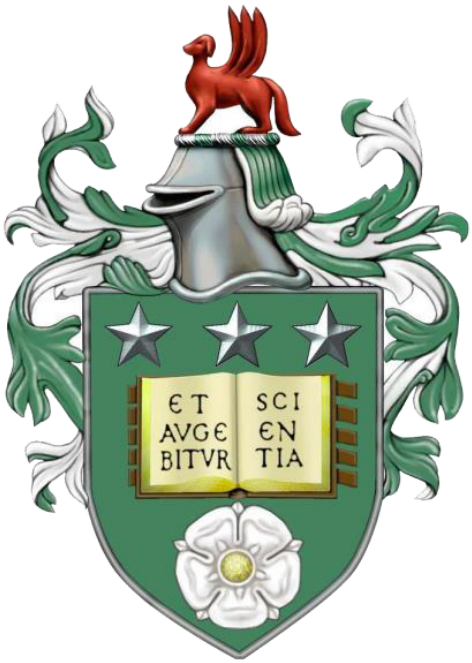
Dietary adherence in phenylketonuria (PKU) and effects on cognitive function and quality of life

by

Denise Leonne Hofman



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Chapter 3:

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In loving memory of Dr. S.B. (Syb) van der Meer (1956-2005)

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Abstract

Phenylketonuria (PKU) is a rare inherited metabolic disorder, characterised by reduced activity of the hepatic enzyme phenylalanine hydroxylase (PAH). PAH is responsible for the conversion of phenylalanine (Phe) to tyrosine (Tyr). Reduced PAH activity results in elevated Phe levels, decreased Tyr levels and an altered Phe:Tyr ratio. When left untreated, PKU causes severe and irreversible neurological impairments. Although early treatment prevents severe cognitive impairments, deficits in cognitive functioning are still observed. The conventional treatment for PKU is a low-protein (Phe-restricted) diet, supplemented with other amino acids and nutrients lacking in the low-protein diet. Due to the restrictive and complex nature of the dietary management, adherence to the PKU diet is poor in a large proportion of early treated adults with PKU (ET AwPKU), who often follow a self-restricted diet without sufficient supplementation with prescribed protein substitutes. To date, the effects of such dietary practice on nutritional status, quality of life (QoL)/wellbeing and cognitive functioning have not been well documented. Hence, this thesis examines cognitive function and QoL/wellbeing in ET AwPKU in relation to dietary adherence. A systematic review identified 22 articles reporting on outcomes from 16 studies which showed that, despite early treatment, ET AwPKU have deficits in sustained attention, working memory, and motor skills compared to healthy controls. In an online study, ET AwPKU (n=27) showed subtle deficits in episodic memory at a younger age than matched healthy controls (n=28) plus impaired speed of response across a range of cognitive tasks. An online survey examining the factors affecting dietary adherence in English, Dutch and German speaking ET AwPKU (n=71) suggested that poor adherence was associated with previous off-diet behaviour and negative attributes of the prescribed protein substitutes (e.g. bitter (after)taste, convenience). To improve dietary practices of semi-adherent ET AwPKU and assess subsequent effects on nutritional status, QoL/wellbeing and cognitive functioning, a 12-week dietary intervention study with a novel, more palatable, casein glycomacropeptide (CGMP)-based protein substitute was carried out in 10 patients. Improvements in QoL were evident with little effect on nutritional status or cognitive function. This new product could improve adherence to the PKU diet with potential long-term effects on nutritional status, QoL/wellbeing and cognitive function.

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List of Abbreviations

AA	Amino Acid(s)
AAM	Amino Acid Mixture
AChE	Acetylcholinesterase
ADHD	Attention deficit hyperactivity disorder
ADM	Attention Diagnostic Method
AE	Adverse Events
AICC	small-sample Akaike's Information Criterion
Ala	Alanine
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ANT	Amsterdam Neurological Tasks
ApoA	Apolipoprotein A
ApoB	Apolipoprotein B
ARA	Arachidonic acid
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
AST	Aspartate transaminase
AwPKU	Adults with Phenylketonuria
BBB	Blood Brain Barrier
BCAA	Branched Chain Amino Acids
BH_2	Dihydrobiopterin
BH_4	Tetrahydrobiopterin
BMD	Bone Mineral Density
BMI	Body Mass Index
BNT	Boston Naming Test
BS	Baseline Speed

BUN	Blood Urea Nitrogen
CANTAB	Cambridge Neuropsychological Test Automated Battery
CBC	Complete Blood Count
CCK	Cholecystokinin
CGMP	Casein Glycomacropeptide
CHO	Carbohydrate
Cit	Citrulline
CoL	Course of Lie
COWAT	Controlled Oral Word Association Test
CRP	C-reactive protein
CRT	Choice Reaction Time
CPT	Conners' Continuous Performance Task
CTEQ	Cognitive Test Evaluation Questionnaire
CTIMP	Clinical Trial for the Investigation of a Medicinal Product
CVLT	California Verbal Learning Test
Cys	Cysteine
DBS	Dried Blood Spot
DG	Dentate Gyrus
DHA	Docosahexaenoic Acid
D-KEFS	Delis-Kaplan Executive Function System
DPE	Dot Pattern Exercise
DSST	Digit Symbol (Substitution) Task
DV	Digit Vigilance
EAA	Essential Amino Acid
ECT	Early and Continuously Treated
EF	Executive Function(s)
EPMT	Elithorn's Perceptual Maze Test
ET	Early Treated
(E)FA	(Essential) Fatty Acids
EPA	Eicosapentaenoic Acid
FAA	Free Amino Acid
FL	Flanker

FI	Feature Integration task
FPT	Faux-Pas Recognition Test
FR	Face Recognition task
fMRI	functional Magnetic Resonance Imaging
FSIQ	Full Scale Intelligence Quotient
FSME	Finger Motor Speed Exercise
FSMP	Food for Special Medical Purposes
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transferase
Glu	Glutamate
Gln	Glutamine
GLP-1	Glucagon-like peptide-1
Gly	Glycine
GMP	Glycomacropeptide
HARU	Human Appetite Research Unit
HC	Healthy controls
HDL-C	High-Density Lipoprotein Cholesterol
His	Histidine
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HPA	Hyperphenylalaninaemia
HRQoL	Health Related Quality of Life
IBD	Inflammatory Bowel Disease
IDC	Index of Dietary Control
IFE	Identification of Facial Emotions test
IMD	Inherited Metabolic Disorder
Iso	Isoleucine
IQ	Intelligence Quotient
LAT-1	L-type Amino Acid Transporter
LCPUFA	Long-Chain Polyunsaturated Fatty Acids
LD	Late Diagnosed
LDL-C	Low-Density Lipoprotein Cholesterol
Leu	Leucine

LNAA	Large Neutral Amino Acids
LPE	Letter Pattern Exercise
LTHT	Leeds Teaching Hospitals NHS Foundation Trust
Lys	Lysine
MANOVA	Multivariate Analysis Of Variance
MAQ	Medication Adherence Questionnaire
Met	Methionine
MF	Medical Food
MLS	Motorische Leistungsserie
MMA	Methylmalonic Acid
MMAS-8	8-item Morisky Medication Adherence Survey
MOT	Motor Screening Test
MR	Magnetic Resonance
MS2D	Memory Search 2-Dimensions task
msec	Milliseconds
MQ	Motor development index
n3	Omega-3 fatty acids
n6	Omega-6 fatty acids
Na^+K^+-ATPase	Sodium-Potassium Adenosine Triphosphatase
$NADP^+$	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate – reduced form
NHS	National Health Service
NRS	Non-Randomized controlled Study
NWM	Numeric Working Memory
Orn	Ornitine
P&P	Pen and Paper
PAH	Phenylalanine Hydroxylase
PAL	Phenylalanine Ammonia Lyase
PEG-PAL	Pegylated Recombinant Phenylalanine Ammonia Lyase
PFC	Prefrontal Cortex
Phe	Phenylalanine
Phe:Tyr	ratio between levels of Phenylalanine and Tyrosine

PIQ	Performance Intelligence Quotient
PKU	Phenylketonuria
PKU-QoLQ	PKU specific Quality of Life Questionnaire
PLC	Profile of Quality of Life in the Chronically Ill
PU	Pursuit
PO4	Alkaline Phosphatase
POI	Perceptual Organization Index
PPVT(-R)	Peabody Picture Vocabulary Test(-Revised)
PPY	Peptide Tyrosine-Tyrosine
PRAL	Potential Renal Acid Load
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
Pro	Proline
PSI	Processing Speed Index
PTH	Parathyroid Hormone
PU	Pursuit
QATSDD	Quality Assessment Tool for Reviewing Studies with Diverse Design
QoL	Quality of Life
R&D	Research and Development
RAVLT	Rey Auditory Verbal Learning test
RBC	Red Blood Cells
RCT	Randomised Controlled Trial
RDI	Recommended Daily Intake
REC	Research Ethics Committee
RME	Reading the Mind in the Eyes test
ROCFT	Rey Österrieth Complex Figure Test
ROS	Rostock-Osretzky Scale
RT	Reaction Time
RVP	Rapid Visual Information Processing
SA	Sustained Attention
SAD	Sustained Attention-Dots
Ser	Serine
SF-36	Medical Outcome Survey 36 item Short Form

SIDM	Society for Inherited Metabolic Disorders
SOC	Stocking of Cambridge
SOPT	Self-Ordered Pointing Test
SRFT	Saford Royal Foundation NHS Trust
SRT	Simple Reaction Time
SSP	Spatial Span
SST	Stop Signal Task
SSV	Shifting Attentional Set-Visual
SVAT	Sonneville Visual Attention Tasks (Precursor ANT)
SWM	Spatial Working Memory
TAAQoL	TNO-AZL Adult Quality of Life
Tau	Taurine
TEA	Test of Everyday Attention
TC	Total Cholesterol
(t)Hcy	(total) Homocysteine
TIQ	Total Intelligence Quotient
TGC	Triglycerides
TMFO	Trimethylamine N-Oxide
TMT	Trail Making Test
TMT-A	Trail Making Test part A
TMT-B	Trail Making Test part B
TOH	Tower of Hanoi
ToL	Tower of London
TR	Tracking
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine
VAS	Visual Analogue Scale
VIQ	Verbal Intelligence Quotient
VSS	Visuo-Spatial Sequencing task
WAIS(-R)	Wechsler Adult Intelligence Scale(–Revised)
WASI	Wechsler Adult Scale of Intelligence Scale

