 mg ♀, 134 mg ♂) were reported in the Nutrition Report 2004 (46). Recently, the dietary intake of EPA and DHA combined in the German population has been estimated to be 141 mg/d among women and 186 mg/d among men (medians) (13). In conclusion, PKU-patients have lower intakes of DHA and EPA compared to healthy adults and lower intakes of PUFA compared with D-A-CH recommendations and results of the Nutrition Report as previously reported in several studies (4;63;130;148;177). These findings must be taken into consideration to ensure an optimal nutritional supply in PKU.

The intake of *carbohydrates* in g per day was about 82 % (♀) and 90 % (♂) in relation to recommendations of D-A-CH. Carbohydrates in % of energy taken in by PKU-patients were about 108 % of D-A-CH whereas the intake of fiber was 43 % lower than the recommended 30 grams per day (17 ♀ and 9 g ♂ per day). Higher intakes than determined in our study were reported among the German population in the Nutrition Report 2004 (108 %) in which also fiber intake exceeded our results (25 g ♀, 22 g ♂). In conclusion, the intake of carbohydrates and fiber was lower than reference values for adults. The proposed 30 g fiber per day often cannot be realized due to a restriction of food assortment in the PKU-diet. Moreover, PKU-patients tend to have high intakes of food rich of sugar and little content of fiber.

Regarding the *vitamin* supply of PKU-patients, intakes and blood levels were determined and compared with reference values of D-A-CH (47), as well as results of the Nutrition Report 2004 (46). The intakes of retinol were about 175 % among women and 150 % among men compared with D-A-CH reference values. The thiamin intake was about twice as much than the reference value (220/142 % ♀/♂), the riboflavin intake was about 183/157% (♀/♂), the pyridoxine intake was 192/153 % (♀/♂) and the daily intake of folic acid about 83 % in relation to the reference values. The intake of ascorbic acid amounted to 162 %, the intake of

vitamin D achieved 92 %, the intake of α-tocopherol was 155 % and the vitamin K intake exceeded the D-A-CH reference values several times (393 % ♀, 413 % ♂). The intake of panthotenic acid was about 115 %, the intake of biotin 246 % and the cobalamin intake amounted to 170 % of the D-A-CH values. Thus, lower levels than reference values were determined for folic acid and vitamin D. In contrast to that, the mean intakes of vitamin A, B-group vitamins, ascorbic acid and α-tocopherol were higher than reference values. In comparison with our results the intake among the German population was for retinol 173/112 % (♀/♂), vitamin B1 136/113 % (♀/♂), vitamin B2 128/111 % (♀/♂), vitamin B6 143/119 % (♀/♂), vitamin D 49/66 % (♀/♂), vitamin E 106/106% (♀/♂) and vitamin K 526/403 % (♀/♂) of the D-A-CH reference values. Determining blood samples in one PKU-patient we found a blood retinol concentration less than the reference range. Three PKU-patients had reduced levels of α-tocopherol, five PKU-patients had higher folic acid and one PKU-patient higher cobalamin levels compared to the reference range. All PKU-patients met the reference intake values for vitamin D. A lack of panthotenic acid, biotin and ascorbic acid as determined in the German population (46) was not found in PKU-patients. These results indicate that adult PKU-patients, mainly with loosened diet and only little restriction of protein intake, as well as without intake of an amino acid mixture, have to pay attention to an adequate intake of vitamins. A substitution might become necessary. Numerous studies showed inadequate intakes of vitamins (41;70;70;144;147;153;158;177) in adult PKU-patients due to a vegetarian diet. We found mainly low intakes of folic acid and vitamin D which was also reported in the general German population, as well as low blood levels of α-tocopherol and retinol (3). However, a lack of B-group vitamins (129), or vitamin B12 (70), vitamin B6 (144), and vitamin B2 (108) was not found.

Regarding the intake of *minerals* and *trace elements*, calcium intake amounted to 108 %, phosphorus 177 %, magnesium 137/133 % (♀/♂), iron 193/121 % (♀/♂), zinc 216 %, iodine 83 %, copper 208 %, chloride 142 %, fluoride 26 % and manganese 393/413 % (♀/♂)in relation to reference values of D-A-CH. Results of the Nutrition Report showed the following supply: calcium 181/90 % (♀/♂), magnesium 142/110 % (♀/♂), iron 101/134 % (♀/♂), zinc 159/111 % (♀/♂), copper 154/147 % (♀/♂), manganese 129/110 % (♀/♂), phosphorus 196/198 % (♀/♂) and iodine 48 %. In contrast to intakes almost 38 % of the PKU-patients had blood iron or zinc concentrations below the laboratory's reference range. Two PKU-patients had low contents of ferritin. A high intake of zinc and iron but low blood levels might be caused by higher requirements or worse utilization of zinc and iron. Schäfer et al. concluded that the growth retardation found in PKU-patients might be caused mainly by poor supply of zinc and other trace elements (149). In 56 % of the subjects, reduced blood levels of selenium were found which were significantly positively correlated with blood Phe-levels throughout life ($r=0.451$, $p=0.024$). As food items rich of selenium are often rich of protein this

might cause a low intake of selenium adhering to a low-protein diet. Regarding blood levels, we revealed a higher Ca level in two of the PKU-patients, one patient had higher blood ferritin and seven higher blood copper concentrations. In conclusion, mean intakes of sodium, potassium, magnesium, iron, zinc, copper, chloride and manganese were found higher than the reference values. Levels of iodine and fluoride turned out to be lower but this was also reported among healthy adults in the Nutrition Report. The blood levels of iron, ferritin, selenium and zinc were lower than reference ranges in some of the PKU-patients but not in other minerals and trace elements as it was shown in numerous studies (5;22;24;44;67;74;110;122).

4.3 Immune status

There are indications that PKU-patients show alterations in the immune system (88;146). Karagoz and colleagues (88) found that children with PKU had lower immunoglobulin levels compared to healthy children. Regarding the concentrations of immunoglobulins no major abnormal results were detected for IgG, IgA and IgM. IgG levels were normal in all patients, elevated IgA was found in one and two PKU-patients had greater IgM values compared with normal ranges. The most significant result was that in about 30 % (n=10) of all patients elevated serum IgE levels were determined. In these patients, an Elisa immunoassay was performed for measurement of special IgE. Searching for correlations significant positive correlations were noticed between IgE and blood Phe-level in the study (p=0.029). Furthermore significantly negative correlations were found between the PUFA content of blood and IgA (p=0.036), as well as IgE (p=0.012). In contrast to our results, Riva et al. (146) revealed significantly decreased serum concentrations of IgG, IgA and IgM in PKU-patients compared with reference values but increased IgE levels without a higher incidence of infections or allergic diseases but a more frequent allergic sensitation. Gropper et al. (68) found significantly lower mean plasma IgG and IgA concentrations in PKU-children compared to values in healthy controls. He reported that IgG levels were positively correlated with intakes of energy, protein and iron. Testing for special IgE, PKU-patients showed positive reactions regarding different allergens of the Elisa immunoassay. Most of the 10 patients with elevated IgE showed allergic reactions with phleum pratense (n=6), secale cereale (n=5), quercus alba (n=4), as well as dermatophagoides pteronyssinus (n=4). Using a questionnaire, atopic disease was suspected if the subject had or ever had had at least one of the diseases associated with atopic disease. Based on these criteria, almost 55 % of all PKU-patients were suffering from atopic disease compared with only 21 % of the age-matched controls, which means that adults with PKU had highly significantly more often atopic disease than the controls. Phenylketonurics had significantly more often hay fever, allergic coryza or allergic conjunctivitis. A trend towards a higher prevalence of asthma in

PKU-patients compared to healthy controls was observed (p=0.053). A running nose and swollen eyes as typical allergic symptoms, as well as neurodermitis, urticaria or food allergy were not significantly but still more often observable in patients compared with controls. Searching for correlations there was no significant correlation between the frequency of allergic diseases and blood Phe, or blood PUFA level observable in PKU-patients. Furthermore, the median arachidonic acid (AA) proportion was found to be higher and median DHA levels lower in PKU-patients compared to healthy omnivores which could explain alterations in the immune status. Thus, Agostoni et al. (7) found that well-compliant PKU-subjects had low AA concentrations in plasma but low compliance with animal food avoidance and higher Phe-levels resulted in elevation of plasma AA. Giovannini et al. (63) reported that the availability of AA from plasma in PKU was related to dietary compliance and seems to influence the synthesis of AA-derived eicosanoids influencing the immune system. Another cause for changes in the immune status in PKU might be the damage of the antioxidative status. We found decreased blood concentrations of selenium, zinc, retinol and α-tocopherol. Van Bakel (177) showed that PKU-patients had lower glutathione peroxidase activity compared with HPA subjects and controls and a lower glutathione concentration than the control group. Schulpis et al. (157) found a high plasma antioxidant status in patients with PKU, especially in those with a good compliance with their diet which is possibly due to the amounts of antioxidants present in their special low-Phe vegetarian diet. Sirtori et al. (164) presented data about increased oxdative stress parameters and decreased total antioxidant reactivity in patients with PKU indicating a stimulation of lipidoxidation and deficient capacity to rapidly handle an increase of reactive species. And finally, a recent multicenter study in the USA found that adult PKU-patients who discontinued their diet show more frequently eczema and bronchial asthma compared to adults who continue their diet (96).

4.4 Cardiovascular risk

BMI, consumption of cigarettes, blood pressure, hypertension, heart rate and blood parameters like homocysteine and the lipoprotein profile were determined as indicators of cardiovascular risk of the PKU-patient. We found significantly greater values for BMI, blood pressure and heart rate in the PKU-patients compared with controls. The adults with PKU were significantly more often obese.

Eleven PKU-patients but only three of the controls had BMI values in the range of 25 to <30. Obesity grade I (BMI 30 - <35) was revealed in 4 of the PKU-patients compared to only 2 of the controls. BMI values of 35 and higher were found solely in two adults with PKU but in none of the controls. But the subjects from the control group tended to smoke more often

than patients with PKU (n.s.). Hypertension was not significantly different in both groups. Six of the PKU-patients but eight of the controls had mild, two patients but six controls moderate and only one control subject had heavy hypertension. Regarding the blood lipid status and blood homocysteine levels of the PKU-patients, elevated levels (compared to the laboratory's reference range) were often observed: 3 % up to 19 % had higher concentrations of blood parameters that are considered as risk factors for cardiovascular disease (CVD). Five PKU-patients (15 %) had elevated total cholesterol and six (18 %) elevated TG levels relative to fasted reference values, our PKU-patients were not fasting before bood sampling. Circulating TG levels have been associated with the severity and progression of atherosclerosis (75) and are considered independent risk factors for coronary heart disease (77), but how many fasting TG values might have been elevated is unknown. Elevated VLDL concentrations relative to fasting levels were detected in six adults with PKU (19 %), but again it is unclear how many subjects might have had elevated fasting levels. In one PKU-patient, the LDL level exceeded the reference range, and two subjects had LDL/HDL ratios above the reference range. Lp (a) was elevated in six PKU-patients (18 %), Apo A1 in six (19 %) and Apo B was elevated in one patient. Homocysteine was found above the reference range in 16 % of the PKU-patients. Schulpis et al. (153) reported that PKU-patients on a strict diet had low vitamin B6, vitamin B12 and folate levels resulting in moderate homocysteinemia, which might influence the risk of coronary artery disease. In another study (153) he showed that TG, as well as larger and less atherogenic LDL particles were associated with a high zinc to copper ratio, which might not be reached in PKU-patients due to a low dietary supply of bioavailable zinc. Previous studies have shown that the risk of coronary heart disease is influenced by dietary fatty acid intake (29;103;191). A higher degree of incorporation of n-3 LCPUFA into myocardial membranes reduces sudden cardiac death following myocardial ischemia (105). A case-control study showed that levels of DHA in plasma phospholipids were inversely correlated with the risk of coronary heart disease in a multivariate model that controlled for the effects of HDL/LDL ratio (163). Kinosian et al. (91;91;92) proposed that the LDL/HDL ratio is a better predictor of risk for coronary heart disease than total cholesterol or LDL plasma levels alone. Omega-3 LCPUFA also have a TG lowering effect in humans (42;45;66;135;136). N-3 LCPUFA were reported to improve blood pressure control in hypertensive patients (25;93;114) and to decrease the ApoA1/HDL (30;66). Testing for correlations between cardiovascular risk factors and blood Phe or blood PUFA level, significant positive correlations were detected between the blood Phe-level in the study and total cholesterol ($p=0.017$), as well as TG ($p=0.050$) and VLDL ($p=0.033$). Additionally, blood DHA content (wt%) was positively related to HDL ($r=0.466$, $p=0.007$), to LDL/HDL ($r=0.371$, $p=0.036$) and to ApoA1 ($r=0.514$, $p=0.003$). In the collaborative study of PKU in adulthood (96) PKU-patients who never discontinued diet rarely suffered from heart disease or

hypertension. It seems likely that low PUFA and DHA intakes in PKU-patients, jointly with elevated blood lipid levels and an elevated LDL/HDL ratio in two patients (mean 2.4, SD 0.9), increased obesity and hypertension, poor antioxidant status and less physical activity in PKU-patients might all contribute to an elevated cardiovascular risk in PKU-patients compared with healthy controls.

4.5 Metabolic status

For determination of differences in metabolic parameters, all patients and controls were asked to wear an accelerometer for three days. The total energy expenditure per day, the number of steps taken, as well as the sleeping duration showed no significant differences between the PKU and the control group. The lying duration tended to be (n.s.) different with 8.6 hours for patients and only 8.1 hours per day for the controls (p=0.051). Significantly higher values for physical activity levels and the daily active energy expenditure were found in the control group. Significant correlations found between accelerometer results and inactivity parameters in all subjects were noticed. Significant positive correlations were found between activity energy expenditure, as well as physical activity level and duration of physical activity per week, but there were significant negative correlations between duration of watching TV per day and activity expenditure as well as physical activity level per day. A significant negative correlation was found between physical activity level per day and obesity, as well as body weight. A significant positive correlation was found between the blood PUFA content (see 3.2.1.) and physical activity level (r=0.371, p=0.040) and the number of steps taken (r=0.406, p=0.023). Healthy adults reported significantly more often sports activities and less frequent television viewing than PKU subjects. The duration of computer use during free time tended to be higher in controls compared to PKU-patients (n.s.) which might result from different educational levels and occupations e.g. to qualify for University entrance one needs to have a computer. There were no significant correlations between cardiovascular risk factors and blood Phe or blood PUFA levels.

Compared with our results, Acosta et al. (6) and McBurnie et al. (119) found a Phe-associated tendency to overweight in patients with PKU. Fisberg et al. (56) found lower height/weight ratios in PKU-children compared with healthy children. These findings might be related to the avoidance of sport club activities due to reported social isolation and anxiety of PKU-patients (167), but there are also further explanations. Schulpis et al. (155) revealed that ghrelin is negatively correlated with plasma Phe content but positively with catecholamine levels and energy intake. In another study (154) these investigators showed that leptin was significantly increased in PKU-patients on a loose diet compared with PKU-patients on a very strict diet. Leptin was negatively correlated with the total energy intake and

positively with the Phe-level. In contrast to these findings, White et al. (192) reported on overweight which was not significantly related to the diet but to socioeconomic status and overweight of parents. Allen et al. (8) investigated resting expenditure in children with PKU and found no evidence of reduced resting energy expenditure or increased weight.

4.6 Neurological status

4.6.1 Neurophysiological findings

The number of failures and durations of the different tasks, as well as fine motor factors like aiming of motion, hand unrest (tremor), arm-hand precision, arm-hand and wrist-finger speed were compared between patients and controls. Adults with PKU committed about three times more failures in the steadiness task, almost one third more in line tracking (highly significant) and approximately twice as much failures in aiming as controls. Regarding the measured duration of failures, there were significant differences for steadiness and highly significant differences for line tracking. The total duration of line tracking and aiming was not significantly different between both groups but significantly different for inserting pins. Controls had a higher rate of hits in tapping and almost as many hits as the PKU-patients in aiming. Significant positive correlation between the blood Phe-level in the study and the number of failures in steadiness ($r=0.370$, $p=0.031$) and aiming ($r=0.398$, $p=0.026$), the duration of failures in aiming ($r=0.469$, $p=0.008$), as well as the total duration of aiming ($r=0.389$, $p=0.031$) should be noticed. Additionally, a significantly negative correlation was found between the blood PUFA content and total duration of inserting pins ($r=-0.361$, $p=0.042$). The results of the PKU-patients are positively correlated with blood Phe-levels during the study. Differences were observed comparing motor factors determined by the MPT task. PKU-patients had worse t-values for every factor compared with controls. There were significant differences in aiming of motion, arm-hand precision, as well as in arm-hand speed, and non significant trends to worse hand unrest and wrist-finger speed. There was a significant negative correlation between the blood Phe-level during the study and aiming of motion, as well as arm-hand speed. These results indicate that fine motor skills appear to be correlated with the strictness of the PKU-diet, with lower blood Phe- and PUFA-levels associated with better results.

From evaluable VEP results (n=23) a mean latency of 110.5 ms was calculated for PKU-patients. In comparison, mean VEP (70') latency in 30 healthy omnivores of comparable age and gender ratio was 100.8 ms. P100 latencies above 115 ms were stated as prolonged. In eight (34.8 %) of all 23 evaluable VEPs prolonged P100 latencies above 115 ms were determined, whereas in none of the 30 omnivores were found prolonged latencies. Nutt et al.

(137) showed significantly shorter P100 latencies after administration of dopamine in healthy adults and Parkinson patients. Ludolph et al. and Korinthenberg et al. (99;113) found significantly prolonged P100 latencies independent of the actual blood Phe-level explained by dysmyelination (14;134). Beblo et al. (15) measured VEPs in 36 patients with early treated PKU (aged 1-11 yrs) and good metabolic control before and after supplementation with LCPUFA from fish oil. After three months, 22 of the PKU-patients with significantly longer P100 latencies had significantly improved P100 latencies but latencies did not change in 12 of the PKU-patients. This might signify that LCPUFA are essential substrates for the nervous system function even beyond infancy.

Patients with PKU seem to be differently vulnerable to alterations in the brain, and a few untreated patients with classical PKU with normal development have been described (48;143;160). Only Bick et al. (18;19) did not find any significant relation between VEP results and blood Phe-level or diet. One of the possible explanations might be dysmyelination of the visual nerve tract and disorders of the neurotransmitter metabolisation (14;82) but administration of L-dopa showed no effect on P100 latencies (175) which might be due to inhibition of dopa intake through blood-brain barrier (94;95). It is unclear whether Phe not only inhibits intake and synthesis of dopa but also interferes with its release (137). Additionally, the role of the density of dopamine receptors is not clear; there might be a compensating postsynaptic upregulation of dopamine receptors (175).

4.6.2 Neurostructural findings

Pathological alterations were found in all of the 16 MRIs performed. The severity of these findings ranged from one up to 6 points. There were no significant correlations between severity of deep white matter alterations and blood Phe, or blood PUFA level. Pietz et al. (139) reported alterations in only 10 % of the PKU-patients altered MRI findings. No relation to MRIs was reported by Cleary et al. (40), whereas Schafer et al. (150) reported improvement after strict diet and administration of transmitters (L-dopa, 5-hydroxy-tryptophan) which explains dependency on transmitters which are decreased in PKU due to inhibition of synthesis of dopamine, noradrenalin, serotonin, as well as inhibition of expression of dopamine receptors (52;80).

Regarding the MRI findings of patients with PKU, alterations in deep white matter were first described in 1989 (180) in two adult, lately treated PKU-patients (after 3rd year of life) with cerebral convulsions or decreased intellectual skills. Both had mainly periventricular occipital but also frontal, subcortical alterations with participation of corpus callosum and brainstem as shown in other studies (18;19). The causes are de- or dysmyelination (134;162) with inserted water (edemas) (19) which were found also in untreated PKU-patients post mortem (14). The exact pathogenesis has not not been clarified but might be caused by increase of total tissue

water or structural myelin alterations and consecutive increase of free, extracellular water (18;19). Similar to other studies (40;139) we found a high proportion of pathological MRI findings in adult PKU-patients. The severity of these findings ranged from one up to 6 points on a scale according to their extension. It was reported that such pathological morphological alterations are also found in PKU-patients without noticeable neurological abnormalities (18;19). Phe has toxic implications on brain (34;79;82) causing disorders of synthesis and breakdown of neuronal proteins, myelin and myelin proteins (81;83;89;171). The alterations are mainly periventricular, but in more severe manifestations also frontal and subcortical, and in some cases atrophy is found. Extension increases with age and poor diet compliance (183), whereas the appearance and severity are individually very different and not related to intelligence, neurophysiological, or neuropsychological findings (40;139). Nevertheless, it was shown that edemas are reversible by adhering to a very strict diet for 3 months (19;40). It was reported that untreated patients with classical PKU had only minimal alterations of deep white matter (187) which indicated that there are further causes like different brain Phe-levels but same blood Phe-levels (190). In conclusion, these findings seem to be caused by dysmyelinisation with insertion of water dependent on the blood Phe-level and therefore reversible by strict diet in adulthood.

4.6.3 Neuropsychological findings

From the variety of neuropsychological tests, the d2 attention test, the color-word-interference-task, the auditory verbal learning test and the number combination test were chosen to apply. In adult patients with PKU there are differences expected but the influence of elevated Phe-levels might have a lower impact on cognitive functions than in children because further brain development decreases with age and perhaps there are changes in the metabolisation of transmitters (84). However, it seems that most of the PKU-patients are not very adversely affected in normal life (145;152), but some are worse (181). The neuropsychological results do not generally worsen with age and higher Phe-levels (32).