WCST	Wisconsin Card Sorting Test
WCST(-PR)	Wisconsin Card Sorting Test(-*Perseverative Responses)*
WHO	World Health Organisation
WMS	Wechsler Memory Scale
WT	Wild Type
ZVT	Zahlen-Verbindungs-Test

Chapter 1 Introduction to phenylketonuria (PKU)

1.1 Aetiology and classification of PKU

Phenylketonuria (PKU) is a rare (on average 1 in 10,000-12,000 live births in Western Europe) inherited metabolic disorder (IMD). IMDs are defined as *"genetically inherited biochemical disorders of specific enzymes or proteins causing a block in a normal metabolic process of protein, carbohydrate (CHO) or fat metabolism"* (Shaw, 2014; p. 385). Mutations in genes coding for these specific enzymes or proteins result in either an absent or abnormal protein or enzyme. Different mutations can have different outcomes in relation to severity of the defect. PKU is the result of mutations in the encoding gene of the hepatic enzyme phenylalanine hydroxylase (PAH) and is characterised by altered PAH activity (Blau, van Spronsen, & Levy, 2010). PAH, together with its cofactor tetrahydrobiopterin (BH4), is responsible for the conversion of the essential amino acid (EAA) phenylalanine (Phe) to tyrosine (Tyr; see Figure 1.1). To date, 1,072 different PAH variants have been identified (Blau, Yue, & Perez, 2015; last accessed 22 September 2018). The most common type of mutations observed in PKU are missense mutations (~60%), usually resulting in protein misfolding and/or impairment of catalytic functions (Shaw, 2015). PKU is an autosomal recessive IMD, meaning that both copies of the PAH-gene have to be mutated for the disorder to be present.

As a result of the large number of PAH variants, there is substantial variation in residual PAH activity and, therefore, the Phe tolerance among individuals with PKU. Depending on which specific mutations (genotype) an individual with PKU has, PAH is either completely inactive, or its activity is reduced. Deficient PAH activity results in an accumulation of Phe in the blood (hyperphenylalaninaemia; HPA) and brain, resulting in increased Phe levels, decreased Tyr levels and a disturbed Phe:Tyr ratio.

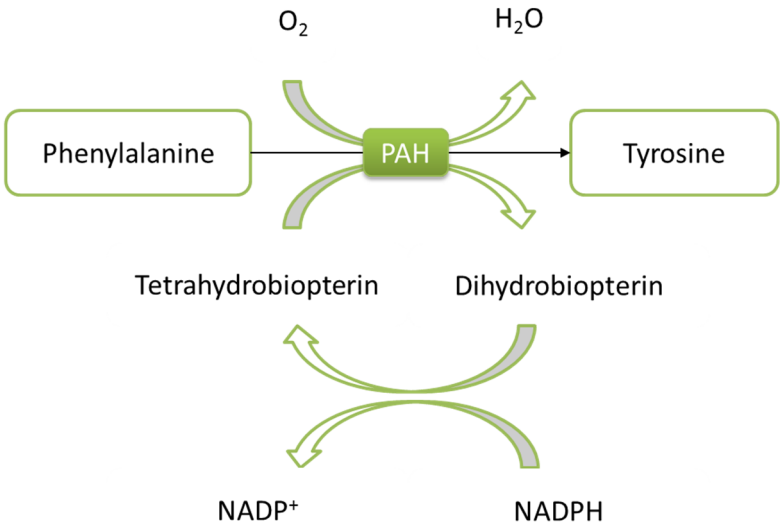


Figure 1.1 Metabolism of phenylalanine (Phe): Phenylalanine hydroxylase (PAH), together with its cofactor tetrahydrobiopterin (BH4), is responsible for the conversion of Phe into tyrosine (Tyr). In phenylketonuria (PKU), partial or complete inactivity of PAH leads to an accumulation of Phe, decreased Tyr levels and an altered Phe:Tyr ratio. During the conversion, BH4 undergoes oxidation to dihydrobiopterin (BH2). Using reductive electrons from the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH, which gets converted to nicotinamide adenine dinucleotide phosphate (NADP+) in the process), BH2 is recycled back to BH4.

Until recently, different phenotypes of PKU were typically classified into classical or severe PKU, moderate PKU, and mild PKU (Shaw, 2015). Classification into these different phenotypes is commonly based on the highest untreated Phe level as well as the Phe tolerance (PAH activity) of individuals with the disorder (see Table 1.1). As a reference, the Phe level in individuals without PKU is usually between 55 and 110 µmol/L (Blau, van Spronsen, et al., 2010).

There is a good correlation between PKU genotype and phenotype: affected individuals with more severe mutations will present with higher untreated Phe levels than those with milder mutations (Shaw, 2015). However, because treatment is initiated as early as possible, patients may not reach their peak untreated Phe levels. Therefore, the new European guidelines for the management of PKU state that patients should be classified based on whether treatment is necessary or not (van Spronsen et al., 2017).

Table 1.1 Traditional classification(s) of PKU phenotypes using the highest untreated blood Phe level and Phe tolerance

PKU phenotype[1] (Blau et al., 2010)	Untreated blood Phe (µmol/L)	PKU phenotype[1] (Shaw et al., 2014)	Untreated blood Phe (µmol/L)	Phe tolerance[2] (mg/day)
Classical/Severe PKU	>1200	Classical/Severe PKU	>1200	≤250
Moderate PKU	900-1200	Moderate PKU	600-1200	200-700
Mild PKU	600-900			
Mild HPA	120-600	Mild PKU	120-600	n/a

Notes: [1] terminology used differs slightly within the literature; [2] to maintain levels <360 µmol/L
Key: HPA: hyperphenylalaninaemia

Untreated PKU is characterised by severe and irreversible intellectual disability and neurological impairments, including microcephaly, developmental problems, behavioural difficulties (e.g. autistic behaviour, self-mutilation, auto-aggression), movement disorders (e.g. spastic paresis, ataxia), motor deficits (e.g. tremors), and epilepsy/seizures (Blau, van Spronsen, et al., 2010; Hoeks, den Heijer, & Janssen, 2009; Saudubray, Baumgartner, & Walter, 2016; van Wegberg et al., 2017). As a consequence, undiagnosed individuals with PKU often require lifelong institutional care.

1.2 Pathogenesis and pathophysiology of PKU

There is some debate regarding the specific mechanism(s) responsible for the neurological impairments observed in PKU, but the general belief is that these deficits are the result of elevated Phe levels (Blau, van Spronsen, et al., 2010; van Spronsen et al., 2017). Two theories on the mechanism of action of the disturbed Phe metabolism in PKU have been developed (Figure 1.2) (de Groot, Hoeksma, Blau, Reijngoud, & van Spronsen, 2010).

The first theory suggests that, because Phe competes with other Large Neutral Amino Acids (LNAA; e.g. Tryptophan (Trp) and Tyr) for transport across the blood-brain barrier (BBB), high levels of Phe saturate the LNAA-transporters, limiting the uptake of other LNAA into the brain. Hence, PKU patients often present with lower brain concentrations of other LNAA such as Trp and Tyr. This is thought to interfere with cerebral protein

synthesis and to inhibit synthesis of the neurotransmitters serotonin, dopamine, and norepinephrine (Burlina et al., 2000; Butler, O'Flynn, Seifert, & Howell, 1981; Feillet et al., 2010; Surtees & Blau, 2000), known to be involved in cognitive functioning (Antenor-Dorsey et al., 2013). Furthermore, it has been suggested that high brain Phe concentrations cause neurotoxicity. More specifically, Phe is believed to inhibit Tyr hydroxylase, responsible for the conversion of Tyr to dopamine, as well as Trp hydroxylase, involved in the production of melatonin. In addition, research has demonstrated that Phe reduces glutamatergic synaptic transmission (Martynyuk et al., 2005), the activity of pyruvate kinase, involved in cerebral glycolysis (Wasserstein, Snyderman, Sansaricq, & Buchsbaum, 2006), and the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), involved in the synthesis of cholesterol (Shefer et al., 2000). Finally, Phe has been found to increase myelin turnover, resulting in white-matter lesions, which may contribute to the neurological issues observed in PKU (Feillet et al., 2010; Surtees & Blau, 2000).

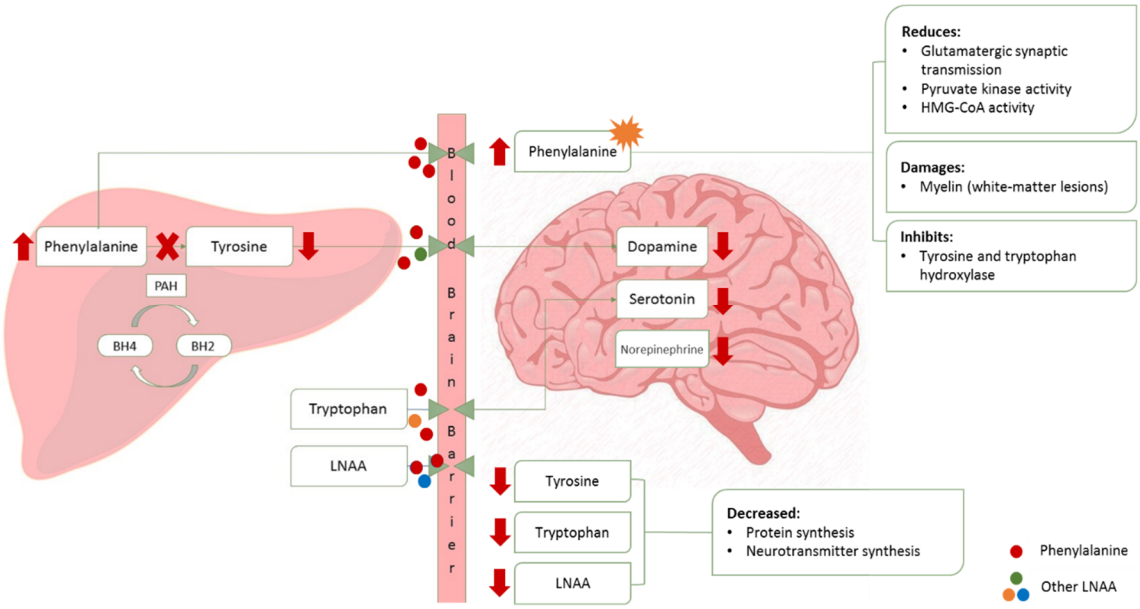


Figure 1.2 Simplified metabolism of phenylalanine (Phe) in the liver and competition with tyrosine (Tyr) and tryptophan (Trp) and other Large Neutral Amino Acids (LNAA) for L-type amino acid transporters (LAT-1; ▶◀) at the blood brain barrier (BBB). Decreased or no activity of the liver enzyme phenylalanine hydroxylase (PAH) leads to an increase of Phe in the blood and saturation of BBB transporters by Phe. Increased Phe brain levels can result in neurotoxicity. Furthermore decreased brain levels of other LNAA (e.g. Tyr, Trp) leads to decreased protein and neurotransmitter synthesis.

Key: BH2: dihydrobiopterin; BH4: tetrahydrobiopterin; HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A; LNAA: Large Neutral Amino Acids

1.3 Brief history of PKU: discovery, diagnosis and first treatment

Three key scientific findings/developments have improved the understanding of PKU in such a way that it can now be detected and treated early. Asbjørn Følling first described PKU in 1934 (Følling, 1934a, 1934b). He unexpectedly detected phenyl ketone bodies (phenyl pyruvic acid) in the urine of two children with intellectual disabilities and hypothesised that the two were related. He further hypothesised that these phenyl ketone bodies were caused by an inability to metabolize Phe. He later successfully identified raised levels of Phe in blood (HPA) as the underlying cause of the neurological deficits observed in the children (Christ, 2003). In the 1950s, Horst Bickel and colleagues introduced the first dietary treatment for PKU: a diet low in Phe (Bickel, Gerrard, & Hickmans, 1953; Bickel, Gerrard, & Hickmans, 1954). They reported a decrease in Phe levels and improvement in the behaviour of a child that was diagnosed with PKU upon initiation of the diet. Finally, Robert Guthrie introduced a diagnostic test suitable for mass screening for HPA (the Guthrie test or heel prick test) in the 1960s (Guthrie & Susi, 1963). This led to the introduction of neonatal screening for PKU.

Nowadays, many countries around the world include a test for PKU in neonatal screening programmes (i.e. the Guthrie test or more modern tests based on tandem mass spectrometry). Early diagnosis and prompt intervention (upon diagnosis) allows individuals with PKU to avoid severe neurological impairments (see 1.4.2).

1.4 Management of PKU

The management of PKU is aimed at (1) maintaining blood Phe levels within safe limits, (2) ensuring normal growth and development, (3) preventing severe neurological impairments and mental retardation and (4) achieving *'optimal cognitive and psychosocial development and wellbeing of the individual with PKU during his/her entire life'* (van Spronsen & Burgard 2008, p673; Giovannini, Verduci, Salvatici, Paci, & Riva, 2012; MacDonald 2000).

As a result of increasing knowledge of PKU and its impact on the development and functioning of those affected, guidelines with respect to target Phe levels and duration of the management of PKU have changed considerably since the introduction of the diet in the 1950s.

In Europe, guidelines for the management of PKU (target Phe levels) tend to vary between, and sometimes even within, countries (Ahring et al., 2009; Blau, Bélanger-Quintana, et al., 2010; Demirkol, Giżewska, Giovannini, & Walter, 2011; Schweitzer-Krantz & Burgard, 2000). There is consensus between the guidelines and recommendations that treatment should start as early as possible (Demirkol et al., 2011). Treatment for life is still a relatively new recommendation (Cockburn et al., 1993), but is included in all current guidelines. Furthermore, there seems to be a consensus amongst guidelines with regards to target Phe levels in the first 10-12 years of life (between 120 and 360/480 µmol/L), but guidelines for adolescent and adult patients vary widely, and are often not evidence-based (van Spronsen & Burgard, 2008). For years, the management of PKU in the UK was aimed at keeping blood Phe levels between 120 and 700 µmol/L from age 13 years onwards (Griffiths, Ward, Harvie, & Cockburn, 1998). In contrast, until recently, adolescent and adult PKU patients were recommended to aim to maintain their Phe levels between 120 and 480 µmol/L in Portugal, between 120 and 600 µmol/L in the Netherlands, and between 120 and 900 (age 13-15 years) and 120 and 1200 (age 15+ years) µmol/L in France and Germany (Blau, van Spronsen, et al., 2010).

To harmonise the management of PKU across Europe, a group of European professionals set out to develop European guidelines for the management of PKU in 2012 (European Society for Phenylketonuria and Allied Disorders, 2012). These guidelines were published in 2017 and recommend that Phe levels should be between 120 and 360 µmol/L in treated PKU patients until 12 years of age and 120 and 600 µmol/L in PKU patients ≥12 years of age (van Spronsen et al., 2017; van Wegberg et al., 2017). In contrast, US and Australasian guidelines recommend Phe levels should stay between 120 and 360 µmol/L throughout life (Inwood et al., 2017; Vockley et al., 2014).

1.4.1 Dietary management of PKU

Despite the development and introduction of novel treatment strategies (e.g. LNAA, sapropterin dihydrochloride (Kuvan®) and phenylalanine ammonia lyase (PAL; Palynziq™); see Table 1.2), the conventional management of PKU consists of a low-protein diet, supplemented with protein substitutes free from (or very low in) Phe. The low-protein diet involves restriction of natural protein intake, based on individual Phe tolerance, and consumption of low-protein foods to meet appetite and energy requirements (MacLeod, Gleason, van Calcar, & Ney, 2009; van Spronsen et al., 2017). Concomitant supplementation with amino acids (AA; other than Phe) is used to meet the age-related protein requirements (World Health Organisation, 2007) of each individual patient. In most individuals with classical PKU, protein substitutes provide approximately 52-80% of their daily protein intake (van Wegberg et al., 2017).

In practice, the reduction of natural protein intake in the management of PKU means that most patients avoid meat, poultry, fish, nuts and dairy foods. These high-protein foods are also sources of several essential vitamins, minerals and trace minerals such as omega 3 and 6 fatty acids, vitamin B12, zinc and selenium. Therefore, to ensure patients receive a nutritionally complete diet, vitamins, minerals and trace minerals are added to the protein substitutes (MacDonald, Rocha, van Rijn, & Feillet, 2011; MacDonald, 2000; MacLeod & Ney, 2010). Dietary management is periodically assessed and changed (if necessary) to maintain adequate nutrition for physical development (MacLeod et al., 2009; van Spronsen et al., 2017; van Wegberg et al., 2017).

Table 1.2 Overview of alternative treatments for the management of PKU (Bélanger-Quintana, Burlina, Harding, & Muntau, 2011; Giovannini et al., 2012)

Treatment	Description/mechanism of action	Notes
Large Neutral Amino Acids (LNAA)	- Reduction in brain Phe; - Reduction in blood (& consequently brain) Phe; - Increase in brain EAA; - Increase in brain neurotransmitters; - Improvement in blood AA profiles (increased levels of Tyr and Trp).	- Used in combination with a (sometimes relaxed) low-protein diet
Phenylalanine ammonia lyase (PAL; Palynziq™)	- Plant-derived enzyme - Breaks down Phe (without producing Tyr) without use of a co-factor	- Pegylated PAL (PEG-PAL; injections) have been approved for use in the management of adults with PKU (AwPKU)
Sapropterin dihydrochloride (Kuvan®)	- Synthetic form of tetrahydrobiopterin (BH4); - increases PAH activity → increase in Phe tolerance	- Only effective in BH4-sensitive PKU phenotypes: >30% reduction of blood Phe observed in approximately 20-60% of patients - Often prescribed in combination with a (relaxed) PKU diet - Minority of responders able to discontinue dietary treatment completely

1.4.2 Outcomes of dietary management in PKU

1.4.2.1 Quality of life (QoL) and wellbeing

Due to the complex and restrictive nature of the PKU diet, it has been suggested that long-term adherence to the dietary management of PKU may negatively affect quality of life (QoL) and wellbeing of patients and their families (Enns et al., 2010; Medford, Hare, Carpenter, et al., 2017). However, research has found that children and adolescents with PKU, as well as their parents, have a QoL that is comparable to that of healthy children, adolescents and their parents, respectively (Landolt, Nuoffer, Steinmann, & Superti-Furga, 2002; ten Hoedt, Maurice-Stam, et al., 2011). In spite of this, Landolt et al. (2002) reported less positive emotions in children with PKU, compared to healthy controls. In addition, compared to parents with healthy children,

parents of the children with PKU reported their children to be less joyful, happy, and confident (Landolt et al., 2002). Furthermore, despite research reporting comparable QoL in parents of children with PKU and parents of healthy children, a recent study reported that 59% of 46 interviewed maternal caregivers of children with PKU had clinical levels of psychological distress (Medford, Hare, Carpenter, et al., 2017). Moreover, it has been suggested that family stress and perceived social support are powerful predictors of parental QoL (Arafa, Zaher, El-Dowaty, & Moneeb, 2008).