Regarding the d2 attention test, subjects from the control group had a significantly higher number of items completed in the whole test, a higher value for number of items completed subtracted by failures as well as a higher concentration performance (number of crossed out characters minus failures) compared to PKU-patients. The number and percentage of failures, as well as the spread of the number of items completed between the lines differed not significantly between PKU-patients and the control group. In PKU-patients (n=30), the number and percentage of failures were significantly positively correlated with the blood Phe-level in the study and throughout life, whereas the number of items completed, the number of items completed minus failures, as well as the concentration performance were significantly negatively correlated with blood Phe-level in the study and throughout life. In addition to that,

the concentration performance was significant negatively correlated with the blood Phe-level throughout life.

Regarding the color-word interference test, highly significant differences were found to be in the reading duration of color-words, color-strokes, as well as of the interference list between PKU-patients and healthy controls. Subjects from the control group had higher t-values in the reading duration of all three tasks, while no differences were observed for nomination and selectivity skills. Significant negative correlations were found between the reading duration of all three different tasks and blood Phe-levels during the study. Elevated Phe-levels at least partly contribute to consecutive impairments of the metabolism of neurotransmitters (52;82;111;112). The frontal brain depends on dopamine (49;50). The color-word interference test is a sensitive test for determination of deficits in frontal brain dependent cognitive functions (16). Significant differences are detectable in the number of immediately recalled words (of 15 presented words) and in the number of recalled words after the fifth recollection between PKU-patients and healthy controls, whereas for the learning and recalling performance, as well as for the loss after distraction and the total number of recognized words no significant differences were found. There were significant negative correlations detectable between the blood Phe-levels during life and recalled words after the fifth recollection, learning performance, as well as recalling performance in PKU-patients. The blood Phe-level during the study showed a significant negative correlation with recalling performance and a significant positive correlation with loss after distraction.

Significant differences regarding the number combination test (NCT) results were found between PKU-patients and healthy controls. PKU-patients needed more time to complete the whole test. The determined IQ equivalent showed much higher values in controls (mean IQ 104) compared with PKU-patients (mean IQ 85). Searching for significant correlations between total duration of the NCT, as well as IQ equivalent and blood Phe-level, blood PUFA content showed a significant relations regarding the Phe-level but none regarding the PUFA content. An explanation for the mental retardation might be the inhibition of the ATP-sulphurylase in oligodendrocytes with decreased synthesis of sulphatides that are needed to protect myelin proteins against breakdown, which finally causes a decrease of synaptic contacts (83). Detailed studies have shown that the IQ is not any more significantly (33;165;166) influenced by the Phe-level after the 10^{th} year of life.

Neuropsychological results might be improved by a stricter diet (32). Weglage et al. (185) investigated 20 early-treated adolescent PKU-patients and 20 healthy controls twice (test 1 and three years later test 2) at mean ages of 11 and 14 years, respectively, for their fine motor abilities (MPT), sustained (d2) and selected (CWIT) attention. He found no significant correlation between changes of neuropsychological test results and the blood Phe-levels during the three years between test times 1 and 2, as well as with the lifetime blood Phe-

level. At the first test, examinations revealed significant blood Phe-correlated neuropsychological deficits in PKU-patients. However, in spite of raised blood Phe-levels during the following three years and significantly elevated concurrent blood Phe-levels, the repeated measurements revealed a significant decrease of patients' deficits compared to controls. These results indicate a decreased vulnerability of the PKU-patients with respect to their neuropsychological functioning against elevated blood Phe-levels on ageing.

Based on results of the PKU Adult Collaborative Study, Brumm et al. (28) suggested that adults with early-treated PKU demonstrate specific cognitive deficits, a number of which are associated with the frontal and temporal area of the brain. Deficits were noted in several domains including executive functioning, attention, verbal memory, expressive naming and verbal fluency. PKU-patients with blood Phe-levels higher than 1000 µmol/l (group 1) scored lower than the group with Phe-level below 1000 µmol/l (group 2) on measures of focused attention, verbal fluency, reaction time, verbal recognition, visual memory and naming. Tests of cognitive functioning were often correlated with measures of treatment during childhood rather than with Phe-level at the time of cognitive testing. Subjects with abnormal MRI scored significantly lower on two cognitive tests (Trails A and CVLT Recognition Memory). The investigators found no significant correlation between current brain Phe-level obtained through MR spectroscopy (n = 10) and neuropsychological function. Future longitudinal investigation with a larger sample size will assist in clarifying the etiology of neuropsychological deficits and association with treatment history.

Feldmann et al. (53) assessed 35 PKU-patients between 13 and 21 years of age and compared them to diabetic patients with respect to IQ, information processing, sustained (d2) and selective (CWIT) attention. The assessments were repeated within a 3-year follow-up. PKU-patients had significantly poorer test results, with reduced performance speed than diabetic patients. He concluded that elevated Phe concentrations seem to exert a global effect on slowing performance speed in adolescence and early adulthood. Regarding the blood Phe-levels, studies showed that these are not related to Phe concentrations in the brain (87;102;124-126) although they are correlated with neurological results. Nevertheless, the Phe-level in the brain seems to be a big confounder for the development of the patients so that blood Phe-levels might not be optimal for dietary control (124;125).

There are many investigations on the pathologic mechanism of the development of the severe mental damage due to high Phe-levels, which indicate that it may be caused by a multifactorial process. Perhaps the inhibition of amino acid transport into brain is not the major cause of structural and functional alterations in the brain. In patients with tyrosinemia type II and histidinemia, similar imbalances of amino acids are observed but do not lead to alterations of neurotransmitters (79). Another cause might be an abnormal glucose metabolism, as Wasserstein et al. (182) found a decreased relative glucose metabolic rate in

cortical regions and an increased activity in subcortical regions of the PKU-patients. The actual Phe-level correlated with abnormal activity in the prefrontal and visual cortices. Since plasma Phe-levels are negatively associated with intelligence and other cognitive functions (60), Channon et al. (37) suggested that continuous dietary management may be a successful strategy to enhance cognitive outcome in adults with PKU.

4.7 Subjective assessment of health status

All subjects were asked about their assessment of their health status. Although there was no significant difference between PKU-patients and controls, there is a possible indication that PKU-patients might be less content with their health compared to controls. The same number of PKU-patients and controls felt content, but about 39 % of the controls compared with only 8 % of the PKU-patients felt very content with their health status. There were more subjects in the group of the PKU-patients who felt less content (12 % compared with 0 %) or even not at all content (6 % compared to 3 %).

Asking for their feelings in more detail, there are again some differences detectable. More than 50 % of the PKU-patients, as well as of the controls feel healthy, but the proportion of the controls feeling very healthy exceeds that one in the group of phenylketonurics (39 % vs. 27 %). Five PKU-patients felt less healthy in contrast to only one from the control group and one of the PKU-patients compared with none of the controls even felt not at all healthy. Expectedly, there was a strong correlation between the feelings about their health and the contentedness with life (fig. 16). Both, patients and controls were more content if they felt healthier. Thus, considering all results there are indications of a worse (felt) physical health status of adults with PKU compared with controls, and concepts should be developed to improve the health of adult PKU-patients.

In the present study, physical health status was investigated by evaluation of nutritional, immune, metabolic and neurological status, as well as the cardiovascular risk. Altogether we found worse results for PKU-patients regarding the nutritional, the immune, the metabolic and the neurological status, as well as the cardiovascular risk compared to controls. Thus, the current concept for treating adult patients with PKU does not ensure a desirable physical health outcome. Together with the results of the interviews for contentedness with health, these data lead us to conclude that better medical, nutritional and continuous psychosocial care is needed to improve the health status of adult PKU-patients and the situation for their families (23;64;187).

It seams that current treatment concepts do not allow to obtain desirable blood Phe-levels in many adult PKU-patients. One PKU-patient had psychological problems but did not receive any adequate treatment. An adequate psychosocial, medical and nutritional care is

suggested to influence the long-term physical health of adult PKU-patients (96;159), independent from the early and strict diet in childhood. The mainly medically orientated outpatient visits in the hospital clinic, under time pressure and usually occurring only once a year, appear not to suffice for adequate coping with the disease and dietary treatment.

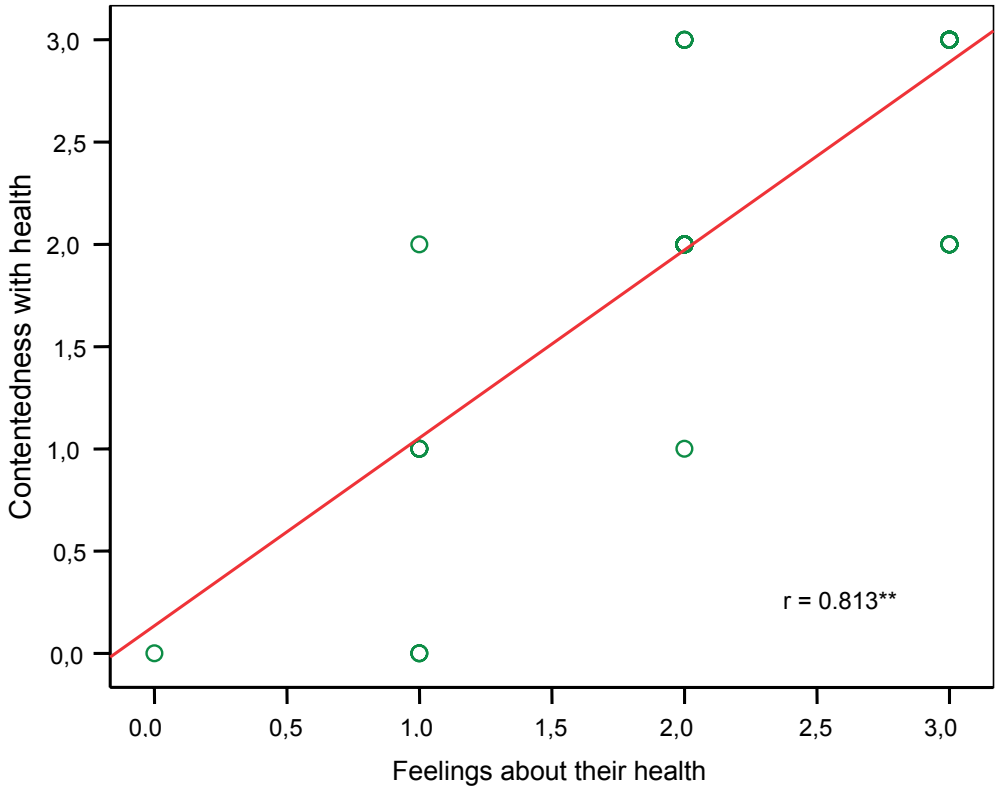


Fig. 16: Spearman-Rho correlation between feeling healthy and being content with life

5 Summary

Background

Adult patients with PKU might benefit from an adequate medical, nutritional and psychological support. In addition to an early and strict diet in childhood, the nutritional and medical support during adolescence and adulthood appears to influence the long-term physical health of adult PKU-patients. Current concepts propose a “diet for life” but studies show that after intensive care during childhood, adult PKU-patients often lack an adequate medical, nutritional and psychosocial supervision. The dietary compliance of the PKU-patients decreases with age, and there is a lack of age-adapted dietary guidelines and nutritional education. Since the health care for adult PKU-patients is not well developed in Germany, studies of this age group are urgently required. The present evaluation deals with the controversial question whether and how strictly adult patients with PKU should be treated, and furthermore which effects and side effects result from a low-Phe diet.

Hypotheses to be tested were that adult PKU-patients exhibit diet induced nutritional deficiencies (e.g. LCPUFA, minerals, trace elements and vitamins), that an unbalanced diet in PKU-patients has implications for their immune system and cardiovascular risk profile, and that compared to healthy age matched subjects, adult PKU-patients show distinct neurological abnormalities.

Design

We carried out a Case-Control study with adult PKU-patients and healthy age-matched controls. Thirty-three adult PKU-patients attending the Department of Metabolic Diseases of the Dr von Hauner Children’s Hospital, University of Munich, as well as 33 healthy subjects of the same gender and age took part in the investigations.

In contrast to healthy age-matched controls, adult PKU-patients showed worse results regarding nutritional, immune, metabolic and neurological status, as well as cardiovascular risk profile. Blood levels of PUFA (DHA, EPA), selenium, zinc, iron, vitamin E, iodine and fluoride were often lower than normal. PKU-patients suffered more often from allergic diseases. Accelerometer data showed a lower physical activity level in patients with PKU. The cardiovascular risk profile of patients with PKU was worse compared with controls, with a higher prevalence of hypertension and obesity, as well as elevated blood lipids. PKU-patients had worse outcomes in neurological testings, all measured MRIs were pathologically changed, and pathological VEPs were observed in more than 50 % of the PKU-patients.

Conclusions

These data verify that there is a lack of adequate treatment of adult PKU-patients, which has detrimental effects upon the nutritional, immunological, metabolic and neurological status as well as the cardiovascular risk profile of adult patients. The development and implementation of improved approaches to dietary, medical and psychosocial treatment is needed to improve the physical health of adult PKU-patients and the situation for the whole family.

6 Zusammenfassung

Hintergrund

Derzeit fehlt ein umfassendes Behandlungskonzept für PKU-Patienten im Erwachsenenalter. Eine altersentsprechende medizinische, ernährungswissenschaftliche sowie psychologische Betreuung für diese Patientengruppe ist in Deutschland nicht sichergestellt. Es fehlen stichhaltige Beweise für dessen Notwendigkeit sowie Vorschläge für ein solches Konzept.

Ziele:

In der vorliegenden Arbeit sollte der Gesundheitszustand von erwachsenen PKU-Patienten ab 25 Jahren untersucht und dem Gesundheitszustand von gleichaltrigen gesunden Erwachsenen gegenüber gestellt werden. Speziell sollten mögliche Unterschiede in der Nährstoffversorgung, immunologischen Parametern, dem kardiovaskulären Risiko, der Stoffwechselsituation sowie dem neurologischen Zustand zwischen den beiden Gruppen festgestellt werden.

Studiendesign

Es wurde eine Fall-Kontroll-Studie mit 33 erwachsenen PKU-Patienten im Alter zwischen 26 und 59 Jahren (12 Männer, 21 Frauen) sowie 33 gleichaltrigen gesunden Kontrollpersonen im Alter zwischen 23 und 49 Jahren (11 Männer, 22 Frauen) an der Dr. von Haunerschen Kinderklinik in München durchgeführt.

Ergebnisse

Die PKU-Patienten wiesen einen schlechteren Versorgungszustand bezüglich einzelner Nährstoffe, vor allem bezüglich mehrfach ungesättigter Fettsäuren (EPA, DHA), Selen, Zink, Eisen, Vitamin E, Jod und Flur auf. Immunologische Parameter weisen auf eine höhere Anfälligkeit für allergische Erkrankungen hin. Das kardiovaskuläre Risiko von erwachsenen PKU-Patienten ist im Vergleich zur Kontrollgruppe aufgrund häufiger vorkommenden Übergewichts, Bluthochdrucks sowie eines ungünstigen Blutlipidstatuses als erhöht einzuschätzen. Hinsichtlich der Stoffwechselsituation wurden bei PKU-Patienten schlechtere Ergebnisse erreicht als bei den Kontrollen. PKU-Patienten scheinen sich weniger häufig körperlich zu bewegen. Darüber hinaus wies die Patientengruppe zahlreiche krankhafte neurologische Veränderungen auf, so waren alle MRT-Befunde auffällig und eine Großzahl der VEP-Befunde als pathologisch zu bewerten, außerdem erzielten PKU-Patienten schlechtere Ergebnisse in neuropsycho- und physiologischen Tests.

Schlussfolgerungen

Anhand der vorliegenden Ergebnisse wurde nachgewiesen, dass erwachsene PKU-Patienten einen schlechteren Gesundheitszustand aufweisen als gleichaltrige gesunde Erwachsene. Dies bedeutet, dass dringend Möglichkeiten und ein entsprechendes Behandlungskonzept geschaffen werden muss, um PKU-Patienten über das Kindesalter hinaus eine adäquate Behandlung und damit einen den Umständen der Erkrankung entsprechenden optimalen Gesundheitszustand gewährleisten zu können.

7 Attachment

7.1 Tables

Tab. A1: Daily nutrient intake of the PKU-patients in Munich

	PKU-patients (7 ♂, 24 ♀)				
	Mean ♀ / ♂	SD ♀ / ♂	Median ♀ / ♂	Reference value* ♀ / ♂	Mean in % of reference value
Energy (kcal)	2283 / 2772	646 / 565	2180 / 2843	2300 / 2900	99 / 96
Energy (kJ)	8988 / 11615	3409 / 2361	8848 / 11927	9500 / 12000	95 / 97
Protein (g)	74 / 90	34 / 31	72 / 93	68.5 / 86.3	108 / 104
Protein (% of energy)	13	4	14	8-10	163
Total fat (g)	82 / 92	41 / 39	72 / 96	74.2 / 93.5	111 / 98
Total fat (% of energy)	32	10	32	30	107
Carbohydrates (g)	259 / 356	79 / 84	297 / 354	314.9 / 397	82 / 90
Carbohydrates (% of energy)	54	12	55	≥ 50	108
Phe (mg)	2362	1755	1904		
Fiber (g)	17	9	16	≥ 30	57
Cholesterol (mg)	241	209	172	≤ 300	80
PUFA (g)	12.5 / 13.4	6.7 / 6.0	10.8 / 13.9	≤ 24.7 / 31.2	51 / 43
MUFA (g)	26.6 / 29.6	18.0 / 17.4	20.1 / 26.3	≥ 24.7 / 31.2	108 / 95
SFA (g)	30.5 / 36.3	19.1 / 19.7	25.8 / 33.1	≤ 24.7 / 31.2	123 / 116
Sodium	2708	1439	2512	2000	135
Potassium	3302	957	3136	2000	165
Calcium (mg)	1084	427	993	1000	108
Phosphorus (mg)	1240	552	1046	700	177
Magnesium (mg)	412 / 472	129 / 127	440 / 421	300 / 350	137 / 135
Iron (mg)	19 / 18	9.1 / 5.6	17.6 / 18.3	15 / 10	193 / 121
Retinol (mg)	1.4 / 1.5	0.8 / 1.1	1.3 / 1.1	0.8 / 1.0	175 / 150
Vitamin B1 (mg)	2.2 / 1.7	1.4 / 0.6	1.9 / 1.6	1.0 / 1.2	220 / 142
Vitamin B2 (mg)	2.2 / 2.2	1.8 / 1.5	1.7 / 1.7	1.2 / 1.4	183 / 157
Vitamin B6 (mg)	2.3 / 2.3	1.2 / 0.9	1.9 / 2.3	1.2 / 1.5	192 / 153
Folic acid (µg)	332	182	288	400	83
Ascorbic acid (mg)	162	73	158	100	162
Vitamin D (µg)	4.6	4.8	2.7	5	92
Vitamin E (mg)	18.6 / 11.9	15.5 / 2.5	15.4 / 11.5	12 / 14	155
Zinc (mg)	15.1 / 15.0	7.5 / 5.4	14.1 / 16.4	7 / 10	216
Iodine (µg)	166	121	146	200	83
Copper (µg)	2604	1083	2453	1250	208
Chloride (mg)	4268	2983	3901	3000	142
Fluoride (µg)	802 / 973	646 / 356	676 / 933	3100 / 3800	26 / 26

Manganese (µg)	4409	2187	3940	3500	126
Vitamin K (µg)	236 / 289	133 / 104	204 / 272	60 / 70	393 / 413
Pantothenic acid (mg)	6.88	4.0	5.5	6	115
Biotin (µg)	111	141	58	45	246
Vitamin B12 (µg)	5.2	3.4	4.7	3	170
Niacin (mg)	31 / 35	20.4 / 18.2	28.2 / 28.0	13 / 16	240 / 216
Linoleic acid (g)	10.4 / 10.5	5.6 / 4.4	8.7 / 10.3	6.2 / 7.8	168 / 135
α-Linolenic acid (g)	1.3 / 1.5	1.0 / 0.8	1.0 / 1.7	1.2 / 1.6	108 / 94
EPA (g)	0.002	0.01	0.0	> 0.23 / 0.29	1.1 / 1.1
DHA (g)	0.042	0.01	0.0	> 0.23 / 0.29	19.7 / 19.5

* reference values derive from D-A-CH recommendation (47); for EPA and DHA from ISSFAL (86)

Tab. A2: Relative fatty acid composition (wt%) of plasma phospholipids in PKU-patients

	PKU-patients in Munich (n=32)		
	Mean (wt%)	SD (wt%)	Median (wt%)
14:0	0,39	0,08	0,39
16:0	27,43	1,51	27,44
17:0	0,34	0,07	0,33
18:0	13,54	1,30	13,38
20:0	0,60	0,11	0,59
22:0	1,48	0,27	1,46
24:0	1,27	0,24	1,24
18-1t	0,18	0,07	0,16
22-1	0,16	0,08	0,14
16-1n-7	0,71	0,28	0,63
17-1n-7	0,23	0,09	0,21
18-1n-7	1,50	0,26	1,44
18-1n-9	10,18	1,37	10,15
20-1n-9	0,15	0,02	0,15
22-1n-9	0,25	0,07	0,23
24-1n-9	2,63	0,51	2,55
20-3n-9	0,18	0,06	0,17
18-2n-6	18,77	2,56	18,55
18-3n-6	0,13	0,07	0,12
20-2n-6	0,37	0,08	0,36
20-3n-6	3,49	0,62	3,61
20-4n-6	10,21	1,14	10,29
22-4n-6	0,48	0,11	0,45
22-5n-6	0,33	0,09	0,32
18-3n-3	0,19	0,11	0,16
20-3n-3	0,12	0,02	0,13
20-5n-3	0,70	0,32	0,65
22-5n-3	1,06	0,28	1,08
22-6n-3	2,79	0,85	2,74