Limited research in PKU has assessed health related quality of life (HRQoL) in adults with PKU (AwPKU; see Table 1.3). HRQoL is defined as *"is a broad concept which can be defined as the patient's subjective perception of the impact of his disease and its treatment(s) on his daily life, physical, psychological and social functioning and well-being"* (Committee for Medicinal Products for Human Use, 2005, p.3). With the exception of HRQoL related to cognitive functioning (Bosch et al., 2007; Demirdas et al., 2013; Huijbregts et al., 2018), none of the studies have reported any differences in (generic) HRQoL between AwPKU and controls or data from a reference population (Bosch et al., 2007, 2015; Cotugno et al., 2011; Demirdas et al., 2013; Huijbregts et al., 2018; Simon et al., 2008). However, adolescent and adult PKU patients often demonstrate increased internalizing disorders such as depressive mood, anxiety, low self-esteem, social withdrawal and decreased positive emotions (Brumm, Bilder, & Waisbren, 2010; Gentile, ten Hoedt, & Bosch, 2010; Huijbregts et al., 2018; Lou Smith et al., 2000). These psychiatric symptoms could influence social behaviour and QoL for individuals with PKU (Simon et al., 2008). Investigators from the German Collaborative Study identified depressive symptoms as the most frequently described psychiatric disturbance associated with PKU in adults, and they appear to be more prevalent in female than male AwPKU (Burgard, Armbruster, Schmidt, & Rupp, 1994; Pietz, Fatkenheuer, Armbruster, Esser, & Schmidt, 1997). Anxiety-related disorders were found to be the second most frequently reported psychiatric complication in AwPKU (Pietz et al., 1997) Furthermore, mental health problems were more frequently observed in off-diet than on-diet patients (41% vs. 22%) (Koch et al., 2002).

Table 1.3 Summary of studies assessing Quality of Life (QoL) in AwPKU

Author (year)	Country	Study sample	Study design	Assessments	Results	Conclusions
Bosch et al. (2007)	the Netherlands	31 ECT AwPKU 22 female, 11 male Mean age 24.6 (±3.6) years (range 18-30) 501 controls no characteristics of comparison group reported	Cross-sectional	Course of Life (CoL) questionnaire <u>Health related Quality of Life (HRQoL)</u>: RAND-36 Health Survey Cognitive scale of the TNO-AZL Adult Quality of Life (TAAQoL) questionnaire	- HRQoL of ECT AwPKU was comparable to that of controls, although there was a trend towards a lower score on the cognitive scale of the TAAQoL - higher percentage of PKU received special education in primary school - educational attainment was comparable for both groups	Although PKU is a chronic disorder with the burden of strict dietary control, ECT AwPKU can have a normal HRQoL and CoL
Bosch et al. (2015)	France, Germany, Italy, the Netherlands, Spain, Turkey and the UK	559 ET PKU patients 104 ET AwPKU (n=67 classical PKU) 66 female, 38 male Mean age 25.8 (±6.6) years (range 18-45) n=15 treated with BH4 *Adult QoL scores were compared to scores obtained in the general US population*.	Prospective cross-sectional	<u>QoL (adults):</u> Medical Outcome Survey 36 item Short Form (SF-36) PKU specific Quality of Life Questionnaire (PKU-QoLQ) – adult version	<u>HRQoL:</u> - mean domain scores of generic HRQoL measures were comparable to the general population <u>PKU-QoLQ:</u> - perceived negative impact of PKU was primarily reported with regards to emotional impact, anxiety about blood Phe levels (in general and during pregnancy) and guilt regarding poor adherence - patients with mild/moderate PKU and those treated with BH4 reported lower emotional and practical impact of the dietary management of PKU	Results support the ability of the PKU-QoLQ to identify HRQoL issues characteristic of the PKU population, and to measure HRQoL differences specific to severity of disease and treatment received.

Author (year)	Country	Study sample	Study design	Assessments	Results	Conclusions
Cazzorla et al. (2014)	Italy	43 PKU patients (n=21 classical PKU, n=22 mild PKU, respondent to BH4) 17 AwPKU (7 on BH4)	Cross-sectional	<u>QoL (adults):</u> - WHOQOL questionnaire-100 (WHOQOL-100) - Predictors of QoL (e.g. age, sex, educational level and employment status) <u>Depression + anxiety:</u> - Beck Depression Inventory (BDI) - State-Trait Anxiety Inventory form Y (STAI-Y)	- QoL scores were significantly lower in patients with classical (compared to mild) PKU, males, in patients with lower education and in those employed or unemployed (as compared to students) - irrespective of treatment (diet or BH4), QoL was better for those who had been adherent to their treatment for a longer period of time at the time of assessment - BDI and STAI-Y scores were within normal ranges	Some specific categories of adult patients (males, those less educated and non-students) reported a significantly lower QoL score and should be investigated more in depth, targeting categories patients with potential low treatment compliance.
Cotugno et al. (2011)	Italy	41 ET PKU (n=30 classical PKU, n=11 mild PKU) 16 female, 25 male Mean age 10.6 (±0.5) years (range 3-24) Adult QoL scores were compared to those of an Italian reference population (aged 18-24 years).	Cross-sectional	3-day food record (Phe intake) Adherence (Phe intake-prescribed Phe) Concurrent blood Phe QoL; for adults: SF-36	- n=23 (56.1%) ET PKU were found to be adherent, possibly associated with mother's educational level (higher education ⇨ better adherence) - metabolic control (blood Phe) only achieved by <50% of ET PKU (when compared to standards of UK, France and Germany) - no association observed between age and adherence - lower than normal QoL observed in ET PKU patients <18 years old - no significant differences between QoL in ET AwPKU and the reference population observed	- Adherence to dietary prescriptions does not necessarily correspond to suggested metabolic control - QoL is reduced in ET children and adolescents, but not adults with PKU*. **different instrument used to measure QoL in ET AwPKU*

Author (year)	Country	Study sample	Study design	Assessments	Results	Conclusions
Demirdas et al. (2013)	the Netherlands	69 ET PKU patients 30 ET AwPKU female, male Mean age 28.5 (±6.1) years Adult HRQoL scores were compared to norm data from the general Dutch population (aged ≥16 years)	Intervention with BH4 only baseline results summarised here	HRQoL: - generic: TAAQoL - chronically ill: modified form of the DISABKIDS chronic generic module	- HRQoL of ET AwPKU comparable to that of the general population, except for a significantly lower HRQoL with respect to cognitive functioning	Lower HRQoL in relation to cognitive functioning in ET AwPKU could potentially be..: - due to lower mean IQ or deficits in EF and sustained attention, observed in previous studies - a reflection of worries about their cognitive functioning
Huijbregts et al. (2018)	the Netherlands	90 ET PKU 58 ET AwPKU 36 female, 22 male Mean age 27.2 (±6.8) years (range 16.5-40.0) 109 controls	Cross-sectional	HRQoL: TAAQoL Metabolic control: historical and concurrent blood Phe	- poorer age-controlled HRQoL was found for the domains cognition, depressive moods, and anger, with a further trend for the domain "pain" - poorer functioning (most notably in the domains cognition, sleep, pain, sexuality and anger) was associated with higher historical and concurrent Phe-levels	This study revealed poorer HRQoL especially for AwPKU compared to controls and suggest HRQoL in adulthood may be negatively associated with metabolic control.
Simon et al. (2008)	Germany	67 ET AwPKU 44 female, 23 male Median age 25 years (range 17-38) QoL scores were compared to those of a German reference	Cross-sectional	QoL and social status: - Profile of Quality of Life in the Chronically Ill (PLC) – measure of physical, psychological and social capacity of performance and well-being.	- no significant differences observed in QoL of ET AwPKU and the reference population - tendency towards lower/delayed autonomy and low rate of forming normal adult relationships and having children	- It is undeniable that PKU places a burden on a high number of patients - 'Normal' QoL in ET AwPKU shows a healthy emotional adjustment is possible when PKU

Author (year)	Country	Study sample	Study design	Assessments	Results	Conclusions
		population (aged 14-92 years).		- Frequency of symptoms observed in PKU (e.g. tremors).	- ET AwPKU >25 years reported more PKU specific neurological symptoms than younger ET AwPKU (n.s.)	is diagnosed early and treated well

Key: CoL: Course of Life; ECT AwPKU: Early and continuously treated adults with phenylketonuria; HRQoL: Health Related Quality of Life; PLC: Profile of Quality of Life in the Chronically Ill; QoL: Quality of Life; SF-36: Medical Outcome Survey 36 item Short Form; TAAQoL: TNO-AZL Adult Quality of Life

1.4.2.2 Cognitive functioning

With early treatment, severe neurological impairments are prevented (Blau, van Spronsen, et al., 2010). Nonetheless, deficits in cognitive functioning in PKU patients are still observed. In childhood, deficits are mainly observed in executive functions (EF), such as working memory and reasoning/planning, attention, and processing speed (Albrecht, Garbade, & Burgard, 2009; DeRoche & Welsh, 2008). In adults, similar deficits have been reported (Bilder et al., 2016). However, the majority of research has focussed on these specific cognitive domains, whereas other cognitive functions have received less attention. There is a lack of a comprehensive overview of cognitive functioning across different cognitive domains in early treated (ET) AwPKU assessing the effectiveness of conventional treatment strategies in achieving optimal cognitive functioning (Palermo et al., 2017). Chapter 3 provides a systematic review of cognitive functioning across different cognitive domains in early treated adults with PKU (ET AwPKU).

1.4.3 Adherence to dietary management

The overall management of PKU is complex, not only requiring adherence to the low-protein diet and protein substitute intake, but also regular collection of blood samples, recording of food intake and regular visits to the metabolic clinic (MacDonald, Van Rijn, Gokmen-Ozel, & Burgard, 2010). Adherence to the diet is especially important during the early childhood years since cognitive outcomes have reported to be closely related to the control of blood Phe levels in this period of life (Huijbregts, 2002; Waisbren, Mahon, Schnell, & Levy, 1987), and should be maintained through adulthood to protect from neurological dysfunction (Bélanger-Quintana et al., 2011; Gassió et al., 2003; Moyle, Fox, Arthur, Bynevelt, & Burnett, 2007). However, the strict low-protein diet and protein substitutes impose a burden on patients and their families and has been associated with dietary non-adherence, especially in adolescents and young adults (Bilginsoy, Waitzman, Leonard, & Ernst, 2005; Gassió et al., 2003; Mütze et al., 2011; Prince, McMurray, & Buist, 1997; Walter et al., 2002).

Various metabolic centres have reported increased loss to follow-up and decreased adherence to dietary recommendations when patients grow older (Crone et al., 2005;

Van Spronsen & Burgard, 2008; Walter et al., 2002) and multiple studies have observed a progressive reduction in metabolic control with age amongst children and adolescents with PKU (Medford, Hare, & Wittkowski, 2017). Moreover, many adolescent and adult PKU patients are reluctant to adhere to their protein substitutes and to maintain a strict low-protein diet once they reach adulthood (Cazzorla et al., 2018; Schulz & Bremer, 1995). In a recent survey of 111 AwPKU, only 42% reported to be strictly following the low-protein diet with protein substitutes (Cazzorla et al., 2018).

1.4.3.1 'Semi-adherent' early treated adults with PKU (ET AwPKU)

Patients who relax their diet are often either wary of, or unused to, naturally protein-rich foods, especially those of animal origin. As a result they tend to follow a self-selected, unmeasured, un-supplemented low-protein diet which may be vegan or vegetarian and limited in food variety (Feillet & Agostoni, 2010; Van Spronsen & Burgard, 2008). Adherence to the low-protein diet and protein substitutes is essential to ensure adequate protein and micronutrient status (Montoya Parra, Singh, Cetinyurek-Yavuz, Kuhn, & MacDonald, 2018). Therefore, such dietary practice may put patients at a significant nutritional risk (Bernstein et al., 2014; MacDonald et al., 2011). At present there has been no systematic study of adult PKU patients following such dietary practices (referred to as 'semi-adherent' PKU patients throughout this thesis) with respect to nutritional status, QoL, and cognitive functioning. Furthermore, previous studies assessing cognitive functioning and QoL of AwPKU either report on 'on-diet' (adherent) PKU patients or 'off-diet' PKU patients (e.g. Burgard, Rey, Rupp, Abadie, & Rey, 1997; Moyle, Fox, Bynevelt, Arthur, & Burnett, 2007), or a mixture of both (e.g. Brumm et al., 2004; Palermo et al., 2017; Pietz et al., 1998; Ris, Williams, Hunt, Berry, & Leslie, 1994), but often do not specify whether these 'off-diet' patients follow a completely 'normal' diet or still follow a self-selected diet as described above. The metabolic team at the Mark Holland Metabolic Clinic at the Salford Royal Foundation NHS Trust (SRFT), however, estimate that about 50% of their adolescent and adult patients could be classed as 'semi-adherent' (personal communication with metabolic team at Salford Royal NHS Foundation Trust, 2015). Hence, semi-adherent ET AwPKU are an understudied group of PKU worthy of more research attention.

1.5 Aims of the thesis

Neurological impairments observed in untreated PKU are prevented with early treatment, but a comprehensive overview of cognitive functioning in ET AwPKU is currently lacking. Furthermore, due to the restrictive and complex nature of the dietary management of PKU, adherence to the PKU diet is poor in a large proportion of ET AwPKU. ET AwPKU with poor dietary adherence often follow a self-restricted diet without sufficient supplementation with prescribed protein substitutes. The (long-term) effects of suboptimal dietary practices on nutritional status, QoL/wellbeing and cognitive functioning of ET AwPKU are not well described in literature. Therefore, the studies reported in this thesis were designed to address the following aims:

1. To identify the demographic characteristics and PKU-related factors which predict adherence to dietary management in ET AwPKU (Study 1, Chapter 2).
2. To systematically review cognitive functioning across different cognitive domains in ET AwPKU and compare this to healthy controls (Systematic review, Chapter 3).
3. To assess cognitive function using appropriate tests of different cognitive domains in ET AwPKU compared to healthy controls (Study 2, Chapter 4).
4. To compare nutritional status, QoL/wellbeing and cognitive functioning in adherent and semi-adherent ET AwPKU (Study 3, part 1, Chapter 5).
5. To examine the adherence to and effect of 12 weeks dietary supplementation with a novel protein substitute on nutritional status, QoL/wellbeing and cognitive functioning in semi-adherent ET AwPKU (Study 3, part 2, Chapter 6).

Chapter 2 Adherence to dietary management in PKU (Study 1)

2.1 Introduction

2.1.1 Adherence to dietary management is not as simple as "on-diet" vs. "off-diet"

As introduced in Chapter 1, many adult patients struggle to adhere to the strict dietary management of PKU (Bilginsoy et al., 2005; Gassió et al., 2003; Mütze et al., 2011; Prince et al., 1997; Walter et al., 2002). Psychological research on ET AwPKU often refers to participants as being either "on-diet" or "off-diet". In addition, as it is believed that cognitive deficits are primarily caused by elevated levels of Phe (see Section 1.4.3), research in this field often includes concurrent, and sometimes also historical, blood Phe concentrations as a measure of dietary adherence. Adherence is defined as *"The extent to which the patient's behaviour matches agreed recommendations from the prescriber"* (Julius, Novitsky, & Dubin, 2009, p.34). In PKU, the dietary advice given by specialist dietitians and consultants is comprised of a specific amount of natural protein (referred to as "exchanges", determined by the patient's Phe tolerance) in combination with a specific amount of protein substitute (see Section 1.4.1). Whilst Phe levels give information about whether or not someone is adhering to individual dietary advice, they are not very informative about the exact dietary practices of those who have poor dietary control (i.e. those with Phe levels outside of target treatment ranges). Patients who are following a close to normal diet will have resulting high Phe levels, but they might also have sufficient dietary intake of other amino acids, vitamins and minerals. However, patients with poor dietary control commonly struggle to adhere to their protein substitutes but, at the same time, still avoid consumption of high protein foods, either by choice, out of habit or as a result of food neophobia. As a result, these patients (referred to as semi-adherent throughout this thesis) could be consuming a nutritionally incomplete diet. In this case, elevated blood Phe levels could be the result of both an elevated intake of dietary Phe as well as an inadequate total protein intake, resulting in muscle catabolism (i.e. the breakdown of muscle tissues, releasing protein into the blood stream). By avoiding high-protein foods, semi-adherent ET AwPKU are at risk of

developing nutritional deficiencies, with consequences for several aspects of their health (see also Chapter 5).

2.1.2 Factors influencing adherence to dietary management in PKU

Numerous contributors to poor adherence to the dietary management of PKU have been described, including restrictions imposed on social life (Bilginsoy et al., 2005; Cazzorla et al., 2018), time constraints and stress associated with food preparation and record-keeping (Bilginsoy et al., 2005). Other potential contributors include low educational achievement of caregivers and patients (Shulman, Fisch, Zempel, Gadish, & Chang, 1991), low family cohesion (e.g. the impact of divorce) (Olsson, Montgomery, & Alm, 2007; Shulman et al., 1991), parent–child conflicts (Bilginsoy et al., 2005; MacDonald, 2000), costs and accessibility of medical (low-protein) foods and protein substitutes (Bilginsoy et al., 2005; Finkelson, Bailey, & Waisbren, 2001), and language or cultural barriers between migrant families and health care providers (Ipsiroglu et al., 2005). In addition, lack of availability or satisfaction with professional support (Bilginsoy et al., 2005) and of a strong support network (Finkelson et al., 2001; Levy & Waisbren, 1994) have been associated with poor adherence to the PKU diet.

The monotonous and unpalatable nature of diet has been identified as another barrier to adherence in the management of PKU (Prince et al., 1997). Issues with the palatability of medical foods (Bilginsoy et al., 2005), and, especially, issues with the palatability, smell, taste, aftertaste, texture and ease of use of protein substitutes have been observed in patients of all ages (Cazzorla et al., 2018; Hoeks et al., 2009; MacDonald et al., 2004, 2010; MacDonald & Asplin, 2006). Even though the palatability, taste and variety of available low-protein foods and protein substitutes has greatly improved since the introduction of the dietary management of PKU (Giovannini et al., 2012; MacDonald et al., 2004), many patients still struggle (Cazzorla et al., 2018).

In addition, it has been suggested that it becomes harder to maintain the PKU diet when patients grow older as they apply fewer constraints and seek more variety in their meals (MacDonald, 2000). In adolescents and young adults, peer pressure, and the need to “fit in” discourages patients from complying (Bilginsoy et al., 2005; MacDonald, 2000).

Furthermore, the importance of both components of the PKU diet (i.e. the restriction of dietary protein intake and the supplementation with protein substitutes) as well as the negative effects of poor adherence are not always fully understood by patients and/or their caregivers (Cazzorla et al., 2018; Crone et al., 2005). However, several studies have not observed any differences in knowledge of PKU between patients with good or poor adherence (Gleason, Michals, Matalon, Langenberg, & Kamath, 1992; Vieira et al., 2015). Even when patients are aware of the issues related to low adherence, some still fail to adhere (Walter et al., 2002). Perceived value of the diet and attitudes towards the diet (Bilginsoy et al., 2005) or coping strategies (Medford, Hare, & Wittkowski, 2017) have been reported to influence patients' behaviour more than their knowledge about PKU.

In addition to barriers to adherence described in the literature, it is likely that past off-diet experience will increase the likelihood of difficulties in subsequent attempts to adhere to the dietary management of PKU. Not only is past behaviour predictive of future behaviour, and frequently overrides the influence of intention (Arafa et al., 2008), patients who are or have been "off-diet" will also have introduced and developed a liking for foods that are not compatible with a strict low-protein diet. As a consequence, the temptation to stray from the low-protein diet is likely to be greater than in those who have not been off-diet and, due to an altered taste appreciation, these individuals may also struggle more with the taste and aftertaste of the protein substitutes than adherent patients.

Most of the research related to predictors of dietary adherence in PKU focuses on children and adolescents, which is an important group to study as good adherence at a younger age has been associated with better adherence later on in life (Jahja, van Spronsen, et al., 2017). However, the majority of the (albeit limited) research on barriers to adherence to the PKU diet in (young) AwPKU is outdated. It is important to understand the barriers to continuous adherence to the dietary management of PKU as little is known about the effects of PKU on the health of ageing AwPKU and the extent to which dietary adherence could mitigate deleterious effects in older age. It is also important to update our understanding of barriers to adherence as eating behaviour changes in relation to social norms and opportunities and the availability, variety and palatability of low-protein foods and protein substitutes improves.