Tab. A3: Reproducibility of fatty acid analysis in plasma PL (mean, CV)

	Intra-assay (n = 8)				Inter-assay (n = 5)			
	mg/l	CV (%)	wt %	CV (%)	mg/l	CV (%)	wt %	CV (%)
SFA								
14:0	4.82	3.07	0.45	3.05	4.10	4.70	0.40	3.94
15:0	IS		IS		IS		IS	
16:0	301.68	1.34	28.28	0.59	281.45	3.42	27.51	1.72
17:0	4.62	1.60	0.43	0.47	3.90	2.77	0.38	1.42
18:0	149.57	1.55	14.00	0.41	130.25	2.95	12.73	1.28
20:0	5.71	2.80	0.53	2.01	6.31	2.47	0.62	2.68
22:0	14.45	1.95	1.35	0.74	14.55	6.88	1.42	4.27
24:0	13.08	1.67	1.22	0.73	13.30	8.74	1.30	5.55
TFA								
14:1t	n.d.		n.d.		n.d.		n.d.	
16:1t	0.05	3.80	0.05	3.55	0.16	17.68	0.02	17.97
18:1t	2.35	3.86	0.22	2.38	2.56	10.36	0.25	9.58
18:2tt	0.58	3.69	0.05	3.56	0.52	21.07	0.05	20.72
22:1t	0.77	3.67	0.07	3.32	1.01	3.32	0.10	2.02
MUFA								
14:1n-5	n.d.		n.d.		n.d.		n.d.	
15:1n-5	n.d.		n.d.		n.d.		n.d.	
16:1n-7	9.48	4.06	0.89	2.53	6.19	4.78	0.61	6.14
17:1n-7	n.d.		n.d.		n.d.		n.d.	
18:1n-7	19.33	2.23	1.81	0.50	14.89	4.54	1.46	4.21
18:1n-9	138.03	3.26	12.91	1.67	103.78	2.10	10.15	1.73
20:1n-9	1.91	8.43	0.18	7.96	1.62	3.74	0.16	1.98
22:1n-9	3.31	18.26	0.31	17.20	3.11	21.2	0.31	23.51
24:1n-9	25.23	1.83	2.36	0.79	29.85	10.31	2.91	6.95
PUFA								
20:3n-9	3.16	1.85	0.30	0.51	2.45	4.38	2.51	2.48
18:2n-6	189.76	1.75	17.76	0.19	188.40	3.28	18.42	0.52
18:3n-6	1.49	3.13	0.14	2.90	1.54	3.85	0.15	14.76
20:2n-6	3.20	2.41	0.30	1.00	3.27	3.81	0.31	2.60
20:3n-6	33.12	1.80	3.10	0.41	25.74	5.20	2.51	2.48
20:4n-6	96.02	1.79	8.98	0.36	127.26	4.91	12.44	2.40
22:2n-6	0.23	8.05	0.02	7.86	0.19	17.43	0.02	17.47
22:4n-6	4.28	2.47	0.40	1.00	5.80	4.55	0.57	1.93
22:5n-6	3.51	2.31	0.34	1.00	4.16	5.31	0.41	3.59
18:3n-3	1.56	3.21	0.15	1.42	1.54	5.40	0.15	2.62
18:4n-3	0.39	17.97	0.04	18.05	0.25	17.67	0.02	18.47
20:3n-3	1.01	8.63	0.09	8.59	1.26	5.30	0.12	4.10
20:5n-3	5.06	2.07	0.47	0.71	7.10	5.19	0.69	3.46
22:5n-3	8.6	2.05	0.80	0.67	9.51	6.85	0.93	4.36
22:6n-3	19.68	2.11	1.84	1.28	25.60	7.11	2.50	5.20

IS = internal standard, n.d. = not detected

Tab. A4: Inter-assay of retinol and α-tocopherol in control plasma

Inter-assay (n=9)	Concentrations (mg/l)	
	Retinol	α-tocopherol
Plasma 1	0,382	8,873
Plasma 2	0,383	8,902
Plasma 3	0,410	8,490
Plasma 4	0,395	9,160
Plasma 5	0,402	8,971
Plasma 6	0,392	8,803
Plasma 7	0,415	8,868
Plasma 8	0,411	9,068
Plasma 9	0,403	9,439
Mean	0,399	8,953
SD	0,012	0,261
CV	2,972	2,916

Tab. A5: Intra-assay of retinol and α-tocopherol in control plasma

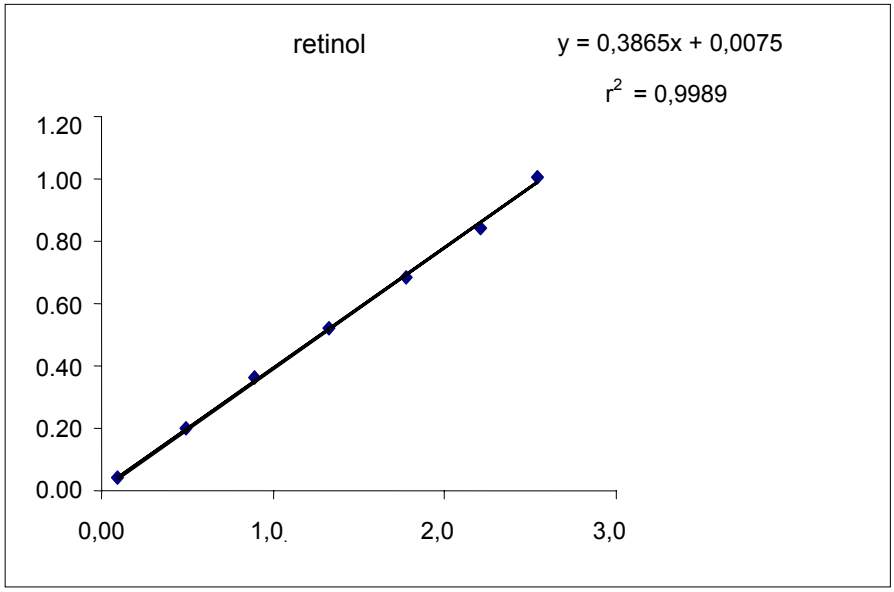
Intra-assay (n=6)	Concentrations (mg/l)	
	Retinol	α-tocopherol
Plasma 1	0,652	9,431
Plasma 2	0,652	9,413
Plasma 3	0,652	9,457
Plasma 4	0,642	9,500
Plasma 5	0,660	9,308
Plasma 6	0,645	9,433
Mean	0,650	9,424
SD	0,006	0,064
CV	0,938	0,680

Tab. A6: Daily nutrient intake of all PKU-patients (Munich and Münster)

	PKU-patients (n=73, 24 ♂, 59 ♀)				
	Mean ♀ / ♂	SD ♀ / ♂	Median ♀ / ♂	Reference value* ♀ / ♂	Mean in % of reference value
Energy (kcal)	2015 / 2436	628 / 671	2009 / 2076	2300 / 2900	89 / 83
Protein (g)	66 / 79	31 / 27	59 / 74	68.5 / 86.3	99 / 98
Total fat (g)	74 / 91	35 / 39	69 / 78	74.2 / 93.5	100 / 97
Carbohydrates (g)	259 / 299	86 / 96	255 / 275	314.9 / 397	84 / 71
Fiber (g)	17 / 20	8 / 9	16 / 19	≥ 30	57 / 63
Cholesterol (mg)	213 / 315	170 / 231	176 / 283	≤ 300	71 / 95
PUFA (g)	11.7 / 13.3	6.0 / 6.8	10.1 / 12.4	≤ 24.7 / 31.2	47 / 44
MUFA (g)	24.8 / 30.4	14.8 / 16.0	20.8 / 25.2	≥ 24.7 / 31.2	101 / 96
SFA (g)	26.9 / 34.1	15.8 / 16.6	24.2 / 32.5	≤ 24.7 / 31.2	108 / 111
Sodium	2382 / 2922	1331 / 1162	2121 / 2816	2000	119 / 144
Potassium	3016 / 3519	927 / 783	2934 / 3424	2000	151 / 173
Calcium (mg)	981 / 1006	475 / 363	879 / 986	1000	102 / 107
Phosphorus (mg)	1075 / 1374	411 / 450	970 / 1334	700	154 / 193
Magnesium (mg)	391 / 458	143 / 142	393 / 437	300 / 350	134 / 135
Iron (mg)	16.1 / 17.0	7.6 / 7.4	14.5 / 14.8	15 / 10	150 / 132
Retinol (mg)	1.8 / 1.5	1.7 / 1.1	1.3 / 1.0	0.8 / 1.0	215 / 149
Vitamin B1 (mg)	1.8 / 1.6	1.1 / 0.5	1.4 / 1.6	1.0 / 1.2	175 / 132
Vitamin B2 (mg)	1.9 / 1.9	1.4 / 1.0	1.5 / 1.6	1.2 / 1.4	158 / 132
Vitamin B6 (mg)	2.1 / 2.5	1.0 / 0.9	1.9 / 2.3	1.2 / 1.5	172 / 163
Folic acid (µg)	287 / 308	161 / 144	239 / 275	400	72 / 77
Ascorbic acid (mg)	144 / 141	75 / 74	140 / 133	100	144 / 140
Vitamin D (µg)	4.9 / 4.5	5.2 / 4.1	2.7 / 2.4	5	98 / 88
Vitamin E (mg)	15.7 / 12.7	11.7 / 3.7	13.0 / 12.0	12 / 14	131 / 90
Zinc (mg)	12.2 / 13.4	6.1 / 5.7	10.6 / 14.8	7 / 10	185 / 139
Iodine (µg)	147 / 127	110 / 77	118 / 96	200	74 / 64
Copper (µg)	2234 / 2558	895 / 896	2188 / 2166	1250	188 / 214
Chloride (mg)	3591 / 4310	2530 / 1592	3022 / 4420	3000	120 / 142
Fluoride (µg)	798 / 928	553 / 413	574 / 833	3100 / 3800	26 / 24
Manganese (µg)	4175 / 4979	2218 / 2816	3537 / 4372	3500	119 / 136
Vitamin K (µg)	312 / 312	287 / 97	251 / 312	60 / 70	520 / 439
Pantothenic acid (mg)	6.0 / 6.5	3.4 / 3.1	4.9 / 5.9	6	100 / 107
Biotin (µg)	96 / 68	120 / 55	44 / 46	45	214 / 150
Vitamin B12 (µg)	4.4 / 5.8	3.3 / 3.6	3.4 / 4.9	3	162 / 185
Niacin (mg)	29.5 / 34.5	15.3 / 13.1	26.0 / 32.8	13 / 16	227 / 215
Linoleic acid (g)	9.9 / 10.5	5.2 / 5.1	8.6 / 10.1	6.2 / 7.8	158 / 142
α-Linolenic acid (g)	1.2 / 1.5	0.8 / 0.7	0.9 / 1.3	1.2 / 1.6	96 / 94
EPA (g)	0.03 / 0.005	0.15 / 0.01	0.00 / 0.00	> 0.23 / 0.29	10 / 8
DHA (g)	0.06 / 0.08	0.16 / 0.10	0.00 / 0.03	> 0.23 / 0.29	27 / 35

* reference values derive from D-A-CH recommendation (47); for EPA and DHA from ISSFAL (86)

7.2 Figures



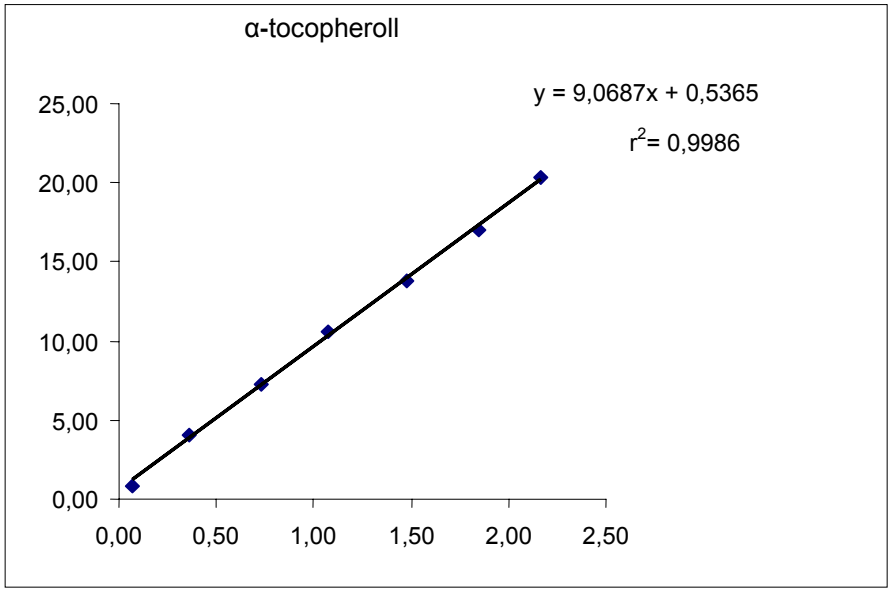


Fig. A1: Seven-point standard curves for retinol and α-tocopherol

7.3 Used information and documentation materials

On the following pages are shown these used information and documentation materials:

- Invitation to participate in the study
- Subject information
- Leaflet with study information
- Informed consent of the subjects
- 3-day dietary protocol
- Protocol for clinical investigation
- Standardized interview
- Atopic disease questionnaire
- Auditory verbal learning test
- d2 attention test
- Number combination test
- Color-word interference test
- MPT finding
- VEP finding
- Accelerator data
- Activity protocol

Lebensqualität und Gesundheit bei Erwachsenen mit Phenylketonurie (PKU)

Sehr geehrte/r PKU-Patient/in,

Wir wenden uns heute an Sie, weil wir Sie über die Entwicklungen bei der Behandlung Ihrer Stoffwechselstörung Phenylketonurie (PKU) unterrichten und Sie zu einer Untersuchung in Ihr Stoffwechselzentrum nach München einladen möchten.

Glaubte man früher, die eiweißarme Diätbehandlung mit ca. 10 Jahren gänzlich beenden zu können, so haben sich in den vergangenen Jahren zunehmend Hinweise ergeben, die zur Vorsicht mahnen und für eine dauerhafte Weiterführung einer gelockerten Diätbehandlung sprechen. Sind die Diätempfehlungen für die ersten 10 Lebensjahre durch wissenschaftliche Untersuchungen sehr gut belegt, so fehlen jedoch systematische Grundlagen für das Erwachsenenalter. In den letzten Jahren wurde jedoch von einzelnen erwachsenen PKU-Patienten berichtet, die seit Jahren die Diät gelockert bzw. aufgegeben hatten und bei denen neurologische Störungen auftraten. Hier handelt es sich um Einzelfälle insbesondere bei Patienten mit Behandlungsbeginn erst nach dem frühen Säuglingsalter. Wir wissen jedoch nicht, ob im Verlauf langer Zeit abhängig von der Diätführung ein nennenswertes Risiko für das Auftreten von Störungen besteht, denn die ältesten frühbehandelten Patienten sind heute erst ca. 45 Jahre alt.

Zurzeit besteht kein Grund zur Beunruhigung. Wir sehen jedoch Anlass, Sie über den Stand der Dinge ausführlich zu informieren und Sie zu einer vorbeugenden und begleitenden Untersuchung in die Dr. von Haunersche Kinderklinik einzuladen (Kinderklinik deshalb, weil es bisher kein eigenes Behandlungszentrum für erwachsene Patienten mit angeborenen Stoffwechselstörungen wie der PKU gibt). Wir werden Sie im Laufe der kommenden Wochen

anrufen, um Ihre Fragen und das Vorgehen mit Ihnen zu besprechen. Gern sprechen wir auch mit Ihrem Hausarzt/Hausärztin, um diese über das Vorgehen und den Sinn der Untersuchungen zu informieren.

Sollten Sie nach unserem Anruf zu einer solchen Untersuchung bereit sein, werden wir Ihnen nochmals schriftlich Dauer und Art der Untersuchungen, Termin und Anfahrtsweg, sowie weitere Einzelheiten mitteilen. Es wird sich zunächst um eine einmalige Untersuchung handeln, die wir ggf. in Abständen von ca. 3 Jahren wiederholen können. Alles Weitere werden wir zunächst telefonisch und dann hoffentlich im persönlichen Gespräch in unserer Klinik besprechen.

Bitte erschrecken Sie nicht über den Inhalt dieses Briefes. Wir sehen es jedoch als unsere Pflicht an, Sie über mögliche mit der PKU verbundene Risiken zu informieren und gemeinsam mit Ihnen das weitere Vorgehen zu überlegen. Für alle weiteren Fragen stehen wir Ihnen jederzeit zu Verfügung.

Mit freundlichen Grüßen

Dipl. oec. troph. J. von Berlepsch
Ernährungswissenschaftlerin

Susanne Bauske
Ärztin

Prof. Dr. B. Koletzko
Projektleiter und Leiter der Abt.
Stoffwechselkrankheiten & Ernährung
Dr. v. Haunersches Kinderspital

Ein Vergleich von Lebensqualität und Gesundheit erwachsener Patienten mit Phenylketonurie (PKU)

Abt. Stoffwechselerkrankungen & Ernährungsmedizin (Leiter: Prof. Dr. med. Berthold Koletzko)
Kinderklinik und Kinderpoliklinik im Dr. von Haunerschen Kinderspital, Klinikum der Universität München, Lindwurmstraße 4, 80337 München, Tel: 089-5160-3486

Sehr geehrte/r PKU-Patient/in,

Als PKU-Patient/in hängen für Sie Lebensqualität und Gesundheit entscheidend von Ihrer Ernährungsweise und Betreuung ab. Langzeituntersuchungen zeigen, dass PKU-Patienten im Jugend- und Erwachsenenalter oftmals keine optimale, regelmäßige Betreuung haben und altersentsprechende Diätpläne und Schulungen fehlen. Um angemessene Betreuungs- und Behandlungskonzepte entwerfen zu können, bedarf es der Erfassung bestehender Risiken und Probleme an einer ausreichend großen Gruppe von erwachsenen PKU-Patienten.

Wir wollen daher in dieser Studie mittels einer Nachuntersuchung die Lebensqualität und Gesundheit bei erwachsenen PKU-Patienten prüfen und speziell untersuchen, welche psychosozialen Einschränkungen (z.B. im Berufsleben) sich für Sie aus Ihrer Erkrankung und der Diät ergeben, wie Sie ernährungswissenschaftlich (professionelle Ernährungsberatung, Diät), psychologisch (Selbsthilfegruppen, Beratungsstellen, psychosomatisch-psychiatrische Betreuung) bzw. neurologisch betreut werden, um Möglichkeiten einer Verbesserung der medizinischen, nutritiven und sozialen Betreuung zu erkennen.

Zur Datenerfassung sollen uns dabei strukturierte Fragebögen, neuropsychologische Testverfahren, verschiedene Untersuchungsmethoden und die Analyse von Blutproben dienen.

Um Ihre Ernährungsweise erfassen zu können, bitten wir Sie in einem Ernährungsprotokoll für drei Tage aufzuschreiben, welche Speisen und Getränke sie an diesen Tagen zu sich genommen haben sowie Angaben zu Ihren Ernährungsgewohnheiten zu machen.

Für die Durchführung der vorgesehenen Untersuchungen, bitten wir Sie nach Ihrer Einverständniserklärung zu einem vereinbarten Termin in unsere Klinik zu kommen und das 3-Tage-Ernährungsprotokoll ausgefüllt sowie den aktuellsten MRT-Befund (falls vorhanden) mitzubringen.

Zu diesem Termin werden Ihnen 18 ml Blut abgenommen und anschließend werden verschiedene Tests durchgeführt sowie Fragen zu Ihrer Lebenssituation gestellt.

Anhand der Blutproben wird festgestellt, wie sich Ihre Ernährungsweise auf den Gehalt an Fettsäuren, Vitaminen, Mineralstoffen und Spurenelementen sowie auf Ihre Blutfette auswirkt. Immunologische Tests geben uns Aufschluss über Veränderungen des Immunsystems. Veränderungen der Feinmotorik, des Verhaltens sowie der Intelligenz und Aufmerksamkeit sollen anhand spezieller Tests festgestellt werden und neurophysiologische Untersuchungen zeigen die Reizleitungsgeschwindigkeit in Ihrem Nervensystem. Dabei werden auf Sie optische Reize ausgeübt und über Elektroden, die auf Ihrer Kopfhaut befestigt werden, das Ankommen der Signale im Gehirn erfasst.

Alle persönlichen Daten werden streng vertraulich behandelt und nicht weitergegeben, sondern nur für die Studie gespeichert. Nach Abschluss der Studie erfolgt die Auswertung der Daten in pseudonymisierter Form.

Falls Sie noch weitere Fragen haben stehen wir Ihnen gerne zur Verfügung.

Mit freundlichen Grüßen

Dipl. oec. troph. J. von Berlepsch
Ernährungswissenschaftlerin

Susanne Bauske
Ärztin

Prof. Dr. B. Koletzko
Projektleiter und Leiter der Abt.
Stoffwechselkrankheiten & Ernährung
Dr. v. Haunersches Kinderspital

Merkblatt

Ein Vergleich von Lebensqualität und Gesundheit erwachsener Patienten mit Phenylketonurie (PKU)

Studienablauf

Vor Aufnahme eines Probanden in die Studie findet telefonisch die Überprüfung der Ein- und Ausschlusskriterien statt. Den geeigneten Personen werden dann per Post eine Einverständniserklärung, ein Aufklärungsbogen, dieses Merkblatt, ein Ernährungsprotokoll sowie 3 Fragebögen zugesandt. Mit Hilfe der **Fragebögen** werden Angaben zur Häufigkeit von Allergien sowie zu Ihrem psychischen Befinden erfasst. Vor dem Untersuchungstermin in unserer Klinik füllen Sie bitte die beigelegten **Fragebögen** sowie an drei aufeinanderfolgenden Tagen (einer davon ein Samstag oder Sonntag) das beiliegende **Ernährungsprotokoll** aus. Dieses Protokoll soll eine allgemeine Übersicht über Ihre tägliche Aufnahme an Nährstoffen geben. Die Ergebnisse sind notwendig, um die gemessenen Parameter genau interpretieren zu können.