2.1.3 Assessment of adherence to dietary management in PKU

2.1.3.1 Metabolic control

As previously introduced (see section 2.1.1), blood Phe concentrations are most commonly used as a measure of dietary adherence in research in PKU, especially in research into cognitive functioning of individuals with PKU. When an individual with PKU adheres to both the low-protein diet and protein substitutes, their Phe levels are expected to be within target treatment ranges. In addition to limitations to the use of Phe as a measure of dietary adherence discussed above (see section 2.1.1), research has suggested that individuals with PKU often change adherence to their dietary management in the days leading up to a blood test, suggesting measured levels of Phe may underrepresent typical Phe levels (Bilginsoy et al., 2005; Weglage et al., 1992). Moreover, as a result of the large variation in Phe tolerance (PAH activity) between patients, some inviduals are able to deviate from their dietary management more than others, whilst still achieving blood Phe concentrations within target treatment ranges (MacDonald, 2000).

2.1.3.2 Other methods

In addition to metabolic control, other methods that are frequently used to assess adherence to the dietary management of PKU include dietary assessments, such as (weighed) food diaries, 24-hour dietary recall, and food frequency questionnaires (MacDonald, 2000). These methods rely on self-report, which has been shown to be an unreliable measure as external factors such as social desirability may lead to under or over reporting of intake of certain foods and / or protein substitutes. Similarly, product distribution and return count, another measure of adherence to the dietary management of PKU (MacDonald, 2000; Prince et al., 1997), could be influenced by factors such as social desirability. This method relies on patients returning medical foods they did not consume. However, individuals may have discarded products instead of returning them and thus, this method likely overestimates product consumption.

2.2 Aims of the study

Study 1 aimed to examine which demographic characteristics and PKU-related factors predict adherence to dietary management of ET AwPKU. The study focussed on adherence to protein substitutes in particular, as they tend to make up 52-80% of a patient's total protein intake (see also Section 1.4.1) and, therefore, are integral to the successful dietary management of PKU (MacDonald, 2000; van Wegberg et al., 2017).

2.3 Methods

2.3.1 Design

Study 1 was a cross-sectional, quantitative study using an online survey.

2.3.2 Participants

English, Dutch, German and Polish speaking AwPKU (n=158), aged 18 years or over were recruited online through social media (e.g. Facebook and Twitter) as well as via patient associations. Respondents were excluded if they were under 18 years of age (n=4), non-PKU (n=0), or if they did not fully complete the survey (n=53). A total of 101 AwPKU completed the survey (see Figure 2.1). Restricting the sample to those reporting to have been diagnosed via neonatal screening and reduced the sample size to 86 ET AwPKU. Further restriction of the sample to those treated by means of dietary management alone (i.e. excluding those on Kuvan® or participating in clinical trials for new therapeutic therapies like Palynziq™) resulted in a sample size of 71 (see Figure 2.1).

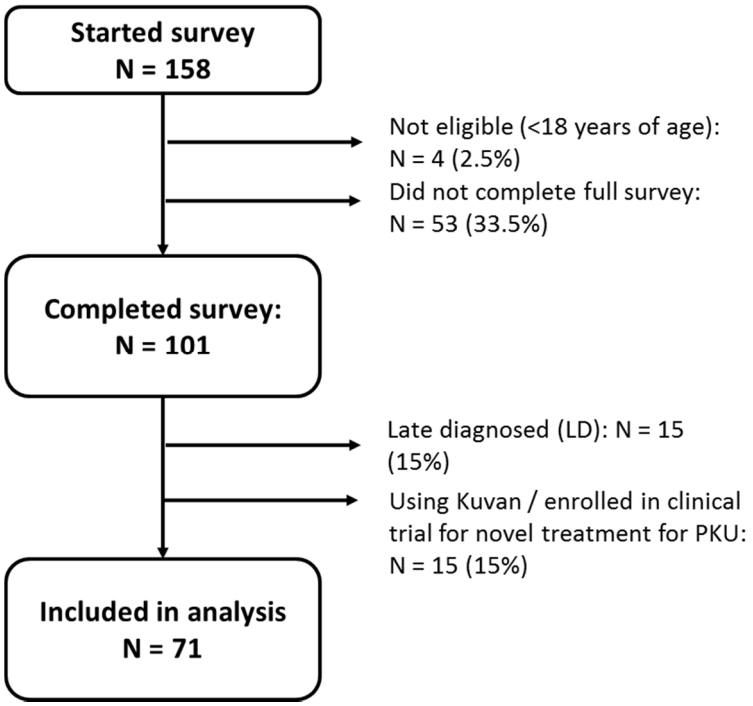


Figure 2.1 Flowchart of participant recruitment

2.3.3 Survey

The survey was administered online using Qualtrics© (Qualtrics, 2017) and was available in English, Dutch, German and Polish. It was comprised of several sections. The first section asked the respondents to provide demographic information (including age, gender, highest completed level of education and occupational status). The second section of the survey required participants to complete information with regards to their PKU (including time of diagnosis and classification of their PKU) and dietary habits (e.g. whether they were following a low-protein diet and/or taking protein substitutes and whether they had discontinued their diet in the past). The final section aimed to assess adherence to protein substitutes (see Appendix A for the full survey). Adherence to protein substitutes was assessed using an adapted version of the 8-item Morisky Medication Adherence Scale (MMAS-8; see Appendix A) (Morisky, Ang, Krousel-Wood, & Ward, 2008).

The first seven items on the MMAS-8 are Yes/No responses while the last item is a 5-point Likert scale response. In contrast to the 4-item Medication Adherence Questionnaire (MAQ) it was based on, this scale provides an insight into medication-taking behaviours, especially related to underuse, such as forgetfulness. Hence, barriers to adherence can be identified more clearly.

Respondents who indicated that they were not taking any protein substitutes were not asked to complete the MMAS-8. Respondents who completed the MMAS-8 obtained an adherence score ranging from 1-8, which reflected either low (1-5), medium (6-7) or high (8) adherence to protein substitutes (see Appendix A for details on scoring).

2.3.4 Ethical considerations

The study was approved by the University of Leeds' School of Psychology Research Ethics Committee (reference number: 15-0398; date: 07/01/2016) and undertaken in accordance with the ethical principles expressed in the Declaration of Helsinki (World Medical Association, 2013). An amendment to the study protocol (for the versions in Dutch, German and Polish) was approved on 06/02/2016 (reference number 16-0034).

Participants were informed about the nature and aims of the study (Appendix B) and were required to provide informed consent (Appendix C) prior to completing the survey. They were also required to create a unique participant ID (Appendix D) to allow data to be stored anonymously but linked so that their response would be identifiable in the event that they wanted to withdraw their responses.

2.3.5 Statistical analysis

Responses were extracted from Qualtrics© into Excel and checked for accuracy. SPSS Version 22 was used for data analysis. Participant characteristics, obtained via the demographic questions were compared using independent t-tests or Chi-squared tests as appropriate. Respondents were split into groups of low, medium or high adherence to their protein substitutes based on responses to the MMAS-8. Predictors of adherence to protein substitutes were explored via discriminant function analysis, which included those factors which best predicted group membership (low, medium, high adherence).

Three initial discriminant function models were performed to examine which variables were able to predict whether an individual was classified as low, medium, or high in terms of adherence to their protein substitutes on the MMAS-8. The ratio of cases to variables for discriminant function analysis (and other parametric analyses such as multiple regression and multivariate analysis of variance (MANOVA)) should be at least 10:1 (Tabachnick & Fidell, 2013). Thus all possible predictors could not be included in one discriminant function analysis and therefore separate analyses were performed. In the first model, predictors included were demographic characteristics of the respondents (i.e. age, gender, highest completed level of education and occupational status). In the second model, factors related to severity of the disorder (i.e. dietary allowance and classification of PKU), were included as predictors. Because not all respondents knew their specific PKU classification only 60 responses were included in this analysis. In the third model, rankings (1-8) of importance of attributes of the protein substitutes (i.e. taste, texture, aftertaste, convenience, smell, volume, appearance and variety) were included as predictors. A final discriminant function model was conducted, which included significant predictors ($p<.05$) and marginally significant predictors ($p<.10$) of group membership from the initial three models. In addition, 'having been off-diet in the past' was included as a predictor in the final model.

2.4 Results

2.4.1 Participant characteristics

Of the 71 ET AwPKU included in the data analysis, 60 reported that they were on-diet at the time of completion of the survey. Nineteen of these ET AwPKU had been off-diet in the past, whilst 41 reported continuous adherence to the low-protein diet. The self-reported dietary protein allowance (i.e. grams of natural protein allowed in the PKU diet, based on individual Phe tolerance) of respondents ranged from 3 to 35 grams of protein per day. The self-reported dietary protein intake ranged from 3 to 50 grams of protein per day. Table 2.1 provides an overview of participant characteristics of the 60 on-diet and 11 off-diet ET AwPKU included in the statistical analysis.

Table 2.1 Participant characteristics of "on-diet" and "off-diet" ET AwPKU included in the data analysis

	"on-diet" ET AwPKU	"off-diet" ET AwPKU	"on-diet" vs. "off-diet"
n	60	11	
Female (n (%))	39 (65)	4 (36)	$X^2(1)=3.19$, $p=.07$
Age (years)			$t(69)=-.48$, $p=.63$
mean (SD)	30.57 (7.73)	31.82 (8.90)	
range	19-47	20-48	
Classification (n (%))			$X^2(5)=3.01$, $p=.70$
Classical/Severe	46 (77)	9 (82)	
Moderate/Mild	5 (8)	1 (9)	
Mild HPA	1 (2)	n/a	
Unknown	8 (13)	1 (9)	
Previously been "off-diet" (n (%))	19 (32)	n/a	
Taking protein substitutes (n (%))	60 (100)	8 (73)	**$X^2(1)=17.09$, $p<.001$**
Occupational status (n (%))			$X^2(3)=7.08$, $p=.27$
employed	48 (80)	10 (91)	
unemployed	1 (2)	1 (9)	
housewife/homemaker	3 (5)	0 (0)	
student	8 (13)	0 (0)	
Educational level[1] (n (%))			$X^2(5)=3.02$, $p=.70$
1	2 (3)	0 (0)	
2	13 (22)	3 (27)	
3	15 (25)	3 (27)	
4	19 (32)	3 (27)	
5	10 (17)	1 (9)	
6	1 (2)	1 (9)	

[1] *Highest completed level of education ranging from 1 (primary education) to 6 (Doctoral degree)*

2.4.2 Adherence to protein substitutes

Of the 71 patients included in the data analysis, 68 reported that they were taking protein substitutes, despite 8 of these reporting that they were not following a low-protein diet. The 3 patients not taking protein substitutes reported that they were off-diet at the time of completing the survey. Two of the 68 ET AwPKU who were taking protein substitutes did not complete the MMAS-8 as they had initially indicated (within the survey) they were not taking protein substitutes. However, from their free text responses it was clear that they were regularly taking 'Phlexy 10 tablets' and 'PKU2 Secunda'.

Only 2 of the 66 ET AwPKU who completed the MMAS-8 questionnaire obtained the highest adherence score (8) on the MMAS-8. Therefore, these respondents were combined with those in the medium adherence group (n=23; MMAS-8 scores 6-7) for analysis. The remaining 41 respondents had low scores on adherence to their protein substitutes (MMAS-8 scores 1-5). Frequency of responses to questions on the MMAS-8 for the low adherence and medium/high adherence groups are shown in Table 2.2. All 8 ET AwPKU who reported being off-diet but still taking protein substitutes were in the low adherence group.

Thirty-three ET AwPKU reported missing their protein substitutes for reasons other than forgetting. The percentage of patients reporting to sometimes intentionally miss their protein substitutes was higher in the off than on-diet ET AwPKU (88% vs. 45%). When asked to give reasons for not taking their protein substitutes, 15 ET AwPKU (45%) reported that they did not like the taste and 14 (42%) said they felt embarrassed taking them. Additionally, 10 respondents (30%) said that preparing and/or taking the protein substitutes was too much effort, with one commenting *"usually I'm just too busy"*. Other reasons for missing protein substitutes included wanting to socially "fit-in" (n=5; 15%), stomach aches/acid reflux (n=4; 12%), not wanting to take them when feeling unwell (n=3; 9%), issues with access (e.g. finances/refill issues, n=2; 6%), high volume of protein substitutes prescribed (n=2; 6%), worries about caloric content (n=1; 3%), and acidity, aftertaste, and inconvenience (n=1; 3%). Finally, respondents commented that the main reasons for forgetting to take their protein substitutes were 1) forgetting to take their protein substitutes with them when leaving the house, but remembering having to take them later on, or 2) forgetting to take them because they were too busy.

Table 2.2 Frequency of responses to the MMAS-8 for "on-diet" and "off-diet" ET AwPKU

Question	"on-diet" ET AwPKU (n=58)		"off-diet" ET AwPKU (n=8)	
	Responses			
	Yes n (%)	No n (%)	Yes n (%)	No n (%)
Do you sometimes forget to take your dietary supplements[1]?	33 (57)	25 (43)	6 (75)	2 (25)
People sometimes miss taking their dietary supplements for reasons other than forgetting. Thinking over the past 2 weeks, were there any days when you did not take your dietary supplements?	26 (45)	32 (55)	7 (88)	1 (12)
Have you ever cut back or stopped taking your dietary supplements without telling your doctor?	12 (21)	46 (79)	3 (38)	5 (62)
When you travel or leave the house, do you sometimes forget to take your dietary supplements?	27 (47)	31 (53)	6 (75)	2 (25)
Did you take all your dietary supplements yesterday?	45 (76)	13 (24)	5 (62)	3 (38)
When you feel like your symptoms are under control, do you sometimes stop taking your dietary supplements?	4 (7)	54 (93)	2 (25)	6 (75)
Have you ever felt distressed for strictly following your dietary supplements?	16 (28)	42 (72)	1 (12)	7 (88)
How often do you have difficulty remembering to take all your dietary supplements?				
Never/rarely	2 (3)		0 (0)	
Once in a while	6 (20)		3 (37.5)	
Sometimes	8 (4)		0 (0)	
Usually	16 (28)		2 (25)	
All the time	26 (45)		3 (37.5)	

Note: [1]Explanation for participants: "By dietary supplement we mean the product or liquid (for example milk, powder) prescribed by your doctor. Your doctor might refer to this as your amino acid mixture. Please note: Kuvan is not a dietary supplement."

2.4.3 Discriminant function analysis

The first model, which included demographic factors (i.e. age, gender, highest level of education and occupational status), accurately predicted 87.8% of the low adherence group (and 76% of the medium/high adherence group (see Figure 2.3). Education was marginally predictive of group membership ($F(1,64)=3.28$, $p=.08$, Wilk's $\Lambda=.95$) and was therefore retained in the final model. None of the other predictors included in the model were significant (see Appendix E).

Table 2.3 Actual and predicted adherence group membership in Model 1

	Actual	Predicted group membership (n (%))	
Adherence group	**membership (n)**	**Low**	**Medium/high**
Low	41	36 (87.8)	5 (12.2)
Medium/high	25	6 (24)	19 (76)

The second model, which included factors related to severity of the disorder (e.g. PKU classification and dietary allowance), accurately predicted 100% of the membership of both groups (see Figure 2.4. However, neither of the predictors in this model were significant (see Appendix E).

Table 2.4 Actual and predicted adherence group membership in Model 2

	Actual	Predicted group membership (n (%))	
Adherence group	**membership (n[1])**	**Low**	**Medium/high**
Low	36	36 (100)	0 (0)
Medium/high	24	0 (0)	24 (100)

Note: [1] lower overall n because not all participants were aware of their PKU classification

In the third model, rankings (1-8) of the importance of attributes of the protein substitutes (taste, texture, aftertaste, convenience, smell, volume, appearance and variety) were included as predictors. Frequencies of rankings of each attribute for both adherence groups are plotted in Figures 2.2-2.9.

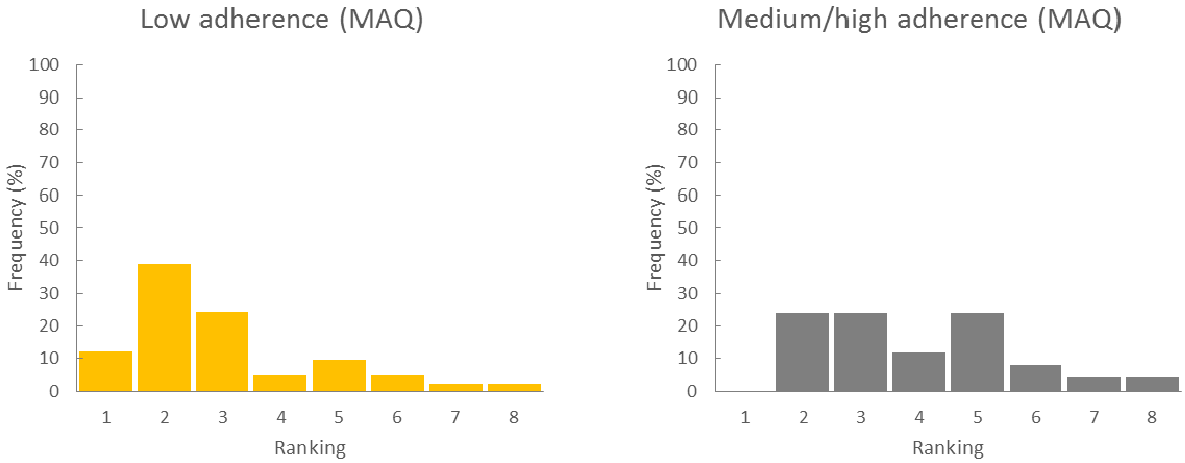


Figure 2.2 Frequency (%) of rankings of importance of 'aftertaste' of protein substitutes in ET AwPKU with low (left) and medium/high adherence (right) (1 = most important, 8 = least important)

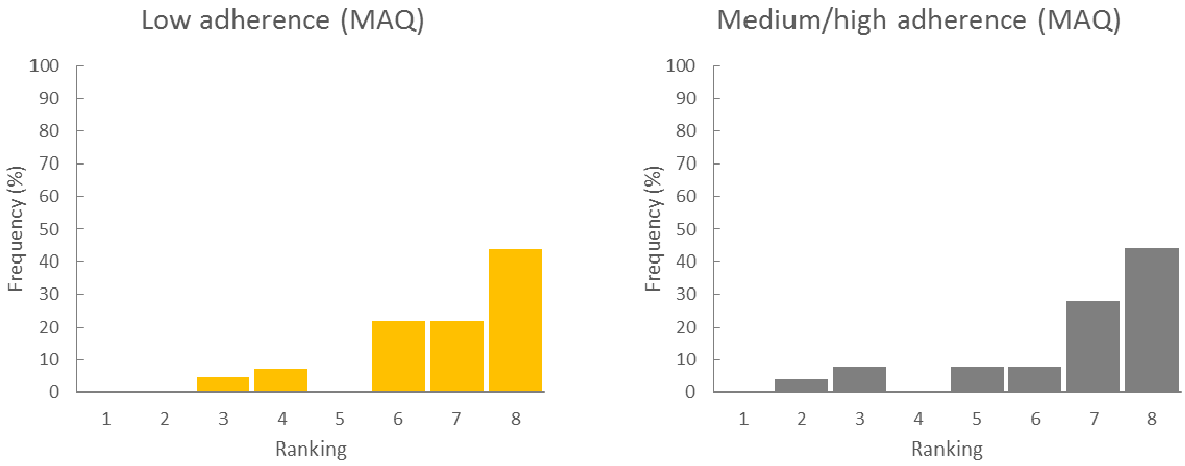


Figure 2.3 Frequency (%) of rankings of importance of 'appearance' of protein substitutes in ET AwPKU with low (left) and medium/high adherence (right) (1 = most important, 8 = least important)