Der Untersuchungstermin wird telefonisch oder schriftlich mit Ihnen abgesprochen. Zu diesem Termin bringen Sie bitte die unterschriebene **Einverständniserklärung**, das **Ernährungsprotokoll**, den **Allergie-Fragebogen**, den **YASR** sowie die **SCL-90** ausgefüllt in die Klinik mit. Der Zeitaufwand beträgt circa 4 Stunden. Es werden dabei folgende Untersuchungen durchgeführt:

- Klinische Untersuchung und Blutentnahme (16 ml)
- Fragen zur Ihrer Lebenssituation
- verschiedene Konzentrations- und Aufmerksamkeitstests
- eine schmerzfreie, neurologische und feinmotorische Untersuchung

Treffpunkt für die Untersuchung
Pforte am Haupteingang des Dr. von Haunerschen Kinderspitals

Wegbeschreibung

Das Dr. von Haunersche Kinderspital befindet sich in der Lindwurmstraße 4 direkt in der Münchner Innenstadt am Goetheplatz (U-Bahn U3/U6; MetroBus 58).

Per Bus/Tram/U-Bahn:
Haltestelle *Goetheplatz*: Bus Linie 58, U-Bahn U3, U6.
Haltestelle *Sendlinger Tor* (Gehstrecke ca. 500 m): Bus Linien 56, 31, Tram Linien 16, 17, 18, 27, U-Bahn U1, U2, U3, U6.
Vom Goetheplatz aus in Richtung Innenstadt circa 50 m die Lindwurmstraße entlanggehen, links am Haupteingang des Kinderspitals an der Pforte melden und nach Frau von BERLEPSCH fragen (Klinikdurchwahl 3486). Sie werden von dort abgeholt.

Mit der Deutschen Bahn:
München Hauptbahnhof, Ausgang Bayerstraße (Südseite), über die gegenüberliegende Goethestraße, ca. 800 m weiter in Richtung Süden bis zur Kreuzung am Goetheplatz, dann links nach ca. 50 m am Haupteingang des Kinderspitals an der Pforte melden und nach Frau von BERLEPSCH fragen (Klinikdurchwahl 3486). Sie werden von dort abgeholt.

Lageplan

Und noch ein paar Hinweise:

- Falls Sie eine Brille oder Kontaktlinsen tragen, bringen Sie diese bitte unbedingt zu den Untersuchungen mit!
- Bitte benutzen am Morgen der Untersuchungen kein Haargel oder ähnliches, da es sonst Probleme mit der Leitung der Elektroden bei den neurologischen Untersuchungen geben kann!

Ein Vergleich von Lebensqualität und Gesundheit erwachsener Patienten mit Phenylketonurie (PKU)

Abt. Stoffwechselerkrankungen & Ernährungsmedizin (Leiter: Prof. Dr. med. B. Koletzko)
Kinderklinik und Kinderpoliklinik im Dr. von Haunerschen Kinderspital, Klinikum der Universität München, Lindwurmstraße 4, 80337 München, Tel: 089-5160-3486

Name des Aufklärenden: _____

Einverständniserklärung

Ich bin mit der Teilnahme an der oben genannten Untersuchung entsprechend dem Inhalt des Aufklärungsbogens einverstanden. Alle aufgetretenen Fragen konnten besprochen werden.

Name: _____ Vorname: _____

Anschrift: _____

_____

_____

_____ _____

Ort, Datum Unterschrift

3-Tage-Ernährungsprotokoll

Ein Vergleich der Lebensqualität und Gesundheit erwachsener Patienten mit Phenylketonurie (PKU)

Name: _____

Datum: _____

Wie wird das 3-Tage-Schätzprotokoll ausgefüllt?

Allgemeiner Hinweis

- Bitte notieren Sie an drei aufeinanderfolgenden Tagen (Donnerstag bis Samstag ODER Sonntag bis Dienstag) vor der Untersuchung **was** und **wie viel** Sie gerade essen und trinken. Notieren Sie das Datum der Tage.

Bitte essen und trinken Sie an diesen Tagen genauso wie immer !!!

Wie soll dies notiert werden?

- **Tragen Sie bitte grundsätzlich alles ein, was Sie verzehren**, d.h. alle Speisen und Getränke (auch Wasser, Milch und Zucker für den Kaffee oder Tee), Süßigkeiten und andere Kleinigkeiten zwischendurch und auch Präparate (z.B. Vitamin-, Mineralstoffpräparate, Stärkungsmittel) sowie Medikamente.

- Suchen Sie ein verzehrtes Lebensmittel in der entsprechenden **Lebensmittelgruppe** (z.B. Marmelade in der Gruppe *Brotbelag*).

- Falls verlangt, benennen Sie bitte Sorte bzw. Fettgehalt genauer. z.B. Mineralwassersorte

- Schätzen Sie die **Menge** bitte gut ab und machen Sie in der Spalte **Anzahl** an dem entsprechenden Tag (1, 2 oder 3) entweder für jede Portion einen Strich oder notieren Sie eine Ziffer (z.B. für 3 Scheiben Graubrot entweder 3 Striche oder eine „3“ eintragen).

- Für die einzelnen Lebensmittel sind jeweils die gewöhnlichen **Maßeinheiten** angegeben. So wird Brot in *Scheiben* angegeben, Kuchen in *Stück*, Kaffee in *Kaffeetassen* und Getränke in *Gläsern* oder *Tassen*. Ändern Sie die Mengenbezeichnungen nicht!
  Hat man eine **kleinere Portion** als angegeben gegessen bzw. getrunken (z.B. ½ Portion Nudeln oder ¼ Apfel) trägt man einfach ½ oder ¼ ein.

- Gerichte/Lebensmittel, die Sie **nicht in der Liste** finden können, tragen Sie bitte unter *Sonstiges* in der jeweiligen Lebensmittelgruppe oder unter *Lebensmittel*, *die nicht aufgeführt sind* mit möglichst genauer Portionsangabe ein.

Tipps:

- Notieren Sie am besten **während oder direkt nach der Mahlzeit.**

- Am einfachsten ist es, wenn man den **Fragebogen immer dabei** hat, auch im Restaurant, bei Verwandten oder Freunden. So kann nichts vergessen werden.

- **Am Abend sollte man noch einmal über den vergangenen Tag nachdenken** - am besten mit der Liste in der Hand - ob etwas vergessen wurde und sollte dies noch nachtragen.

Verzehr über drei Tage

Brot • Brötchen		**Anzahl**		
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Graubrot	Scheibe 45 g			
Weißbrot	Scheibe 35 g			
Toastbrot	Scheibe 20 g			
Vollkornbrot	Scheibe 50 g			
Knäckebrot	Scheibe 10 g			
Zwieback	Scheibe 10 g			
Hefezopf	Scheibe 45 g			
Normales Brötchen	Stück 45 g			
Vollkornbrötchen	Stück 55 g			
Croissant	Stück 50 g			
Sonstiges:				

Brotbelag • Butter • Margarine		**Anzahl**		
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Butter	TL 5 g			
Margarine	Sorte: ……. TL 5 g			
Margarine halbfett	Sorte: ..... TL 5 g			
Marmelade, Konfitüre, Gelee	EL 20 g			
Honig	EL 20 g			
Nuss-Nougat-Creme	EL 20 g			
Vegetarischer Brotaufstrich	Sorte: .... EL 30 g			
Frischkäse	Fettgehalt (% i. Tr.) …… EL 30 g			
Quark	Fettgehalt (% i. Tr.) …… EL 20 g			
Schmelzkäse	Fettgehalt (% i. Tr.) …… Portion 30 g			
Schnittkäse	Fettgehalt (% i. Tr.) …… Scheibe 30 g			
Weichkäse	Fettgehalt (% i. Tr.) …… Scheibe 30 g			
Bierschinken	Scheibe 25 g			
Corned Beef	Portion 25 g			
Fleischwurst	Scheibe 20 g			
Fleischkäse (Aufschnitt)	Scheibe 30 g			
Fleischsalat	Portion 50 g			
Leberwurst	Portion 30 g			
Mettwurst	Portion 30 g			
Teewurst	Portion 30 g			
Salami/Cervelatwurst	Scheibe 20 g			
Schinken roh	Scheibe 15 g			
Schinken gekocht	Scheibe 30 g			
Speck	Portion 30 g			

Sonstiges:			

Frühstücksallerlei				**Anzahl**		
		Einheit		**Tag 1**	**Tag 2**	**Tag 3**
Gekochtes Ei		Stück	55 g			
Cornflakes trocken	Sorte:	EL	4 g			
Cornflakes trocken	**gezuckert**/geröstet	EL	6 g			
Sonstige Frühstückscerealien	Sorte:	EL	6 g			
Haferflocken trocken		EL	10 g			
Müsli trocken		EL	15 g			
Sonstiges:						
Milch • Milchprodukte • Sojaprodukte				**Anzahl**		
		Einheit		**Tag 1**	**Tag 2**	**Tag 3**
Buttermilch		Glas	200 g			
Joghurt natur	Fettarm (**1,5**% Fett)	Becher	150 g			
Joghurt natur	Vollfett (**3,5** % Fett)	Becher	150 g			
Joghurt mit Frucht	Fettarm (**1,5** % Fett)	Becher	150 g			
Joghurt mit Frucht	Vollfett (**3,5** % Fett)	Becher	150 g			
Milch	Fettarm (**1,5** % Fett)	Glas	200 g			
Milch	Vollfett (**3,5** % Fett)	Glas	200 g			
Kakao/Trinkschokolade	Fettgehalt (%)	Glas	200 g			
Sojamilch	Sorte:	Glas	200 g			
Tofu	Sorte:	Portion	100 g			
Sahne	Fettgehalt (%)	EL	10 g			
Kondensmilch	Fettgehalt (%)	Portion	12 g			
Sonstiges:	Fettgehalt (%)					
	Fettgehalt (%)					
	Fettgehalt (%)					
Obst				**Anzahl**		
		Einheit		**Tag 1**	**Tag 2**	**Tag 3**
Brombeere, Erdbeere, Himbeere, Heidelbeere, ..	Beerenobst Sorte:.....	Portion	125 g			
Weintrauben		Portion	150 g			

Apfel, Birne, Quitte,...	Kernobst Sorte:.....	Portion 150 g			
Aprikosen, Kirschen, Mirabellen, Pflaumen, Pfirsich,...	Steinobst Sorte:	Portion 150 g			
Banane		Stück 120 g			
Ananas, Kiwi, Mango, Maracuja,...	Südfrüchte Sorte:.....	Portion 150 g			
Grapefruit, Mandarine, Orange, Zitrone, ...	Zitrusfrüchte Sorte:.....	Portion 150 g			
Rosinen, Trockenobst	Sorte:	Portion 50 g			
Kompott, Obstkonserven	Sorte:	Portion 150 g			
Sonstiges*:*					
Gemüse • Salate				**Anzahl**	
		Einheit	***Tag 1***	**Tag 2**	**Tag 3**
Blattsalat (Dressing bitte unter „Soßen, Fette und Öle“)	Sorte:	Portion 50 g			
Rohkost / Salatgemüse (Dressing bitte unter „Soßen, Fette und Öle“)	Sorte:	Portion 50 g			
Mangold, Spinat	Blattgemüse Sorte:	Portion 200 g			
Grüne Bohnen		Portion 200 g			
Aubergine, Gurke, Paprika, Tomate, Zucchini, ...	Fruchtgemüse Sorte:	Portion 200 g			
Gemüsemais		Portion 200 g			
Blumenkohl, Broccoli, Kohl (Rot-, Grün-, Weiß-), Kohlrabi, Rosenkohl, Wirsing,...	Kohlgemüse Sorte:	Portion 200 g			
Sauerkraut		Portion 150 g			
Fenchel, Lauch, Spargel,...	Sprossengemüse Sorte:	Portion 200 g			
Möhre, Radieschen, Rettich, Rote Bete, Rüben, Sellerie, Schwarzwurzel,...	Wurzel- und Knollengemüse Sorte:	Portion 200 g			
Pilze		Portion 120 g			
Zwiebel		Stücke 30 g			
Küchenkräuter	Sorte:	EL 1g			
Gewürzgurken		Port. 50 g			

Sonstiges:					
Hülsenfrüchte				**Anzahl**	
		Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Weiße / Rote Bohnen gegart		Portion 200 g			
Erbsen gegart		Portion 200 g			
Linsen gegart		Portion 200 g			
Sonstiges					
Beilagen (Kartoffeln, Nudeln, Reis,...)				**Anzahl**	
		Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Salzkartoffeln		Portion 200 g			
Pellkartoffeln		Portion 200 g			
Bratkartoffeln		Portion 200 g			
Kartoffelbrei		Portion 200 g			
Kartoffelknödel		Stück 100 g			
Kartoffelsalat		Portion 250 g			
Pommes Frites		Portion 200 g			
weißer Reis	gekocht	Portion 180 g			
Natur-Reis	gekocht	Portion 180 g			
Nudeln eifrei	gekocht	Portion 180 g			
Eiernudeln	gekocht	Portion 180 g			
Vollkornnudeln eifrei	gekocht	Portion 180 g			
Vollkornnudeln mit Ei	gekocht	Portion 180 g			
Spätzle	gekocht	Portion 200 g			
Beilagen (Kartoffeln, Nudeln, Reis,...)				**Anzahl**	
		Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Schupfnudeln		Portion 200 g			
Sonstiges:					
Soßen • Fette • Öle				**Anzahl**	
		Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Joghurt-Salatsoße	Sorte:	Portion 40 g			
Essig-Öl-Marinade		Portion 20 g			
Bechamelsoße		Portion 75 g			
Grundsoße		Portion 75 g			
Hackfleischsoße		Portion 100 g			
Jägersoße		Portion 75 g			
Käsesoße		Portion 75 g			
Tomatensoße		Portion 75 g			
Grillsauce		Portion 20 g			

Tomatenketchup		Portion 20 g			
Tomatenmark		TL 6 g			
Senf		TL 6 g			
Mayonnaise	Sorte:	EL 12 g			
Kokosfett, Butterschmalz	Sorte:	EL 10 g			
Pflanzenöl	Sorte:	EL 10 g			
Sonstiges:					

Fleisch • Fisch					
		Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Hackfleisch		Portion 125 g			
Kalbfleisch		Portion 125 g			
Rindfleisch		Portion 125 g			
Schweinefleisch		Portion 125 g			
Innereien		Portion 125 g			
Kotelett		Portion 125 g			
Schnitzel paniert		Portion 125 g			
Würstchen		Portion 125 g			
Brathähnchen (1/2)		Portion 370 g			
Geflügel		Portion 125 g			
Fisch	Sorte:	Portion 150 g			
Fischfilet paniert	Sorte:	Portion 170 g			
Fischkonserve abgetropft	Sorte:	Portion 65 g			
Sonstiges:					

Suppen • Eintöpfe	***Anzahl***			
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
als Vorsuppe				
Suppe klar	Portion 200 g			
Suppe gebunden	Portion 200 g			
Cremesuppe	Portion 200 g			
Gulaschsuppe	Portion 200 g			
Nudelsuppe mit Huhn	Portion 200 g			
als Hauptgericht				
Gemüsesuppe	Portion 400 g			
Kartoffelsuppe	Portion 400 g			
Linseneintopf	Portion 400 g			
Sonstiges:				

Fertig- und Schnellgerichte		**Anzahl**		
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Bratwurst (ohne Brötchen)	Stück 150 g			
Currywurst (mit Brötchen)	Portion 160 g			
Hamburger	Stück 100 g			
Cheeseburger	Stück 120 g			
Big Mac	Stück 200 g			
Maultaschen/Ravioli	Portion 200 g			
Pizza	Portion 370 g			
Lasagne	Portion 250 g			
Pfannkuchen	Portion 180 g			
Sonstiges:				
Dessert • Kuchen • Gebäck • Süßes		**Anzahl**		
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Pudding	Portion 150 g			
Eiscreme	Kugel 50 g			
Obstkuchen	Stück 120 g			
Cremetorte	Stück 150 g			
Rührkuchen	Stück 60 g			
Plätzchen, Kekse	Stück 10 g			
Schokolade	Riegel 20 g			
Praline	Stück 10 g			
Bonbon, Hartkaramelle	Stück 3 g			
Fruchtgummi	Portion 50 g			
Zucker	TL 5 g			
Gummibonbons Fantasie Zauberstäbchen (SHS)				
Gummibonbons Fanatsie Bärchen (SHS)				
Gummibonbons Fantasie Magic-Cola (SHS)				
Zitronentaler (SHS)				
Schoko-Dupletten (SHS)				
Vanille-Waffeln (SHS)				
Waliser Plätzchen (SHS)				
Lp-bar (Milupa)				
Lp-ringlets (Milupa)				
Knabbereien • Nüsse		**Anzahl**		
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Nüsse Sorte:	EL 20 g			
Erdnüsse gesalzen	Tasse 100 g			
Erdnussflips	Tasse 50 g			
Chips	Tasse 30 g			
Salzstangen	Portion 30 g			
Lp-chips (Milupa)				
Sonstiges:				

Wasser • Säfte • alkoholfreie Getränke			**Anzahl**		
		Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Mineralwasser	Sorte:	Glas 200 g			
Leitungswasser		Glas 200 g			
Fruchtsaft, 100 % Frucht	Sorte:	Glas 200 g			
Fruchtnektar	Sorte:	Glas 200 g			
Gemüsesaft	Sorte:	Glas 200 g			
Multivitaminsaft	Sorte:	Glas 200 g			
Limonade	Sorte:	Glas 200 g			
Colagetränke	Sorte:	Glas 200 g			
Diätgetränke (mit Süßstoff)	Sorte:	Glas 200 g			
Sonstiges:					

Kaffee • Tee		**Anzahl**		
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Kaffee (koffeinhaltig)	Tasse 150 g			
Kaffee (entkoffeiniert)	Tasse 150 g			
Malzkaffee / Zichorienkaffee	Tasse 150 g			
Schwarzer Tee	Tasse 150 g			
Kräutertee, Früchtetee	Tasse 150 g			
Sonstiges:				

Alkoholische Getränke		**Anzahl**		
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Bier	Glas 330 g			
Bier alkoholfrei	Glas 330 g			
Weizenbier	Glas 500 g			
Weißwein	Glas 200 g			
Rotwein	Glas 200 g			
Sekt	Glas 100 g			
Likör	Glas 40 g			
Schnaps, Branntwein	Glas 20 g			
Sonstiges:				

Aminosäuremischungen und Phe-arme Lebensmittel		**Anzahl**		
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
PAM 3	SHS			
PAM maternal	SHS			
p-am Anamix	SHS			
p-am Easiphen	SHS			
PKU 3 - advanta	Milupa			
PKU 3 - tablets	Milupa			
PKU 3 - shake	Milupa			
PKU 3 (für Schwangere)	Milupa			
Lp-fruity	Milupa			
Lp-drink	Milupa			
Lp-flakes	Milupa			
Lp-pasta	Milupa			
Weißbrot, ohne Salz	Hammermühle			
Kastanienbrot ohne Salz	Hammermühle			
Waffelbrot eiweißarm	Hammermühle			
Aproten-Knusperbrot	Hammermühle			
Aproten-Zwieback	Hammermühle			
Aprotenkekse	Hammermühle			
Aproten Spaghetti	Hammermühle			
Spiralnudeln (Phe-arm)	Hammermühle			
Harifen PKU-Milch	Hammermühle			
Loprofin PKU-Milch	Hammermühle			
Mehlmischung (Phe-arm)	Hammermühle			
Spezialgrieß	Hammermühle			
Backmix eiweißarm	MetaX			

Sonstige Lebensmittel, die nicht aufgeführt sind	**Anzahl**		
Einheit	**Tag 1**	**Tag 2**	**Tag 3**

KLINISCHER UNTERSUCHUNGSBOGEN PKU-Erwachsenen-Untersuchung	Seite 116 von 168	Probandennr.	Datum

O PKU-Patient

O Kontrollperson

Auffälligkeiten des internen Befundes:

RR:
HF:
Größe:
Gewicht:

Neurologische Untersuchung:

1. MER rechts links
BSR
PSR
Grobe Kraft

2. LR Pupillen O isokor O anisokor
Direkt
Indirekt

3. Tremor Ruhetremor
Haltetremor
Intentionstremor

4. Ataxie Standataxie: Romberg
Unterberger
Gangataxie: Linienlauf
Blindlauf

5. Muskeltonus O normal O erhöht O sehr erhöht

6. Sensibilität Zahlen auf Handrücken
Fingerstellung

7. Koordination Finger-Nase Versuch
Finger-Folge-Versuch
Diadochinese

8. Sprachauffälligkeiten: Wortfindung
Artikulation
Syntax

STANDARDISIERTES INTERVIEW	Seite 117 von 168	Probandennr.	Datum
PKU-Erwachsenen-Studie			

„Entwicklung erwachsener Patienten mit PKU“

Standardisiertes Interview

FRAGEN TEIL 1

FRAGEN TEIL 2

A bis F wie oben für Kontrollperson Fragen 1 bis 59

A Angaben zum Patienten

1. Geburtsdatum: .... 19 .....

2. Geschlecht: ❑ weiblich
❑ männlich

3. Telefon:

4. Adresse:

5. Bekannte krankheitsbezogene Daten:

6. Familienstand: ❑ ledig
❑ verheiratet
❑ mit festem Partner zusammenlebend
❑ geschieden
❑ getrennt lebend
❑ verwitwet
❑ anders, nämlich .....

7. Kinder: ❑ Nein
❑ Ja. Wenn ja, wie viele eigene Kinder: .....
und viele angenommene Kinder: .....

8. Maternale PKU-Problematik (nur Frauen)

Kinder: ❑ Nein
❑ Ja. Wenn ja:
Schwangerschaft ❑ ohne Diät
❑ mit Diät
Sind die Kinder ❑ gesund
❑ krank (Klartext:)

9. Höchster Schulabschluß bzw. zur Zeit noch besuchte Schulform der Kinder:

	Kind1	Kind2	Kind3	Kind4
Haupt- (Volksschule)	❑	❑	❑	❑
Realschule	❑	❑	❑	❑
Gymnasium /FOS	❑	❑	❑	❑
Sonderschule für Lernbehinderte	❑	❑	❑	❑
Sonderschule für geistig Behinderte	❑	❑	❑	❑
Sonderschule für Sehbehinderte	❑	❑	❑	❑
Sonderschule für Schwererziehbare	❑	❑	❑	❑
Sonderschule für Körperbehinderte	❑	❑	❑	❑
Ohne Schulabschluß	❑			
Anders, und zwar:	❑	❑	❑	❑

B Wohnsituation des Patienten

9. Wo ist der Patient aufgewachsen?

- ❑ Familie, beide Eltern
- ❑ Familie, ein Elternteil
- ❑ bei Verwandten
- ❑ Adoptivfamilie
- ❑ Pflegefamilie
- ❑ Heim, Name .....
- ❑ anders, nämlich .....
  .....

10. Aktuelles Wohnumfeld des Patienten (mit Zeitangabe, auch Mehrfachangaben)

- ❑ Allein
- ❑ Mit Partner
- ❑ Mit Partner und Kindern
- ❑ Wohngemeinschaft
- ❑ Familie, beide Eltern
- ❑ Familie, ein Elternteil
- ❑ bei Verwandten
- ❑ Adoptivfamilie
- ❑ Pflegefamilie
- ❑ Heim, Name .....
  .....
- ❑ Betreutes Wohnen
- ❑ Psychiatrie, Name .....
  .....
- ❑ anders, nämlich .....
  .....
  .....

11. Mit wie vielen Personen wohnt der Patient zusammen?
    Patient mit eingerechnet: .....

C Ausbildung und Beruf des Patienten

Schule

12. Alter bei Einschulung:

    Dabei Rückstellung?
    - ❑ Nein
    - ❑ Ja, Anzahl Jahre: ....

13. Wechsel des Schultyp (z.B. Von Gymnasium 6. Klasse nach Realschule 6. Klasse). Alle Wechsel eintragen.

Von (Schultyp)	Klasse	nach (Schultyp)	Klasse

14. Klassen wiederholt? ❑ Nein
❑ Ja. Wenn ja welche: ....

15. Höchster Schulabschluss bzw. zur Zeit noch besuchte Schulform:

	Abgeschlossen	zur Zeit besucht
Haupt- (Volksschule)	❑	❑
Realschule	❑	❑
Gymnasium /FOS	❑	❑
Sonderschule für Lernbehinderte	❑	❑
Sonderschule für geistig Behinderte	❑	❑
Sonderschule für Sehbehinderte	❑	❑
Sonderschule für Schwererziehbare	❑	❑
Sonderschule für Körperbehinderte	❑	❑
Ohne Schulabschluß	❑	
Anders, und zwar:	❑	❑

Beruf

16. Name und Art der letzten Berufsausbildung, die bereits abgeschlossen wurde oder der Patient zur Zeit noch absolviert:

Berufsausbildung zum/zur: .....

Abgeschlossen	zur Zeit besucht
❑	❑

17. Art der Ausbildung:
- ❑ schulische Berufsausbildung
- ❑ Lehre
- ❑ Lehre mit Meister
- ❑ Studium
- ❑ ohne abgeschlossene Berufsausbildung
- ❑ Anders, und zwar: .....

18. Abgebrochene Berufsausbildungen:
❑ Nein
❑ Ja. Wenn ja wie oft: .....
Warum abgebrochen? .....

19. Derzeitige/r Tätigkeit/Beruf:

..... ❑ als Angestellter

❑ als Selbständiger

20. Wann hat der Patient zuletzt gearbeitet, falls derzeit keine Tätigkeit? ....

21. Dabei wöchentliche Arbeitszeit (in Stunden) .....

22. Arbeitsfähigkeit im letzten halben Jahr:

- ❑ immer arbeitsfähig
- ❑ zeitweise arbeitsunfähig
- ❑ überwiegend arbeitsunfähig
- ❑ immer arbeitsunfähig
- ❑ (Früh-)Rentner
- ❑ Anders, und zwar: .....

23. Selbsteinschätzung zur beruflichen Leistungsfähigkeit

- ❑ Voll
- ❑ Eingeschränkt
- ❑ Gar nicht
- ❑ Anders, nämlich:

D Gesundheit

Allgemein

24. Lebenszufriedenheit

Beruf

- ❑ Sehr zufrieden
- ❑ Zufrieden
- ❑ Wenig zufrieden
- ❑ Gar nicht zufrieden

Partnerschaft

- ❑ Sehr zufrieden
- ❑ Zufrieden
- ❑ Wenig zufrieden
- ❑ Gar nicht zufrieden

Gesundheit

- ❑ Sehr zufrieden
- ❑ Zufrieden
- ❑ Wenig zufrieden
- ❑ Gar nicht zufrieden

25. Gesundheitliches Befinden (subjektives Gefühl)

- ❑ Sehr gesund
- ❑ Gesund
- ❑ Weniger gesund
- ❑ Gar nicht gesund

26. War/ist der Patient *in den letzten 5 Jahren* erkrankt?

Erkrankung	Behandlung	Arzt	Medikation	Erfolg?

27. Aktuelle gesundheitliche Beschwerden (Klartext)

28. Aktuelle Medikation (Klartext)

29. Wurde der Patient psychotherapeutisch oder psychiatrisch behandelt?

❑ Nein ❑ Ja. Wenn ja:

Von - bis: Grund: Art der Einrichtung:

30. Möchte der Patient sonstige Angaben zu seinem Gesundheitszustand machen?

Diät

31. Beginn der Diät, Dauer der Diät und Angaben zur Diätänderung

	Diät *gelockert oder abgebrochen*?	Angabe von *Gründen*:
Beginn und Dauer Von - bis:		

32. Einnahme der AS-Mischung

	Einnahme der AS-Mischung *abgebrochen*?	Angabe von *Gründen*:
Beginn und Dauer Von - bis:		

33. Angaben zur Betreuung (Arzt, Zentrum)

	Warum *abgebrochen*?	Datum der letzten Vorstellung:	Wo vorgestellt?
Beginn und Dauer Von - bis:			

34. Angaben zum Phe-Spiegel

Datum:

Wie hoch?

Subjektive Angaben zur Diät

35. Wie belastend empfanden oder empfinden Sie die Diät?

Gar nicht belastend	Etwas belastend	Ziemlich belastend	Sehr belastend
❑	❑	❑	❑

36. a Wie stark war/ist Ihr Wunsch, die Diät zu beenden?

Gar nicht stark	Etwas stark	Ziemlich stark	Sehr stark
❑	❑	❑	❑

36. b Falls ja, warum haben Sie die Diät weitergeführt?

.....
.....

37. Wie schätzen Sie Ihren bisherigen Diätverlauf ein?

- ❑ Sehr zufrieden
- ❑ Zufrieden
- ❑ Weniger zufrieden
- ❑ Gar nicht zufrieden
- ❑ Anders, nämlich:

38. Einschränkungen durch die Diät in

Schule, Beruf

Gar nicht stark	Etwas stark	Ziemlich stark	Sehr stark
❑	❑	❑	❑

Partnerschaft

Gar nicht stark	Etwas stark	Ziemlich stark	Sehr stark
❑	❑	❑	❑

allgemeiner Lebensgestaltung

Gar nicht stark	Etwas stark	Ziemlich stark	Sehr stark
❑	❑	❑	❑

E Angaben zum altersmäßig nächstliegenden Geschwister

Angaben zur Person des Geschwister

39. Geburtsdatum: .... 19 .....

40. Geschlecht:
- ❑ weiblich
- ❑ männlich

41. Familienstand:
- ❑ ledig
- ❑ verheiratet
- ❑ mit festem Partner zusammenlebend
- ❑ geschieden
- ❑ getrennt lebend
- ❑ verwitwet
- ❑ anders, nämlich .....

42. Kinder:
- ❑ Nein
- ❑ Ja. Wenn ja, wie viele eigene Kinder: .....
  und viele angenommene Kinder: .....

Wohnsituation des Geschwister

43. Wo ist das leibliche Geschwister aufgewachsen?

- ❑ Familie, beide Eltern
- ❑ Familie, ein Elternteil
- ❑ bei Verwandten
- ❑ Adoptivfamilie
- ❑ Pflegefamilie
- ❑ Heim, Name .....
- ❑ anders, nämlich .....
  .....

44. Aktuelles Wohnumfeld des leiblichen Geschwister:

- ❑ Allein
- ❑ Mit Partner
- ❑ Wohngemeinschaft
- ❑ Familie, beide Eltern
- ❑ Familie, ein Elternteil
- ❑ bei Verwandten
- ❑ Adoptivfamilie
- ❑ Pflegefamilie
- ❑ Heim, Name .....
- ❑ Betreutes Wohnen
- ❑ Psychiatrie, Name .....
- ❑ JVA .....
- ❑ anders, nämlich .....

Schulbesuch des Geschwister

45. Alter bei Einschulung:

46. Wechsel des Schultyp (z.B. Von Gymnasium 6. Klasse nach Realschule 6. Klasse). Alle Wechsel eintragen.

Von (Schultyp)	Klasse	nach (Schultyp)	Klasse

47. Klassen wiederholt? ❑ Nein
❑ Ja. Wenn ja welche: ....

48. Höchster Schulabschluß bzw. zur Zeit noch besuchte Schulform:

	Abgeschlossen	zur Zeit besucht
Haupt- (Volksschule)	❑	❑
Realschule	❑	❑
Gymnasium /FOS	❑	❑
Sonderschule für Lernbehinderte	❑	❑
Sonderschule für geistig Behinderte	❑	❑
Sonderschule für Sehbehinderte	❑	❑
Sonderschule für Schwererziehbare	❑	❑
Sonderschule für Körperbehinderte	❑	❑
Ohne Schulabschluß	❑	
Anders, und zwar:	❑	❑

Beruf des Geschwister

49. Name und Art der letzten Berufsausbildung, die bereits abgeschlossen wurde oder der Patient zur Zeit noch absolviert:

Berufsausbildung zum/zur: .....

Abgeschlossen	zur Zeit besucht
❑	❑

50. Art der Ausbildung:
- ❑ schulische Berufsausbildung
- ❑ Lehre
- ❑ Lehre mit Meister
- ❑ Studium
- ❑ ohne abgeschlossene Berufsausbildung
- ❑ Anders, und zwar: .....

51. Abgebrochene Berufsausbildungen:
❑ Nein
❑ Ja. Wenn ja wie oft: .....
Warum abgebrochen? .....

52. Derzeitige/r Tätigkeit/Beruf des Geschwister:
..... ❑ als Angestellter
❑ als Selbständiger

Gesundheit des Geschwister

53. War/ist das Geschwister *in den letzten 5 Jahren* erkrankt?

Erkrankung	Behandlung	Arzt	Medikation	Erfolg?

54. Aktuelle gesundheitliche Beschwerden (Klartext)

55. Aktuelle Medikation (Klartext)

56. Wurde das Geschwister psychotherapeutisch oder psychiatrisch behandelt?

❑ Nein ❑ Ja. Wenn ja:

Von - bis: Grund: Art der Einrichtung:

57. Möchte der Patient sonstige Angaben zu seinem Gesundheitszustand machen?

F Eltern des Patienten

58. Höchster Schulabschluß der biologischen Eltern:

	Vater	Mutter
Haupt- (Volksschule)	❑	❑
Realschule	❑	❑
Gymnasium /FOS	❑	❑
Sonderschule	❑	❑
ohne Schulabschluß	❑	❑
Anders, und zwar:	.....	.....

59. Namen und die Art der Berufsausbildung der Eltern:

Berufsausbildung des Vaters: .....

Berufsausbildung der Mutter: .....

Art der Ausbildung:

	Vater	Mutter
schulische Berufsausbildung	❑	❑
Lehre	❑	❑
Lehre mit Meister	❑	❑
Studium	❑	❑
ohne abgeschlossene Berufsausbildung	❑	❑
Anders, und zwar:	.....	.....

A Angaben zur KONTROLLPERSON

1. Geburtsdatum: .... 19 .....

2. Geschlecht: ❑ weiblich
❑ männlich

3. Telefon:

4. Adresse:

6. Familienstand: ❑ ledig
❑ verheiratet
❑ mit festem Partner zusammenlebend
❑ geschieden
❑ getrennt lebend
❑ verwitwet
❑ anders, nämlich .....

7. Kinder: ❑ Nein
❑ Ja. Wenn ja, wie viele eigene Kinder: .....
und viele angenommene Kinder: .....

9. Höchster Schulabschluss bzw. zur Zeit noch besuchte Schulform der Kinder:

	Kind1	Kind2	Kind3	Kind4
Haupt- (Volksschule)	❑	❑	❑	❑
Realschule	❑	❑	❑	❑
Gymnasium /FOS	❑	❑	❑	❑
Sonderschule für Lernbehinderte	❑	❑	❑	❑
Sonderschule für geistig Behinderte	❑	❑	❑	❑
Sonderschule für Sehbehinderte	❑	❑	❑	❑
Sonderschule für Schwererziehbare	❑	❑	❑	❑
Sonderschule für Körperbehinderte	❑	❑	❑	❑
Ohne Schulabschluss	❑			
Anders, und zwar:	❑	❑	❑	❑

B Wohnsituation des Probanden

9. Wo ist der Proband aufgewachsen?

❑ Familie, beide Eltern
❑ Familie, ein Elternteil
❑ bei Verwandten
❑ Adoptivfamilie
❑ Pflegefamilie
❑ Heim, Name .....
❑ anders, nämlich .....
.....

10. Aktuelles Wohnumfeld des Probanden (mit Zeitangabe, auch Mehrfachangaben)

❑ Allein
❑ Mit Partner

❑ Mit Partner und Kindern
❑ Wohngemeinschaft
❑ Familie, beide Eltern
❑ Familie, ein Elternteil
❑ bei Verwandten
❑ Adoptivfamilie
❑ Pflegefamilie
❑ Heim, Name .....
.....
❑ Betreutes Wohnen
❑ Psychiatrie, Name .....
.....
❑ anders, nämlich .....
.....
.....

11. Mit wie vielen Personen wohnen Sie zusammen?
(sich selbst mit eingerechnet) .....

C Ausbildung und Beruf des Probanden

Schule

12. Alter bei Einschulung:

Dabei Rückstellung? ❑ Nein
❑ Ja, Anzahl Jahre: ....

13. Wechsel des Schultyp (z.B. Von Gymnasium 6. Klasse nach Realschule 6. Klasse). Alle Wechsel eintragen.

Von (Schultyp)	Klasse	nach (Schultyp)	Klasse

14. Klassen wiederholt? ❑ Nein
❑ Ja. Wenn ja welche: ....

15. Höchster Schulabschluss bzw. zur Zeit noch besuchte Schulform:

	Abgeschlossen	zur Zeit besucht
Haupt- (Volksschule)	❑	❑
Realschule	❑	❑
Gymnasium /FOS	❑	❑
Sonderschule für Lernbehinderte	❑	❑
Sonderschule für geistig Behinderte	❑	❑
Sonderschule für Sehbehinderte	❑	❑
Sonderschule für Schwererziehbare	❑	❑
Sonderschule für Körperbehinderte	❑	❑
Ohne Schulabschluß	❑	
Anders, und zwar:	❑	❑

Beruf

16. Name und Art der letzten Berufsausbildung, die bereits abgeschlossen wurde oder der Patient zur Zeit noch absolviert:

    Berufsausbildung zum/zur: .....

Abgeschlossen	zur Zeit besucht
❑	❑

17. Art der Ausbildung:
    - ❑ schulische Berufsausbildung
    - ❑ Lehre
    - ❑ Lehre mit Meister
    - ❑ Studium
    - ❑ ohne abgeschlossene Berufsausbildung
    - ❑ Anders, und zwar: .....

18. Abgebrochene Berufsausbildungen:
    - ❑ Nein
    - ❑ Ja. Wenn ja wie oft: .....

    Warum abgebrochen? .....

19. Derzeitige/r Tätigkeit/Beruf:

    ..... ❑ als Angestellter

    ❑ als Selbständiger

20. Wann hat der Proband zuletzt gearbeitet, falls derzeit keine Tätigkeit?
    ....

21. Dabei wöchentliche Arbeitszeit (in Stunden) .....

22. Arbeitsfähigkeit im letzten halben Jahr:
    - ❑ immer arbeitsfähig
    - ❑ zeitweise arbeitsunfähig
    - ❑ überwiegend arbeitsunfähig
    - ❑ immer arbeitsunfähig
    - ❑ (Früh-)Rentner
    - ❑ Anders, und zwar: .....

23. Selbsteinschätzung zur beruflichen Leistungsfähigkeit
    - ❑ Voll
    - ❑ Eingeschränkt
    - ❑ Gar nicht
    - ❑ Anders, nämlich:

D Gesundheit

Allgemein

24. Lebenszufriedenheit

Beruf

- ❑ Sehr zufrieden
- ❑ Zufrieden
- ❑ Wenig zufrieden
- ❑ Gar nicht zufrieden

Partnerschaft

- ❑ Sehr zufrieden
- ❑ Zufrieden
- ❑ Wenig zufrieden
- ❑ Gar nicht zufrieden

Gesundheit

- ❑ Sehr zufrieden
- ❑ Zufrieden
- ❑ Wenig zufrieden
- ❑ Gar nicht zufrieden

25. Gesundheitliches Befinden (subjektives Gefühl)

- ❑ Sehr gesund
- ❑ Gesund
- ❑ Weniger gesund
- ❑ Gar nicht gesund

26. War/ist der Proband *in den letzten 5 Jahren* erkrankt?

Erkrankung	Behandlung	Arzt	Medikation	Erfolg?

27. Aktuelle gesundheitliche Beschwerden (Klartext)

28. Aktuelle Medikation (Klartext)

29. Wurden Sie psychotherapeutisch oder psychiatrisch behandelt?

❑ Nein ❑ Ja. Wenn ja:

Von - bis: Grund: Art der Einrichtung:

30. Möchte der Patient sonstige Angaben zu seinem Gesundheitszustand machen?

E Angaben zum altersmäßig nächstliegenden Geschwister

Angaben zur Person des Geschwister

39. Geburtsdatum: .... 19 .....

40. Geschlecht:
- ❑ weiblich
- ❑ männlich

41. Familienstand:
- ❑ ledig
- ❑ verheiratet
- ❑ mit festem Partner zusammenlebend
- ❑ geschieden
- ❑ getrennt lebend
- ❑ verwitwet
- ❑ anders, nämlich .....

42. Kinder:
- ❑ Nein
- ❑ Ja. Wenn ja, wie viele eigene Kinder: .....
  und viele angenommene Kinder: .....

Wohnsituation des Geschwister

43. Wo ist das leibliche Geschwister aufgewachsen?

- ❑ Familie, beide Eltern
- ❑ Familie, ein Elternteil
- ❑ bei Verwandten
- ❑ Adoptivfamilie
- ❑ Pflegefamilie
- ❑ Heim, Name .....
- ❑ anders, nämlich .....
  .....

44. Aktuelles Wohnumfeld des leiblichen Geschwister:

- ❑ Allein
- ❑ Mit Partner
- ❑ Wohngemeinschaft
- ❑ Familie, beide Eltern
- ❑ Familie, ein Elternteil
- ❑ bei Verwandten
- ❑ Adoptivfamilie
- ❑ Pflegefamilie
- ❑ Heim, Name .....
- ❑ Betreutes Wohnen
- ❑ Psychiatrie, Name .....
- ❑ JVA .....
- ❑ anders, nämlich .....

Schulbesuch des Geschwister

45. Alter bei Einschulung:

46. Wechsel des Schultyp (z.B. Von Gymnasium 6. Klasse nach Realschule 6. Klasse). Alle Wechsel eintragen.

Von (Schultyp)	Klasse	nach (Schultyp)	Klasse

47. Klassen wiederholt? ❑ Nein
❑ Ja. Wenn ja welche: ....

48. Höchster Schulabschluss bzw. zur Zeit noch besuchte Schulform:

	Abgeschlossen	zur Zeit besucht
Haupt- (Volksschule)	❑	❑
Realschule	❑	❑
Gymnasium /FOS	❑	❑
Sonderschule für Lernbehinderte	❑	❑
Sonderschule für geistig Behinderte	❑	❑
Sonderschule für Sehbehinderte	❑	❑
Sonderschule für Schwererziehbare	❑	❑
Sonderschule für Körperbehinderte	❑	❑
Ohne Schulabschluß	❑	
Anders, und zwar:	❑	❑

Beruf des Geschwister

49. Name und Art der letzten Berufsausbildung, die bereits abgeschlossen wurde oder der Patient zur Zeit noch absolviert:

Berufsausbildung zum/zur: .....

Abgeschlossen	zur Zeit besucht
❑	❑

50. Art der Ausbildung:
- ❑ schulische Berufsausbildung
- ❑ Lehre
- ❑ Lehre mit Meister
- ❑ Studium
- ❑ ohne abgeschlossene Berufsausbildung
- ❑ Anders, und zwar: .....

51. Abgebrochene Berufsausbildungen:
❑ Nein
❑ Ja. Wenn ja wie oft: .....
Warum abgebrochen? .....

52. Derzeitige/r Tätigkeit/Beruf des Geschwister:

..... ❑ als Angestellter
❑ als Selbständiger

Gesundheit des Geschwister

53. War/ist das Geschwister *in den letzten 5 Jahren* erkrankt?

Erkrankung	Behandlung	Arzt	Medikation	Erfolg?

54. Aktuelle gesundheitliche Beschwerden (Klartext)

55. Aktuelle Medikation (Klartext)

56. Wurde das Geschwister psychotherapeutisch oder psychiatrisch behandelt?

❑ Nein ❑ Ja. Wenn ja:

Von - bis: Grund: Art der Einrichtung:

57. Möchte der Patient sonstige Angaben zu seinem Gesundheitszustand machen?

F Eltern des Probanden

58. Höchster Schulabschluss der biologischen Eltern:

	Vater	Mutter
Haupt- (Volksschule)	❑	❑
Realschule	❑	❑
Gymnasium /FOS	❑	❑
Sonderschule	❑	❑
ohne Schulabschluß	❑	❑
Anders, und zwar:	.....	.....

59. Namen und die Art der Berufsausbildung der Eltern:

Berufsausbildung des Vaters: .....

Berufsausbildung der Mutter: .....

Art der Ausbildung:

	Vater	Mutter
schulische Berufsausbildung	❑	❑
Lehre	❑	❑
Lehre mit Meister	❑	❑
Studium	❑	❑
ohne abgeschlossene Berufsausbildung	❑	❑
Anders, und zwar:	.....	.....

ATOPIE-FRAGEBOGEN PKU-Erwachsenen-Untersuchung	Seite 134 von 168	Probandennr.	Datum

1. Haben oder hatten Sie jemals **Asthma**? ja O nein O
2. Wurde dies durch einen Arzt bestätigt? ja O nein O
3. Hatten Sie in den letzten 12 Monaten einen **Asthmaanfall**? ja O nein O
4. Wurde dies durch einen Arzt bestätigt? ja O nein O
5. Nehmen Sie derzeit **Medikamente gegen Asthma** (Inhalationen, Spray oder Tabletten)? Wenn ja, welche und wie häufig? ja O nein O

   _____
   _____
   _____

6. Haben oder hatten Sie jemals **Heuschnupfen**, **allergischen Schnupfen** oder **allergische Bindehautentzündung**?
7. Wurde dies durch einen Arzt bestätigt? ja O nein O
8. Leiden Sie derzeit - ohne dabei erkältet zu sein - an einer verstopften oder laufenden **Nase** und/oder an geschwollenen, juckenden **Augen** und zwar regelmäßig im Frühjahr oder Sommer oder fast immer beim Umgang mit bestimmten fell- oder federtragenden Tieren? ja O nein O ja O nein O
9. Litten Sie jemals - ohne dabei erkältet zu sein - an einer verstopften oder laufenden **Nase** und/oder an geschwollenen, juckenden **Augen** und zwar regelmäßig im Frühjahr oder Sommer oder fast immer beim Umgang mit bestimmten fell- oder federtragenden Tieren? ja O nein O
10. Wurde dies durch einen Arzt bestätigt?
11. Leiden Sie derzeit an einer **Neurodermitis** (auch endogenes Ekzem oder atopische Dermatitis genannt)?
12. Litten Sie jemals an einer **Neurodermitis** (auch endogenes Ekzem oder atopische Dermatitis genannt)? ja O nein O ja O nein O
13. Wurde dies durch einen Arzt bestätigt?
14. Hatten Sie wiederholt **Nesselsucht**, auch Urtikaria genannt, mit Quaddeln wie nach Brennnesselkontakt und/oder Schwellungen der Lippen und Augen? ja O nein O
15. Wurde dies durch einen Arzt bestätigt? ja O nein O
16. Reagieren Sie regelmäßig **auf bestimmte Nahrungsmittel allergisch** (Nesselsucht, Verschlimmerung eines Ekzems, Übelkeit, Erbrechen, Durchfall, Asthma)? ja O nein O ja O nein O
17. Wurde dies durch einen Arzt bestätigt?

18. Nehmen Sie regelmäßig **Medikamente**? Wenn ja, welche und wie häufig? ja O nein O

_____
_____
_____ ja O nein O
_____
_____

ja O nein O

19. Rauchen Sie? Wenn ja, was und wie viel pro Tag?

_____
_____

ja O nein O

Verbaler Lern- und Merkfähigkeitstest (VLMT)

Name _____ Testdatum

Geburtstag

Alter

Jahr	Monat	Tag

Wortliste A	A1	A2	A3	A4	A5	Wortliste B	B1	A6	A7	Wortliste A
Trommel						Tisch				Trommel
Vorhang						Förster				Vorhang
Glocke						Vogel				Glocke
Kaffee						Schuh				Kaffee
Schule						Ofen				Schule
Eltern						Berg				Eltern
Mond						Handtuch				Mond
Garten						Brille				Garten
Hut						Wolke				Hut
Bauer						Boot				Bauer
Nase						Lamm				Nase
Truthahn						Gewehr				Truthahn
Farbe						Bleistift				Farbe
Haus						Kirsche				Haus
Fluß						Arm				Fluß
Score						Interferenz				

Vergleichswerte: 6,7+/-2,7 9,7+/-2,2 11,7+/-2,3 12,6+/-2 13,2 +/-1,9 (Erwachsene) 7,0+/-2,1 12,0+/-2,4

Wortliste C (nach dreißig Minuten vorgeben)						Score C (Treffer-False Al.)			
Glocke		Tisch		*Vorhang*		Maus		Schuh	
Fenster		*Mond*		Sonne		*Eltern*		blau	
Hut		Mut		*Farbe*		warten		Ofen	
Förster		Locke		Arm		Wolke		Wolle	
Nase		Vogel		Gewehr		*Haus*		*Trommel*	
Himmel		Berg		Kakao		Schur		Vase	
Schule		*Kaffee*		Kirsche		Kinder		Lamm	
Mord		Mauer		*Truthahn*		*Garten*		*Bauer*	
Bleistift		*Fluß*		Zwerg		Brille		Herd	
Mund		Handtuch		Boot		Stuhl		Bach	

Auswertung:

		Erwachsene	Kinder
unmittelbare Gedächtnisleistung (Score A1)		6,7+/-2,7	5,74+/-1,82
Lernleistung (Score A5 minus Score A1)		6,3+/-2,0	5,04+/-2,69
Wiedergewinnungsleistung (Score A6)		12,0+/-2,4	9,13+/-3,38
Verlust durch Interferenz (A5 minus A6)		1,1+/-1,1	1,65+/-1,77
Wiedererkennungsleistung (Score C)		13,7+/-1,8	10,7+/-5,78

Testbogen d2

Datum: _____ Nr.: _____

Name: _____ Alter: _____ Jahre

Vorname: _____ Geschlecht: ☐ männlich ☐ weiblich

Schulart/Klasse: _____

Beruf: _____ Händigkeit: ☐ rechtshändig ☐ linkshändig

Lesebrille benutzt? ☐ ja ☐ nein

Beispiele: d″ d d″ d′

Übungszeile:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
d″	p″	d′	d′	d″	d″	p″	d″	d″	p′	d′	d″	d″	d′	p″	p″	d″	d″	d″	p″	d′	d′

	RW	PR*	SW*
GZ			
F			
F%			
GZ-F			
KL			

F_2 =	F-Vert. 1./2. Hälfte: ___/___	SB =	Ü-Syndr. ☐

* Normierung: Eichstichprobe ☐; Stichprobe BfA ☐; andere Normen ☐: _____

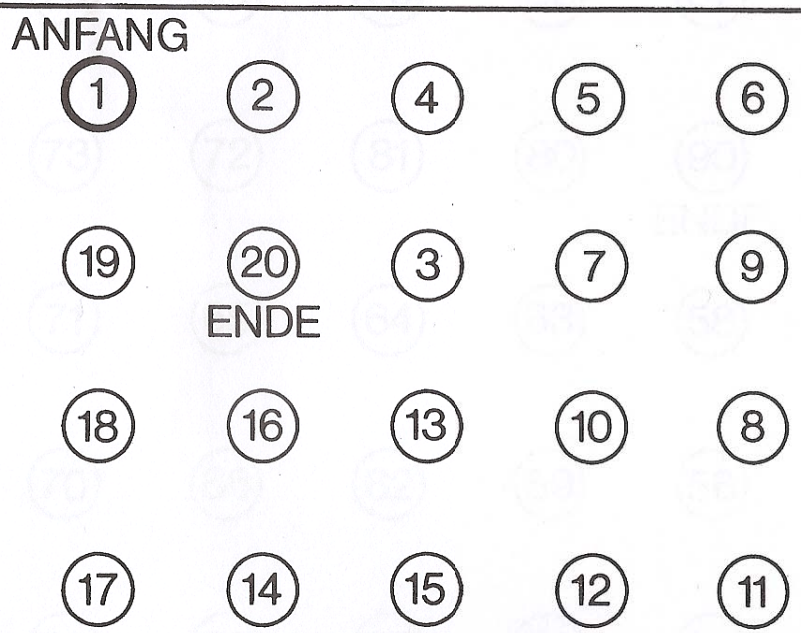
Name	
Vorname	
Alter	
Geburtsdatum	
Schule	
Klasse	
Beruf	
Datum	

Bitte diesen Teil nicht ausfüllen!					**ZVT**
Einzelv.			A		
Gruppenv.			B		Bemerk.:
T			C		
PR			D		
C			Σ		
IQ				: 4	
SW			RW		

ÜBUNGSAUFGABE 1:

Aufgabe: Verbinde die Zahlen in fortlaufender Folge:

1 – 2 – 3 – 4 – 5 – 6 usw. . . .

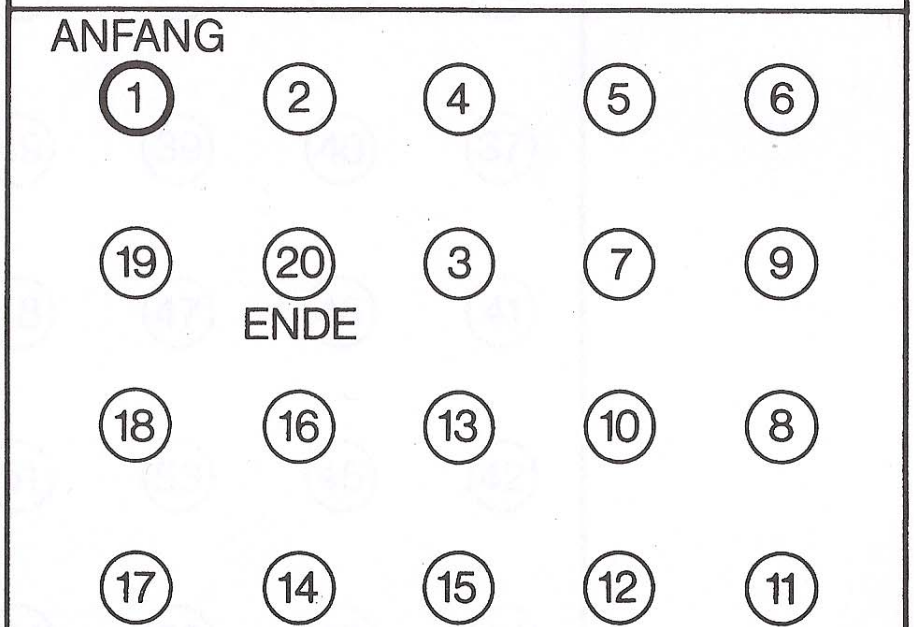


AUSWERTUNGS-/ÜBUNGSBOGEN

ÜBUNGSAUFGABE 2:

Aufgabe: Verbinde die Zahlen in fortlaufender Folge:

1 – 2 – 3 – 4 – 5 – 6 usw. . . .



ANFANG

ENDE

ZVT A

*

Best.-Nr. 01 066 03

PROTOKOLLBOGEN ZUM FARBE-WORT-INTERFERENZTEST (STROOP)
nach G. Bäumler (1. Auflage)

Name: _____ Vorname: _____ Geschlecht: _____

Geburtsdatum _____ Testdatum: _____ Alter: _____

Ausbildung, Beruf: _____

Sonstiges: _____

Itemfolge (Farbnamen) der Interferenztafeln

Tafel 3			*Tafel 6*			*Tafel 9*		
blau	grün	gelb	blau	grün	blau	rot	grün	gelb
grün	gelb	rot	grün	blau	rot	gelb	gelb	grün
gelb	blau	blau	rot	rot	grün	grün	rot	rot
rot	grün	gelb	blau	grün	gelb	blau	blau	gelb
grün	rot	grün	grün	gelb	rot	rot	grün	blau
blau	gelb	rot	gelb	rot	grün	grün	gelb	grün
gelb	grün	blau	rot	blau	blau	gelb	blau	rot
rot	rot	grün	grün	gelb	gelb	rot	grün	blau
blau	blau	rot	blau	grün	grün	blau	rot	grün
gelb	grün	gelb	rot	blau	rot	grün	blau	gelb
grün	gelb	blau	gelb	gelb	gelb	gelb	gelb	rot
rot	rot	grün	blau	grün	blau	blau	rot	grün
gelb	blau	rot	rot	rot	grün	grün	grün	blau
blau	gelb	blau	grün	gelb	gelb	rot	blau	rot
grün	rot	gelb	gelb	blau	rot	gelb	rot	gelb
rot	grün	grün	rot	rot	blau	blau	gelb	grün
gelb	gelb	blau	blau	grün	gelb	grün	blau	rot
grün	blau	rot	gelb	gelb	grün	gelb	grün	blau
blau	rot	gelb	grün	rot	rot	rot	gelb	gelb
rot	gelb	blau	blau	blau	gelb	blau	blau	grün
grün	blau	grün	gelb	gelb	blau	gelb	rot	blau
blau	rot	gelb	grün	rot	grün	grün	gelb	gelb
gelb	grün	rot	rot	blau	rot	blau	grün	rot
rot	blau	grün	gelb	grün	blau	rot	rot	blau

Summe unkorrigierter Fehler (/):	Summe korrigierter Fehler (X):

Nr.	Tafelart	Rep.	Zeit	Nr.	Tafelart	Rep.	Zeit	Nr.	Tafelart	Rep.	Zeit
1	FWL – a			2	FSB – a			3	INT – a		
4	FWL – b			5	FSB – b			6	INT – b		
7	FWL – c			8	FSB – c			9	INT – c		
	Median FWL:				Median FSB:				Median INT:		

Best.-Nr. 01 005 04

geb. 10.09.1970, männlich, 34,10 Jahre, Bildungsgrad 2

Motorische Leistungsserie (MLS)

Verfahren zur Messung der Feinmotorik
Testform S3 - Kurzform nach Vassella
Testdurchführung: 11.07.2005 - 16:41 - 16:54, Dauer: 13 min
Rechts/Linkshänder: 1 (Rechtshändig)

Testergebnisse - Kinder und Jugendliche - Rechtshänder (Vassella - Inselspital Bern) (13-20 Jahre):

Testvariable	Rohwert	PR	T
Rechte Hand			
Aiming Fehlerzahl	2	29	44
Aiming Trefferzahl	**19**	**16**	**40**
Aiming Fehlerdauer (in Sekunden)	0.06	100	80
Aiming Gesamtdauer (in Sekunden)	**7.24**	**70**	**55**
Steadiness Fehlerzahl	9	20	42
Steadiness Fehlerdauer (in Sekunden)	**0.48**	**41**	**48**
Liniennachfahren Fehlerzahl	23	35	46
Liniennachfahren Fehlerdauer (in Sekunden)	**1.25**	**78**	**58**
Liniennachfahren Gesamtdauer (in Sekunden)	33.75	63	52
Lange Stifte einstecken Gesamtdauer (in Sekunden)	**40.42**	**16**	**40**
Tapping Trefferzahl	189	67 (48-82)	54 (45-59)
Linke Hand			
Aiming Fehlerzahl	8	8	36
Aiming Trefferzahl	**20**	**90**	**63**
Aiming Fehlerdauer (in Sekunden)	0.34	13	39
Aiming Gesamtdauer (in Sekunden)	**7.30**	**73**	**56**
Steadiness Fehlerzahl	2	78	58
Steadiness Fehlerdauer (in Sekunden)	**0.03**	**100**	**80**
Liniennachfahren Fehlerzahl	27	50	50
Liniennachfahren Fehlerdauer (in Sekunden)	**1.66**	**87**	**61**
Liniennachfahren Gesamtdauer (in Sekunden)	25.87	75	57
Lange Stifte einstecken Gesamtdauer (in Sekunden)	**41.19**	**35**	**46**
Tapping Trefferzahl	215	99 (95-100)	73 (67-79)
Feinmotorikfaktoren der rechten Hand			
Faktor 1 - Zielgerichtetheit des Verhaltens		82	59
Faktor 2 - Handunruhe, Tremor		**31**	**45**
Faktor 3 - Präzision (Arm, Hand)		58	52
Faktor 5 - Geschwindigkeit (Arm, Hand)		**69**	**55**
Faktor 6 - Geschwindigkeit (Handgelenk, Finger)		67	54

Anmerkung(en): Prozentrang (PR) und T-Wert (T) ergeben sich durch Vergleichmit einem Teil (Auswahl nach Alter) der Stichprobe 'Kinder und Jugendliche - Rechtshänder (Vassella - Inselspital Bern)'
ACHTUNG: Der Vergleich mit dieser Stichprobe ist nicht oder nur eingeschränkt zulässig, da der Proband nicht im Geltungsbereich (13-20 Jahre) liegt!
Die hinter den jeweiligen Normwerten in Klammern angegebenen Vertrauensintervalle sind mit 5%iger Irrtumswahrscheinlichkeit behaftet.

Profil - Kinder und Jugendliche - Rechtshänder (Vassella - Inselspital Bern) (13-20 Jahre):

Anmerkung(en): Der hervorgehobene Bereich stellt den Durchschnittsbereich der Normwertskala dar.
Rechte Hand
Linke Hand

Testergebnisse - Kinder und Jugendliche - Linkshänder (Vassella - Inselspital Bern) (limitierter Geltungsbereich):
Kein Normvergleich, Proband liegt nicht im Geltungsbereich dieser Stichprobe!

Friedrich-Baur-Institut

Patienten Information:

Patienten ID:517
Name:
Geschlecht:Weiblich
Geburtsdatum:16.10.1973
Ableitdatum:02.02.2006

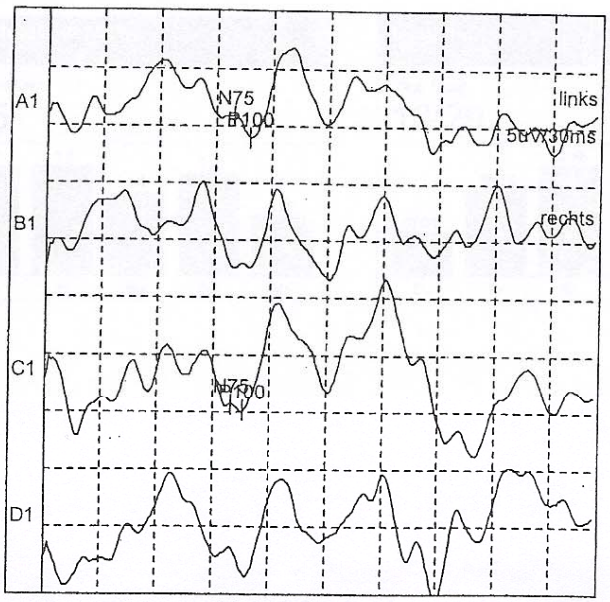
Alter:32
Grösse:0,00 m
Gewicht:0 kg
Arzt:Dr. Reilich
Untersucht durch:]

Bemerkung:

VEP:

		N75	P100
A1	links	101,7 ms	110,7 ms
C1	rechts	101,7 ms	108,3 ms

A1	Amplitude	N75-P100	2.386uV
B1	Amplitude	N75-P100	
C1	Interval	N75-P100	6.6ms
D1	Amplitude	N75-P100	



02.02.2006

InnerView Protokoll

enabled by BODYMEDIA

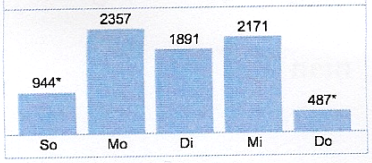
Arzt/Berater	Krankenhaus/Institut	Praxis/Abteilung

Name	Alter	Geschlecht	Gewicht	Größe	Händigkeit	Raucher	BMI
	32	weiblich	64.9 kg	170 cm	Rechts	Nein	22.46

Start	Stopp	Anzeigedauer	Tragedauer
So 9 Okt 2005 16:37	Do 13 Okt 2005 07:27	3 Tage 14 Stdn 50 Min	3 Tage 13 Stdn 39 Min (98.6%)

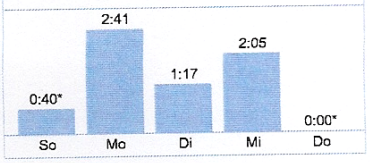
Gesamter Energieumsatz

Tägl. Mittel 1570 kal — Total 7849



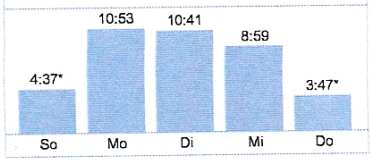
Dauer körperl. Aktivität (2.5 METS)

Tägl. Mittel 1:21 — Total 6 Stdn 43 Min



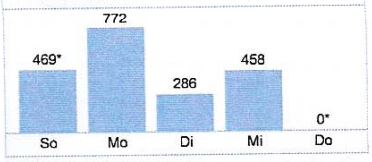
Liegenddauer

Tägl. Mittel 7:47 — Total 1 Tag 14 Stdn 57 Min



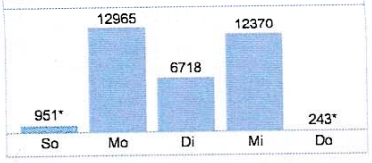
Aktiver Energieumsatz (2.5 METS)

Tägl. Mittel 397 kal — Total 1985



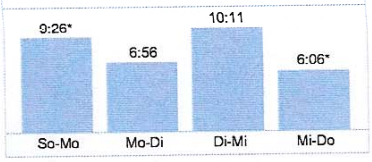
Anzahl Schritte

Tägl. Mittel 6649 Schritte — Total 33247



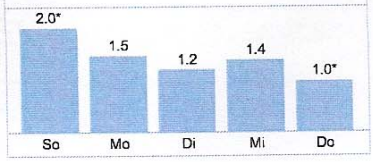
Schlafdauer (12.00-12.00 Uhr)

Tägl. Mittel 8:10 — Total 1 Tag 8 Stdn 39 Min



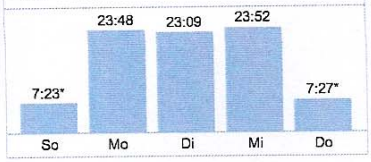
Durchschnitt METs

Tägl. Mittel 1.4



Tragedauer

Tägl. Mittel 17:08 — Total 3 Tage 13 Stdn 39 Min



* Unvollständiger Tag. Werte sind für einen 24 Stunden Zeitraum nicht repräsentativ.

Tätigkeitsprotokoll

Name, Vorname: _____

1 Wie viele Stunden haben Sie von gestern auf heute geschlafen?
Bitte rechnen Sie den gestrigen Tagschlaf mit ein!

_____Std.:_____Min.:____
_____Std.:_____Min.:____

2a Haben Sie gestern Sport getrieben?

ja nein

2b Wenn ja, welchen Sport haben Sie gestern ausgeübt?

_____Std.:_____Min.:____
_____Std.:_____Min.:____
_____Std.:_____Min.:____

3 Wie viel Zeit verbrachten Sie in den vergangenen 12 Monaten durchschnittlich pro Woche mit Sport? (Gymnastik, Aerobic, Joggen, Walken, Wandern, Schwimmen usw.)
Bitte machen Sie Angaben in Stunden bzw. Minuten pro **Woche**!

_____
_____

4 Wie viel Zeit haben Sie in den vergangenen 12 Monaten durchschnittlich in Ihrer **Freizeit**

pro **Tag** vor dem Fernseher oder am PC verbracht?

Fernseher: Std.:_____Min.:____ sehe nicht Fernsehen

PC: Std.:_____Min.:____ benutze keinen PC

7.4 Acknowledgements

First, I would like to sincerely thank my doctoral adviser Professor Dr. Berthold Koletzko for enabling this work, as well as for his constant encouragement.
For the financial support of this study, without which we would not have been able to carry out all these examinations, I would like to deeply thank the SHS Gesellschaft für klinische Ernährung mbH, Heilbronn.
Special thanks to Dr. Andrè Michael Toschke and Dr. Johann Demmelmair for their professional competence and help with all my questions and problems.
I am deeply grateful to my co-operating partners Professor Dr. Dr. Joseph Weglage and Dr. Reinhold Feldmann from Münster for their great forthrightness and cooperativeness.
I would also like to express my gratitude to Dr. Thomas Pfluger, Professor Dr. Wolfgang Müller-Felber, Dr. Dominic Hartl, Dr. Katharina Dokoupil, Ms. Susanne Bauske, Ms. Julia Geppert, Ms. Monika Rachl, as well as all other colleagues from the Division of Metabolic Diseases and Nutritional Medicine for their invaluable support and assistance.
Finally and most importantly, I want to thank all the patients and those in the control group, who gave their consent to participate in this study and so enabled us to perform all these investigations.

7.5 Publication of the obtained data

2006	Garmann K, Feldmann K, Berlepsch Jv, Koletzko B, Weglage J, Feldmann R (2006) Kognitive Defizite bei Erwachsenen mit frühbehandelter PKU. Monatsschr Kinderheilkd 154 (Suppl. 2): ABSTRACT, CD-Beilage
2006	Feldmann K, Garmann K, Berlepsch Jv, Koletzko B, Weglage J, Feldmann R (2006) Soziale Störungen und emotionale Probleme bei frühbehandelter PKU. Monatsschr Kinderheilkd 154 (Suppl. 2): ABSTRACT, CD-Beilage
2009	Weglage J, Kloska, S, van Teeffelen-Heithoff A, Möller HE, von Berlepsch J, Koletzko B, Feldmann R Neuropsychological impairment in adult patients with treated phenylketonuria, submitted for publication
In preparation	Berlepsch Jv, Feldmann R, Koletzko B Determinants of obesity risk in adult patients with phenylketonuria, in preparation
In preparation	Berlepsch Jv, Hartl D, Feldmann R, Koletzko B Atopic disease in patients with phenylketonuria: possible relation to polyunsaturated fatty acid, vitamin and mineral intake, in preparation
In preparation	Berlepsch Jv, Feldmann R, Weglage J, Koletzko B High cardiovascular risk in patients with phenylketonuria, in preparation
In preparation	Berlepsch Jv, Feldmann R, Mueller-Felber W, Koletzko B Motor function and visual evoked potentials in adult patients with phenylketonuria, in preparation
In preparation	Berlepsch Jv, Feldmann R, Weglage J, Koletzko B Dietary intake and blood markers of nutrient situation in adult patients with phenylketonuria, in preparation

8 References

1. Richtlinien zur Organisation und Durchfuehrung des Neugeborenenscreenings auf angeborene Stoffwechselstoerungen und Endokrinopathien in Deutschland. Mschr Kinderheilk 2002;150:1424-40.

2. Statistisches Jahrbuch 2005. Für die Bundesrepublik Deutschland. Wiesbaden: Statistisches Bundesamt (StBA), 2005.

3. Acosta PB. Nutrition studies in treated infants and children with phenylketonuria: vitamins, minerals, trace elements. Eur J Pediatr 1996;155 Suppl 1:S136-S139.

4. Acosta PB, Alfin-Slater RB, Koch R. Serum lipids in children with phenylketonuria (PKU). J Am Diet Assoc 1973;63:631-5.

5. Acosta PB, Fernhoff PM, Warshaw HS et al. Zinc and copper status of treated children with phenylketonuria. JPEN J Parenter Enteral Nutr 1981;5:406-9.

6. Acosta PB, Yannicelli S. Protein intake affects phenylalanine requirements and growth of infants with phenylketonuria. Acta Paediatr Suppl 1994;407:66-7.

7. Agostoni C, Marangoni F, Riva E, Giovannini M, Galli C. Plasma arachidonic acid and serum thromboxane B2 concentrations in phenylketonuric children negatively correlate with dietary compliance. Prostaglandins Leukot Essent Fatty Acids 1997;56:219-22.

8. Allen JR, Humphries IR, Waters DL et al. Decreased bone mineral density in children with phenylketonuria. Am J Clin Nutr 1994;59:419-22.

9. Anderson AE, Avins L. Lowering brain phenylalanine levels by giving other large neutral amino acids. A new experimental therapeutic approach to phenylketonuria. Arch Neurol 1976;33:684-6.

10. Aoki K, Siegel FL. Hyperphenylalaninemia: disaggregation of brain polyribosomes in young rats. Science 1970;168:129-30.

11. Arbeitsgemeinschaft für Pädiatrische Stoffwechselstörungen (APS). Therapie von Patienten mit Phenylketonurie. 2006.
Ref Type: Report

12. Arnold GL, Vladutiu CJ, Orlowski CC, Blakely EM, DeLuca J. Prevalence of stimulant use for attentional dysfunction in children with phenylketonuria. J Inherit Metab Dis 2004;27:137-43.

13. Bauch A, Lindtner O, Mensink GB, Niemann B. Dietary intake and sources of long-chain n-3 PUFAs in German adults. Eur J Clin Nutr 2006;60:810-2.

14. Bauman M, Kemper T. Morphologic and histoanatomic observations of the brain in untreated human phenylketonuria. Acta Neuropathol (Berl) 1982;58:173-80.

15. Beblo S, Reinhardt H, Muntau AC, Mueller-Felber W, Roscher AA, Koletzko B. Fish oil supplementation improves visual evoked potentials in children with phenylketonuria. Neurology 2001;57:1488-91.

16. Bench CJ, Frith CD, Grasby PM et al. Investigations of the functional anatomy of attention using the Stroop test. Neuropsychologia 1993;31:907-22.

17. Bernegger C, Blau N. High frequency of tetrahydrobiopterin-responsiveness among hyperphenylalaninemias: a study of 1,919 patients observed from 1988 to 2002. Mol Genet Metab 2002;77:304-13.

18. Bick U, Fahrendorf G, Ludolph AC, Vassallo P, Weglage J, Ullrich K. Disturbed myelination in patients with treated hyperphenylalaninaemia: evaluation with magnetic resonance imaging. Eur J Pediatr 1991;150:185-9.

19. Bick U, Ullrich K, Stober U et al. White matter abnormalities in patients with treated hyperphenylalaninaemia: magnetic resonance relaxometry and proton spectroscopy findings. Eur J Pediatr 1993;152:1012-20.

20. Bickel H, Gerrard J, Hickmans EM. The influence of phenylalanine intake on the chemistry and behaviour of a phenyl-ketonuric child. Acta Paediatr 1954;43:64-77.

21. Blau N. The Hyperphenylalaninemias. A Differential Diagnosis and International Database of Tetrahydrobiopterin Deficiencies. 1996.

22. Bodley JL, Austin VJ, Hanley WB, Clarke JT, Zlotkin S. Low iron stores in infants and children with treated phenylketonuria: a population at risk for iron-deficiency anaemia and associated cognitive deficits. Eur J Pediatr 1993;152:140-3.

23. Bohles H. Die Führung des stoffwechselkranken Jugendlichen. Sozialpäd 1988;10:170-6.

24. Bohles H, Ullrich K, Endres W, Behbehani AW, Wendel U. Inadequate iron availability as a possible cause of low serum carnitine concentrations in patients with phenylketonuria. Eur J Pediatr 1991;150:425-8.

25. Bonaa KH, Bjerve KS, Straume B, Gram IT, Thelle D. Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension. A population-based intervention trial from the Tromso study. N Engl J Med 1990;322:795-801.

26. Bremer HJ, Anninos A, Schulz B. Amino acid composition of food products used in the treatment of patients with disorders of the amino acid and protein metabolism. Eur J Pediatr 1996;155 Suppl 1:S108-S114.

27. Broesicke HG, Labahn S, de Veer I, Moench E, Helge H. 13C phenylalanine breath test to distinguish heterozygotes of classical phenylketonuria and hyperphenylalaninemia. Mschr Kinderheilk 1992;141:S42.

28. Brumm V, Azen C, Moats R et al. Neuropsychological outcome of subjects participating in the PKU adult collaborative study: A preliminary review. J Inherit Metab Dis 2004;27:549-66.

29. Bucher HC, Hengstler P, Schindler C, Meier G. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. Am J Med 2002;112:298-304.

30. Buckley R, Shewring B, Turner R, Yaqoob P, Minihane AM. Circulating triacylglycerol and apoE levels in response to EPA and docosahexaenoic acid supplementation in adult human subjects. Br J Nutr 2004;92:477-83.

31. Burgard P, Bremer HJ, buhrdel P et al. Rationale for the German recommendations for phenylalanine level control in phenylketonuria 1997. Eur J Pediatr 1999;158:46-54.

32. Burgard P, Rey F, Rupp A, Abadie V, Rey J. Neuropsychologic functions of early treated patients with phenylketonuria, on and off diet: results of a cross-national and cross-sectional study. Pediatr Res 1997;41:368-74.

33. Burgard P, Schmidt E, Rupp A, Schneider W, Bremer HJ. Intellectual development of the patients of the German Collaborative Study of children treated for phenylketonuria. Eur J Pediatr 1996;155 Suppl 1:S33-S38.

34. Burri R, Steffen C, Stieger S, Brodbeck U, Colombo J, Herschkowitz N. Reduced myelinogenesis and recovery in hyperphenylalaninemic rats. Correlation between brain phenylalanine levels, characteristic brain enzymes for myelination and brain development. Mol Chem Neuropathol 1990;13:57-69.

35. Camomme M, Vanderpas J, Francois B et al. Effects of selenium supplementation on thyroid hormone metabolism in phenylketonuria subjects on a phenylalanine restricted diet. Biol Trace Elem Red 1995;47:349-53.

36. Carnielli VP, Pederzini F, Vittorangeli R et al. Plasma and red blood cell fatty acid of very low birth weight infants fed exclusively with expressed preterm human milk. Pediatr Res 1996;39:671-9.

37. Channon S, Mockler C, Lee P. Executive functioning and speed of processing in phenylketonuria. Neuropsychology 2005;19:679-86.

38. Christensen R, Kolvraa S, Jensen T. Manipulation of the phenylalanine metabolism in human keratinocytes by retroviral mediated gene transfer. Cells Tissues Organs 2005;179:170-8.

39. Clayton BE, Jenkins P, Round JM. Pediatric Chemical Pathology - Clinical Tests and Reference Range. Pädiatrie in Praxis und Klinik. Stuttgart: Fischer&Thieme 1980:1163ff.

40. Cleary M. Magnetic resonance imaging of the brain in phenylketonuria. Lancet 1994;92:255-62.

41. Colome C, Artuch R, Vilaseca MA et al. Lipophilic antioxidants in patients with phenylketonuria. Am J Clin Nutr 2003;77:185-8.

42. Conquer JA, Holub BJ. Supplementation with an algae source of docosahexaenoic acid increases (n-3) fatty acid status and alters selected risk factors for heart disease in vegetarian subjects. J Nutr 1996;126:3032-9.

43. Daniel PM, Moorhouse SR, Pratt OE. Amino acid precursors of monoamine neurotransmitters and some factors influencing their supply to the brain. Psychol Med 1976;6:277-86.

44. Darling G, Mathias P, O'Regan M, Naughten E. Serum selenium levels in individuals on PKU diets. J Inherit Metab Dis 1992;15:769-73.

45. Davidson MH, Maki KC, Kalkowski J, Schaefer EJ, Torri SA, Drennan KB. Effects of docosahexaenoic acid on serum lipoproteins in patients with combined hyperlipidemia: a randomized, double-blind, placebo-controlled trial. J Am Coll Nutr 1997;16:236-43.

46. Deutsche Gesellschaft für Ernährung e.V.(DGE). Ernährungsbericht 2004. Bonn: 2004.

47. DGE, OeGE, SGE, SVE. D-A-CH Referenzwerte für die Naehrstoffzufuhr DGE. Frankfurt am Main: Umschau/Braus, 2001.

48. Dhont J, Farriaux J. Atypical cases of phenylketonuria. Eur J Pediatr 1987;146:38-43.

49. Diamond A. Phenylalanine levels of 6-10 mg/dl may not be as benign as once thought. Acta Paediatr Suppl 1994;407:89-91.

50. Diamond A, Ciaramitaro V, Donner E, Djali S, Robinson MB. An animal model of early-treated PKU. J Neurosci 1994;14:3072-82.

51. Dyer CA, Kendler A, Philibotte T, Gardiner P, Cruz J, Levy HL. Evidence for central nervous system glial cell plasticity in phenylketonuria. J Neuropathol Exp Neurol 1996;55:795-814.

52. Dyer R, Howell W, Mcphail R. Dopamine depletion slows retinol transmission. Exp Neurol 1981;71:326-40.

53. Feldmann R, Denecke J, Grenzebach M, Weglage J. Frontal lobe-dependent functions in treated phenylketonuria: blood phenylalanine concentrations and long-term deficits in adolescents and young adults. J Inherit Metab Dis 2005;28:445-55.

54. Fingerhut, R. Unter 1.000.000 gescreenten Neugeborenen in Deutschland fanden sich 99 mit PKU (1:10.100) und 101 mit Hyperphenylalaninaemie (1:9.900). 2005.
Ref Type: Personal Communication

55. Fingerhut R, Roschinger W, Muntau AC et al. Hepatic carnitine palmitoyltransferase I deficiency: acylcarnitine profiles in blood spots are highly specific. Clin Chem 2001;47:1763-8.

56. Fisberg RM, da Silva-Fernandes ME, Schmidt BJ, Fisberg M. Nutritional evaluation of children with phenylketonuria. Sao Paulo Med J 1999;117:185-91.

57. Fisch RO, Gravem HJ, Feinberg SB. Growth and bone characteristics of phenylketonurics. Comparative analysis of treated and untreated phenylketonuric children. Am J Dis Child 1966;112:3-10.

58. Fölling A. Über Ausscheidung von Phenylbrenztraubensäure in den Harn alsStoffwechselanomalie in Verbindung mit Imbezillität. Zeitschr Physiol Chem 1934;227:169-76.

59. Funk-Wentzel, P. Stellungnahme der Arbeitsgemeinschaft für pädiatrische Diätetik (APD): Proteinzufurhempfehlungen für Patienten mit Phenylketonurie. 2001.
Ref Type: Report

60. gassio r, artuch r, vilaseca m et al. Cognitive functions in classic phenylketonuria and mild hyperphenylalaninaemia: experience in a pediatric population. Dev Med Child Neurol 2005;47:443-8.

61. Geppert, J. Unpublished data. 2006.
Ref Type: Unpublished Work

62. Giovannini M, Agostoni C, Biasucci G et al. Fatty acid metabolism in phenylketonuria. Eur J Pediatr 1996;155 Suppl 1:S132-S135.

63. Giovannini M, Biasucci G, Agostoni C, Luotti D, Riva E. Lipid status and fatty acid metabolism in phenylketonuria. J Inherit Metab Dis 1995;18:265-72.

64. Gleason LA, Michals K, Matalon R, Langenberg P, Kamath S. A treatment program for adolescents with phenylketonuria. Clin Pediatr (Phila) 1992;31:331-5.

65. Göbel Y, Schaffer C, Koletzko B. Simultaneous determination of low plasma concentrations of retinol and tocopherols in preterm infants by a high-performance liquid chromatographic micromethod. J Chromatogr B Biomed Sci Appl 1997;688:57-62.

66. Grimsgaard S, Bonaa KH, Hansen JB, Nordoy A. Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids. Am J Clin Nutr 1997;66:649-59.

67. Gropper SS, Acosta PB, Clarke-Sheehan N, Wenz E, Cheng M, Koch R. Trace element status of children with PKU and normal children. J Am Diet Assoc 1988;88:459-65.

68. Gropper SS, Chaung HC, Bernstein LE, Trahms C, Rarback S, Weese SJ. Immune status of children with phenylketonuria. J Am Coll Nutr 1995;14:264-70.

69. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 1963;32:338-43.

70. Hanley WB, Feigenbaum AS, Clarke JT, Schoonheyt WE, Austin VJ. Vitamin B12 deficiency in adolescents and young adults with phenylketonuria. Eur J Pediatr 1996;155 Suppl 1:S145-S147.

71. Harding C, Wild K, Chang D, Messing A. Metabolic engineering as therapy for inborn errors of metabolism-development of mice with phenylalanine hydroxylase expression in muscle. Gene Ther 1998;5:677-83.

72. Hennermann JB, Vetter B, Wolf C et al. Phenylketonuria and hyperphenylalaninemia in Eastern Germany: Acharacteristicmolecular profile and 15 novel mutations. Hum Mutat 2002;15:254-60.

73. Hess D, Keller HE, Oberlin B, Bonfanti R, Schuep W. Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. Int J Vitam Nutr Res 1991;61:232-8.

74. Hillman L, Schlotzhauer C, Lee D et al. Decreased bone mineralization in children with phenylketonuria under treatment. Eur J Pediatr 1996;155 Suppl 1:S148-S152.

75. Hodis HN. Triglyceride-rich lipoprotein remnant particles and risk of atherosclerosis. Circulation 1999;99:2852-4.

76. Hoerster F, Schwab MA, Sauer S et al. Phenylalanin hemmt die Synaptogenese in neuronalen Zellkulturen. Mschr Kinderheilk 2004;152.

77. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 1996;3:213-9.

78. Holm VA, Kronmal RA, Williamson M, Roche AF. Physical growth in phenylketonuria: II. Growth of treated children in the PKU collaborative study from birth to 4 years of age. Pediatrics 1979;63:700-7.

79. Hommes FA. The role of the blood-brain barrier in the aetiology of permanent brain dysfunction in hyperphenylalaninaemia. J Inherit Metab Dis 1989;12:41-6.

80. Hommes FA. Loss of neurotransmitter receptors by hyperphenylalaninemia in the HPH-5 mouse brain. Acta Paediatr 1994;407:121-2.

81. Hommes FA, Eller AG, Taylor EH. The effect of phenylalanine on myelin metabolism in adolescent rats. Inborn errors of metabolism in humans. Lancaster: 1982:193-9.

82. Hommes FA, Lee J. The control of 5-hydroxytryptamine and dopamine synthesis in the brain: a theoretical approach. J Inh Metab Dis 1990;13:37-57.

83. Hommes FA, matsuo k. On a possible mechanism of abnormal brain development in experimental hyperphenylalaninemia. Neurochem 1987;11:1-10.

84. Huttenlocher PR. Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res 1979;163:195-205.

85. International Society for Hypertension (ISH). Classification of hypertension according to WHO/ISH. London health science centre 1999. Internet: http://www.lhsc.on.ca/rss/document/jobaid/GGTSPU-hydra2.fw.med.uni-muenchen.de-28025-128214-DAT/classifi.pdf

86. International Society for the Study of Fatty Acids and Lipids (ISSFAL). Recommendations for intake of polyunsaturated fatty acids in healthy adults. 2004. Brighton.
Ref Type: Conference Proceeding

87. Johannik K, Van HP, Francois B et al. Localized brain proton NMR spectroscopy in young adult phenylketonuria patients. Magn Reson Med 1994;31:53-7.

88. Karagoz T, Coskun T, Ozalp I, Ozkaya E, Ersoy F. Immune function in children with classical phenylketonuria and tetrahydrobiopterin deficiencies. Indian Pediatr 2003;40:822-33.

89. Kaufman S. An evaluation of the possible neurotoxity of metabolites of phenylalanine. J Pediatr 1989;114:895-900.

90. Kay M, Nakai H. Looking into the safety of AAV vectors. Nature 2003;424:251.

91. Kinosian B, Glick H, Garland G. Cholesterol and coronary heart disease: predicting risks by levels and ratios. Ann Intern Med 1994;121:641-7.

92. Kinosian B, Glick H, Preiss L, Puder KL. Cholesterol and coronary heart disease: predicting risks in men by changes in levels and ratios. J Investig Med 1995;43:443-50.

93. Knapp HR, FitzGerald GA. The antihypertensive effects of fish oil. A controlled study of polyunsaturated fatty acid supplements in essential hypertension. N Engl J Med 1989;320:1037-43.

94. Knudsen G, Hasselbalch S, Toft P, Christensen E, Paulson O, Lou H. Blood-brain barrier transport of amino acids in healthy controls and in patients with phenylketonuria. J Inher Metab Dis 1995;18:653-64.

95. Knudsen G, Pettigrew K, Patlak C, Paulson O. The double-indicator method for blood-brain barrier measurements following intavenous injection. Am J Physiol 1994;266:987-99.

96. Koch R, Burton B, Hoganson G et al. Phenylketonuria in adulthood: a collaborative study. J Inherit Metab Dis 2002;25:333-46.

97. Koch R, Moseley KD, Yano S, Moats RA, Nelson M. Large neutral amino acid therapy and phenylketonuria: a promising approach to treatment. Mol Genet Metab 2003;79:110-3.

98. Kolarovic L, Fournier NC. A comparison of extraction methods for the isolation of phospholipids from biological sources. Anal Biochem 1986;156:244-50.

99. Korinthenberg R, Ullrich K, Füllenkemper F. Evoked potentials and electroencephalography in adolescents with phenylketonuria. Neuropediatr 1988;19:175-8.

100. Kraft, V. Unpublished data. 2006.
Ref Type: Unpublished Work

101. Krauch G, Mueller E, Anninos A, Bremer H. Comparison of the protein qualityof dietetically treated phenylketonuria patients with the resommendations of the WHO expert consultation. Eur J Pediatr 1996;155:153-7.

102. Kreis R, Pietz J, Penzien J, Herschkowitz N, Boesch C. Identification and quantitation of phenylalanine in the brain of patients with phenylketonuria by means of localized in vivo 1H magnetic-resonance spectroscopy. J Magn Reson B 1995;107:242-51.

103. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002;106:2747-57.

104. Kure S, Hou D-C, Ohura T et al. Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency - A novel clinical entity. J Inher Metab Dis 2000;23:45A.

105. Leaf A, Kang JX, Xiao Y, Billman GE. Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 2003;107:2646-52.

106. Ledley FD. Clinical application of genotypic diagnosis for phenylketonuria: theoretical considerations. Eur J Pediatr 1991;150:752-6.

107. Leuzzi V, Rinalduzzi S, Chiarotti F, Garzia P, Trasimeni G, Accornero N. Subclinical visual impairment in phenylketonuria. A neurophysiological study (VEP-P) with clinical, biochemical, and neuroradiological (MRI) correlations. J Inherit Metab Dis 1998;21:351-64.

108. Lipinska L, Laskowska-Klita T, Cabalska B. Riboflavin status in phenylketonuric patients in the course of dietary treatment. J Inherit Metab Dis 1994;17:242.

109. Litov RE, Combs GF, Jr. Selenium in pediatric nutrition. Pediatrics 1991;87:339-51.

110. Lombeck I, Jochum F, Terwolbeck K. Selenium status in infants and children with phenylketonuria and in maternal phenylketonuria. Eur J Pediatr 1996;155 Suppl 1:S140-S144.

111. Lou HC, Guttler F, Lykkelund C, Bruhn P, Niederwieser A. Decreased vigilance and neurotransmitter synthesis after discontinuation of dietary treatment for phenylketonuria in adolescents. Eur J Pediatr 1985;144:17-20.

112. Lou H, Lykkelund C, Gerdes A, Udessen H, Bruhn P. Increased vigilance and dopamine synthesis by large dosis of tyrosine or phenylalanine restriction in phenylketonuria. Acta Pediatr Scand 1987;76:560-5.

113. Ludolph A, Vetter U, Ullrich K. Studies of multimodel evoked potentials in treated phenylketonuria: the pattern of vulnerability. Eur J Pediatr 1996;155:64-8.

114. Lungershausen YK, Abbey M, Nestel PJ, Howe PRC. Reduction of blood pressure and plasma triglycerides by omega-3 fatty acids in treated hypertensives. J Hypertens 1994;12:1041-5.

115. MacDonald A. Diet and compliance in phenylketonuria. Eur J Pediatr 2000;159 Suppl 2:S136-S141.

116. Matalon KM, Acosta PB, Azen C. Role of nutrition in pregnancy with phenylketonuria and birth defects. Pediatrics 2003;112:1534-6.

117. Matalon, K. M., Acosta, P. B., Castiglioni, L., Austin, V., Rohr, F., Wenz, E., and Funk-Wentzel, P. Protocol for nutrition support of maternal PKU. 1998. U.S. National Institute of Child Health and Human Development.
Ref Type: Report

118. Matalon R, Surendran S, Matalon K et al. Future role of large neutral amino acids in transport of phenylalanine into the brain. Pediatrics 2003;112:1570-4.

119. McBurnie MA, Kronmal RA, Schuett VE, Koch R, Azeng CG. Physical growth of children treated for phenylketonuria. Ann Hum Biol 1991;18:357-68.

120. McDonnell GV, Esmonde TF, Hadden DR, Morrow JI. A neurological evaluation of adult phenylketonuria in Northern Ireland. Eur Neurol 1998;39:38-43.

121. Medical Research Council Working Party on Phenylketonuria. Phenylketonuria due to phenylalnine hydroxylase deficiency: an unfolding story. Brit Med J 1993;306:115-9.

122. Miranda da Cruz BD, Seidler H, Widhalm K. Iron status and iron supplementation in children with classical phenylketonuria. J Am Coll Nutr 1993;12:531-6.

123. Moats RA, Moseley K, Koch R, Nelson M. Brain phenylalanine concentrations in phenylketonuria: research and treatment of adults. Pediatrics 2003;112:1575-9.

124. Moeller H, Vermathen P, Ullrich K, Weglage J, Koch H, Peters P. In-vivo NMRspectroscopy in patients with phenylketonuria: changes of cerebralphenylalanine levls under dietary treatment. Neuropediatr 1995;26:199-202.

125. Moeller H, Weglage J, Wiedermann D, Vermathen P, bick U, Ullrich K. Kinetics of phenylalanine transport at the human blood-brain-barrier investigated in vivo. Brain Research 1997.

126. Moeller, HE, Wiedermann, D., Weglage, J, Stoeber, U, Vermathen, P, bick, U, Ullrich, K, and Peters, PE. Clinical significance of interindividual differences in brain phenylalanine concentrations in patients with phenylketonuria:aninvivo 1H MRS study. 5th Scientific Meeting and Exhibition. 1208. 1997. Vancouver, International Society of Magnetic Resonance in Medicine.
Ref Type: Conference Proceeding

127. Moench E, Herrmann M, Broesicke H, Schoeffer A, Keller M. Utilisation of amino acid mixtures in adolescents with phenylketonuria. Eur J Pediatr 1996;155:115-20.

128. Moench E, Kneer J, Jakobs C, Arnold M, Diehl H, Batzler U. Examination of urine metabolites in the newborn period and during protein loading tests at 6 months of age. Eur J Pediatr 1990;149:s17-s24.

129. Moench E, Link R. Diagnostik und Therapie bei angeborenen Stoffwechselstoerungen. Heilbronn: SPS Verlagsgesellschaft, 2002.

130. Moseley K, Koch R, Moser AB. Lipid status and long-chain polyunsaturated fatty acid concentrations in adults and adolescents with phenylketonuria on phenylalanine-restricted diet. J Inherit Metab Dis 2002;25:56-64.

131. Mueller E. Praktische Diaetetik in der Paediatrie, Grundlagen für die Ernaehrungstherapie. Heilbronn: SPS Verlagsgesellschaft, 2003.

132. Muntau AC, Beblo S, Koletzko BK. PKU und Hyperphe. Mschr Kinderheilk 2000;2:179-93.

133. Nagatsu T, Levitt M, Udenfriend S. Tyrosine hydroxylase. The initial step in norepinephrine biosynthesis. J Biol Chem 1964;239:2910-7.

134. Naumann M, Kemer T. Morphologic and histoanatomic observations of the brain in untreated humans with phenylketonuria. Acta Neuropathol 1982;58:55.

135. Nelson GJ, Schmidt PC, Bartolini GL, Kelley DS, Kyle D. The effect of dietary docosahexaenoic acid on plasma lipoproteins and tissue fatty acid composition in humans. Lipids 1997;32:1137-46.

136. Nestel P, Shige H, Pomeroy M, Cehun M, Abbey M, Raederstorff D. The n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans. Am J Clin Nutr 2002;76:326-30.

137. Nutt J, Woodward W, Hammerstad J, Carter J, Anderson J. The "on-off" phenomenon in parkinson's disease. Relation to levodopa adsorption and transport. N Engl J Med 1984;310:483-8.

138. Oh H, Park E, Kang S, Jo I. Long-term enzymatic and phenotypic correction in the phenylketonuria mouse model by adeno-associated virus vector-mediated gene transfer. Pediatr Res 2004;56:278-84.

139. Pietz J, Kreis R, Schmidt H, Meyding-Lamade U, Rupp A, Boesch C. Phenylketonuria: findings at MR imaging and localized in vivo H-1 MR spectroscopy of the brain in patients with early treatment. Radiology 1996;201:413-20.

140. Pietz J. Neurological aspects of adult phenylketonuria. Curr Opin Neurol 1998;11:679-88.

141. Pietz J, Fatkenheuer B, Burgard P, Armbruster M, Esser G, Schmidt H. Psychiatric disorders in adult patients with early-treated phenylketonuria. Pediatrics 1997;99:345-50.

142. Podskarbi T. Molekulargenetik des Phenalaninhydroxylase-Mangels (PAH). Screening auf angeborene endokrine und metabole Störungen. Wien: Springer 2002.

143. Primrose DA. Phenylketonuria with normal intelligence. J Ment Defic Res 1983;27 (Pt 4):239-46.

144. Prince AP, Leklem JE. Vitamin B-6 status of school-aged patients with phenylketonuria. Am J Clin Nutr 1994;60:262-8.

145. Ris M, Williams S, Hunt M, Berry H, Leslie N. Early-treated phenylketonuria: adult neuropsychologic outcome. J Pediatr 1994;124:388-92.

146. Riva E, Fiocchi A, Agostoni C et al. PKU-related dysgammaglobulinaemia: the effect of diet therapy on IgE and allergic sensitization. J Inherit Metab Dis 1994;17:710-7.

147. Robinson M, White FJ, Cleary MA, Wraith E, Lam WK, Walter JH. Increased risk of vitamin B12 deficiency in patients with phenylketonuria on an unrestricted or relaxed diet. J Pediatr 2000;136:545-7.

148. Sanjurjo P, Perteagudo L, Rodriguez SJ, Vilaseca A, Campistol J. Polyunsaturated fatty acid status in patients with phenylketonuria. J Inherit Metab Dis 1994;17:704-9.

149. Schaefer F, Burgard P, Batzler U et al. Growth and skeletal maturation in children with phenylketonuria. Acta Paediatr 1994;83:534-41.

150. Schafer E, McKean C. Evidence that monoamines influence human eviked potentials. Brain Res 1975;99:49-58.

151. Schaffer, C. Bewertung der Plasmaspiegel der fettlöslichen Vitamine A und E bei Frühgeborenen mit zwei verschiedenen parenteralen Substitutionsregimes. 1997. LMU München.
Ref Type: Thesis/Dissertation

152. Schmidt E, Rupp A, Burgard P, Pietz J, Weglage J, de Sonneville L. Sustained attention in adult phenylketonuria: the influence of the concurrent phenylalanine-blood-level. J Clin Exp Neuropsych 1994;16:681-8.

153. Schulpis KH, Karikas GA, Papakonstantinou E. Homocysteine and other vascular risk factors in patients with phenylketonuria on a diet. Acta Paediatr 2002;91:905-9.

154. Schulpis KH, Papakonstantinou ED, Tzamouranis J. Plasma leptin concentrations in phenylketonuric patients. Horm Res 2000;53:32-5.

155. Schulpis KH, Papassotiriou I, Vounatsou M, Karikas GA, Tsakiris S, Chrousos GP. Morning preprandial plasma ghrelin and catecholamine concentrations in patients with phenylketonuria and normal controls: evidence for catecholamine-mediated ghrelin regulation. J Clin Endocrinol Metab 2004;89:3983-7.

156. Schulpis KH, Scarpalezou A. Triglycerides, cholesterol, HDL, LDL, and VLDL cholesterol in serum of phenylketonuric children under dietary control. Clin Pediatr (Phila) 1989;28:466-9.

157. Schulpis KH, Tsakiris S, Karikas GA, Moukas M, Behrakis P. Effect of diet on plasma total antioxidant status in phenylketonuric patients. Eur J Clin Nutr 2003;57:383-7.

158. Schulz B, Bremer HJ. Nutrient intake and food consumption of adolescents and young adults with phenylketonuria. Acta Paediatr 1995;84:743-8.

159. Schwarz M, Harms E, Wendel U, Berger M, Abholz HH. Stoffwechselkrankheiten im Saeuglingsalter - Ignoranz im Erwachsenenalter. Deutsches Ärzteblatt 2002;99:2030-2.

160. Schwenke W, Anke A, Knapp A. Über einen Fall von "klassischer" Phenylketonurie mit durchschnittlicher Intelligenz. Klein Wochenschau 1969;47:1051-3.

161. Scriver CR, Kaufman S. Hyperphenylalaninemia:Phenylalanine Hydroxylase Deficiency. The Metabolic and Molecular Bases of Inherited Disease. New York: 2001:1667-724.

162. Scriver CR, Kaufman S, Eisensmith RC, Woo SCL. The hyperphenylalaninemias. The metabolic and molecular bases of inherited disease. New York: McGraw-Hill 1995:1015-53.

163. Simon JA, Hodgkins ML, Browner WS, Neuhaus JM, Bernert JT, Jr., Hulley SB. Serum Fatty Acids and the Risk of Coronary Heart Disease. Am J Epidemiol 1995;142:469-76.

164. Sirtori LR, Dutra-Filho CS, Fitarelli D et al. Oxidative stress in patients with phenylketonuria. Biochim Biophys Acta 2005;1740:68-73.

165. Smith I, Beasley MG, Ades AE. Intelligence and quality of dietary treatment in phenylketonuria. Arch Dis Child 1990;65:472-8.

166. Smith I, Beasley MG, Ades AE. Effect on intelligence of relaxing the low phenylalanine diet in phenylketonuria. Arch Dis Child 1991;66:311-6.

167. Smith I, Beasley MG, Wolff OH, Ades AE. Behavior disturbance in 8-year-old children with early treated phenylketonuria. Report from the MRC/DHSS Phenylketonuria Register. J Pediatr 1988;112:403-8.

168. Smith I, Knowles J. Behaviour in early treated phenylketonuria: a systematic review. Eur J Pediatr 2000;159 Suppl 2:S89-S93.

169. Sourkes TL. Effect of alpha-methyl-tryptopan on tryptophan, 5-hydroxy-tryptamine and protein metabolism in the brain. Ciba Foundation Symposium 22 1974;361-78.

170. Sweetman L. Organic acid analysis. Tchniques in diagnostic human biochemical genetics. NewYork: Wiley-Liss 1991:143-76.

171. Taylor E, Hommes F. Effect of experimental hyperphenylalaninemia on myelin metabolism at later stages of brain development. Inter J Neuroscience 1983;20:217-28.

172. Terwolbeck K, Behne D, Meinhold H, Menzel H, Lombeck I. Increased plasma T4-levels in children with low selenium state due to reduced type I iodothyronine 5'deiodinase activity? J Trace Elem Electrolytes Health Dis 1993;7:53-5.

173. Thompson AJ, Smith I, Kendall BE, Youl BD, Brenton D. Magnetic resonance imaging changes in early treated patients with phenylketonuria. Lancet 1991;337:1224.

174. Trefz F. Stellungnahme der APS: Prophylaxe und Behandlung der maternalen Phenylketonurie. Mschr Kinderheilk 1995;143:898-9.

175. Ullrich K, Weglage J, Oberwittler C et al. Effect of L-dopa on pattern visual evoked potentials (P-100) and neuropsychological tests in untreated adult patients with phenylketonuria. J Inher Metab Dis 1994;17:349-52.

176. Ullrich K. Therapie von Patienten mit Phenylketonurie. Mschr Kinderheilk 1997;9:961-2.

177. van Bakel MM, Printzen G, Wermuth B, Wiesmann UN. Antioxidant and thyroid hormone status in selenium-deficient phenylketonuric and hyperphenylalaninemic patients. Am J Clin Nutr 2000;72:976-81.

178. van Teeffelen-Heithoff A. Diätbehandlung bei Phenylketonurie (PKU). Akt Ernähr-Med 1999;24:123-8.

179. Verkerk PH, van Spronsen FJ, Smit GP, Sengers RC. Impaired prenatal and postnatal growth in Dutch patients with phenylketonuria. The National PKU Steering Committee. Arch Dis Child 1994;71:114-8.

180. Villasana D, Butler IJ, Williams JC, Roongta SM. Neurological deterioration in adult phenylketonuria. J Inherit Metab Dis 1989;12:451-7.

181. Waisbren S, Zaff J. Personality disorders in young women with treated PKU. J Inher Metab Dis 1994;17:592.

182. Wasserstein MP, Snyderman SE, Sansaricq C, Buchsbaum MS. Cerebral glucose metabolism in adults with early treated classic phenylketonuria. Mol Genet Metab 2006;87:272-7.

183. Weglage J. Diätbehandlung bei Phenylketonurie. Indikationen, Wirkungen und Nebenwirkungen. Göttingen: Hogrefe, 2000.

184. Weglage J, Oberwittler C, Marquardt T et al. Neurological deterioration in adult phenylketonuria. J Inherit Metab Dis 2000;23:83-4.

185. Weglage J, Pietsch M, Denecke J et al. Regression of neuropsychological deficits in early-treated phenylketonurics during adolescence. J Inherit Metab Dis 1999;22:693-705.

186. Weglage J, Pietsch M, Funders B, Koch HG, Ullrich K. Neurological findings in early treated phenylketonuria. Acta Paediatr 1995;84:411-5.

187. Weglage J, Schuierer G, Kurlemann G, Bick R, Ullrich K. Different degrees of white matter abnormalities in untreated phenylketonurics: findings in magnetic resonance imaging. J Inherit Metab Dis 1993;16:1047-8.

188. Weglage J, Ullrich K, Pietsch M, Funders B, Zass R, Koch HG. Untreated non-phenylketonuric-hyperphenylalaninaemia: intellectual and neurological outcome. Eur J Pediatr 1996;155 Suppl 1:S26-S28.

189. Weglage J, Wiedermann D, Denecke J et al. Individualblood-brain barrier phenylalanine transport in siblings with classical phenylketonuria. J Inherit Metab Dis 2006;25:431-6.

190. Weglage J, Wiedermann D, Moller H, Ullrich K. Pathogenesis of different clinical outcomes in spite of identical genotypes and comparable blood phenylalanine concentrations in phenylketonurics. J Inherit Metab Dis 1998;21:181-2.

191. Whelton SP, He J, Whelton PK, Muntner P. Meta-analysis of observational studies on fish intake and coronary heart disease. Am J Cardiol 2004;93:1119-23.

192. White JE, Kronmal RA, Acosta PB. Excess weight among children with phenylketonuria. J Am Coll Nutr 1982;1:293-303.

193. World Health Organisation (WHO). Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation. Tech Rep Ser 1985;724.

194. Zschocke J. Phenylketonuria mutations in Europe. Hum Mutat 2003;21:345-56.

195. Kühweider B. Die Informationsseite über Multiple Sklerose. Nuglar, 2008. Internet: http://www.kabelschaden.de/was_ist_ms/diagnose.htm

196. Bäumler G. Farbe-Wort-Interferenztest (FWIT) nach J. R. Stroop. Handanweisung. Göttingen: Hogrefe, 1985.

9 Curriculum vitae