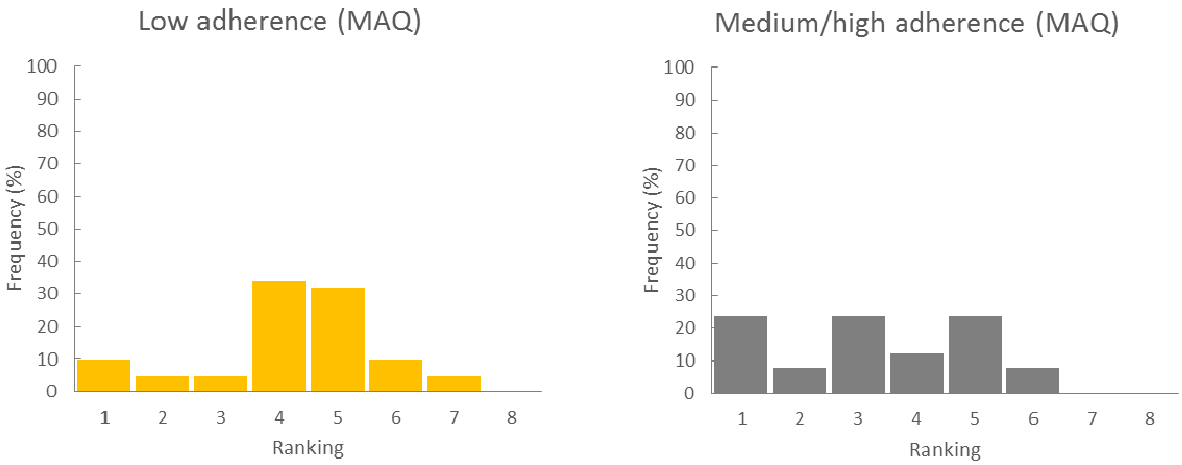


Figure 2.4 Frequency (%) of rankings of importance of 'convenience' of protein substitutes in ET AwPKU with low (left) and medium/high adherence (right) (1 = most important, 8 = least important)

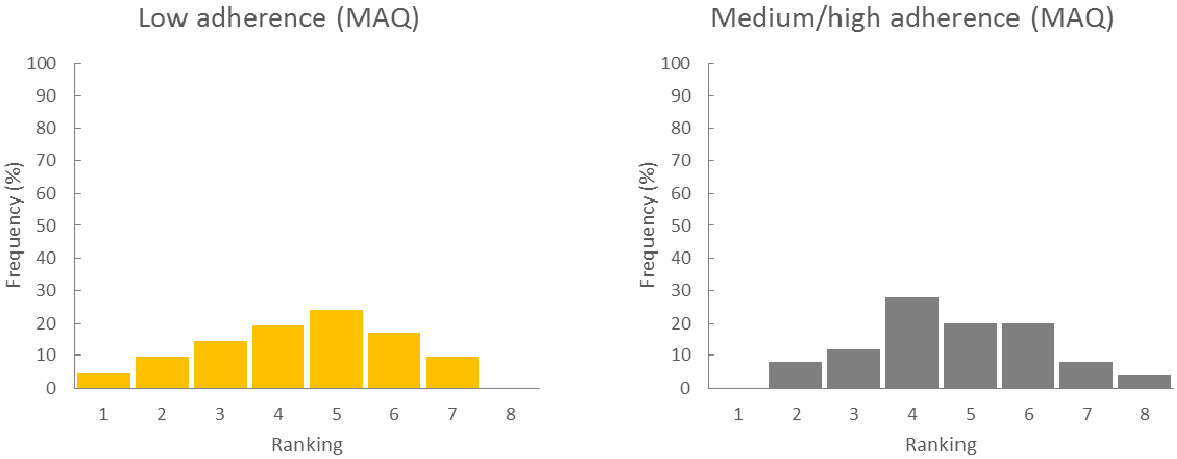


Figure 2.5 Frequency (%) of rankings of importance of 'smell' of protein substitutes in ET AwPKU with low (left) and medium/high adherence (right) (1 = most important, 8 = least important)

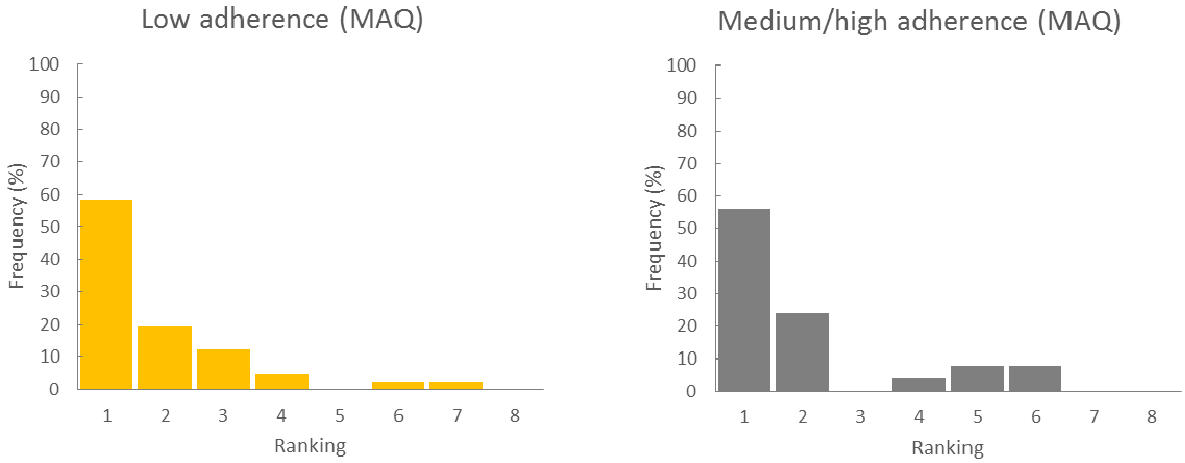


Figure 2.6 Frequency (%) of rankings of importance of 'taste' of protein substitutes in ET AwPKU with low (left) and medium/high adherence (right) (1 = most important, 8 = least important)

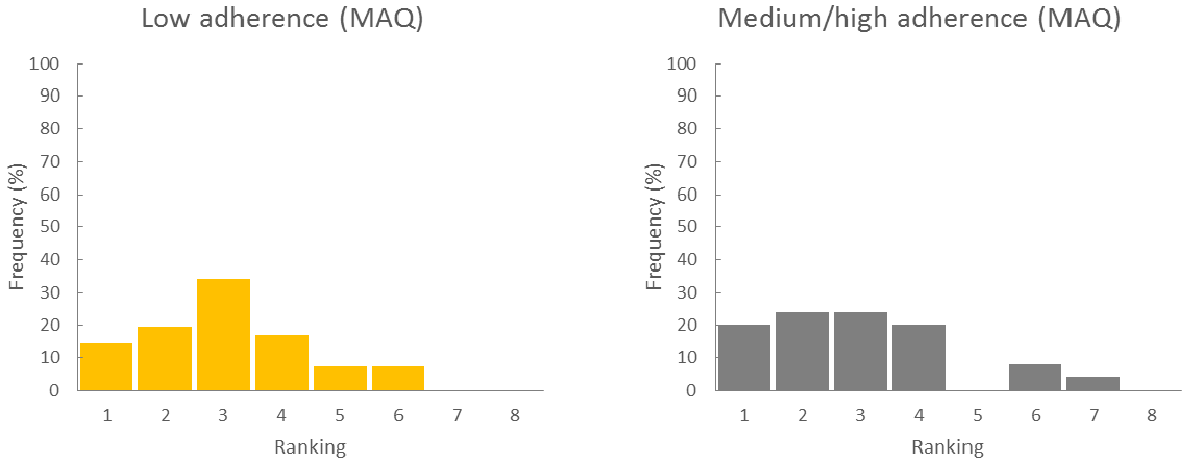


Figure 2.7 Frequency (%) of rankings of importance of 'texture' of protein substitutes in ET AwPKU with low (left) and medium/high adherence (right) (1 = most important, 8 = least important)

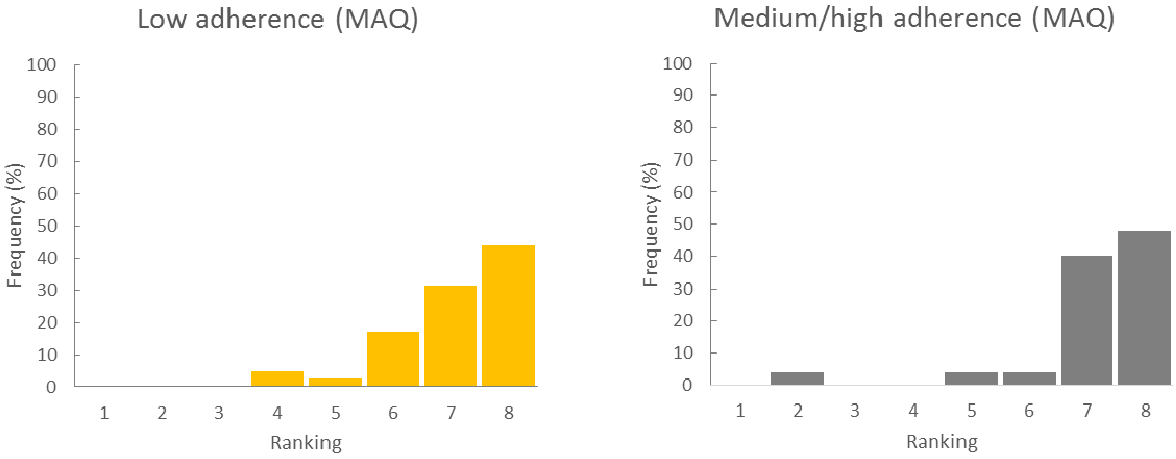


Figure 2.8 Frequency (%) of rankings of importance of 'variety' of protein substitutes in ET AwPKU with low (left) and medium/high adherence (right) (1 = most important, 8 = least important)

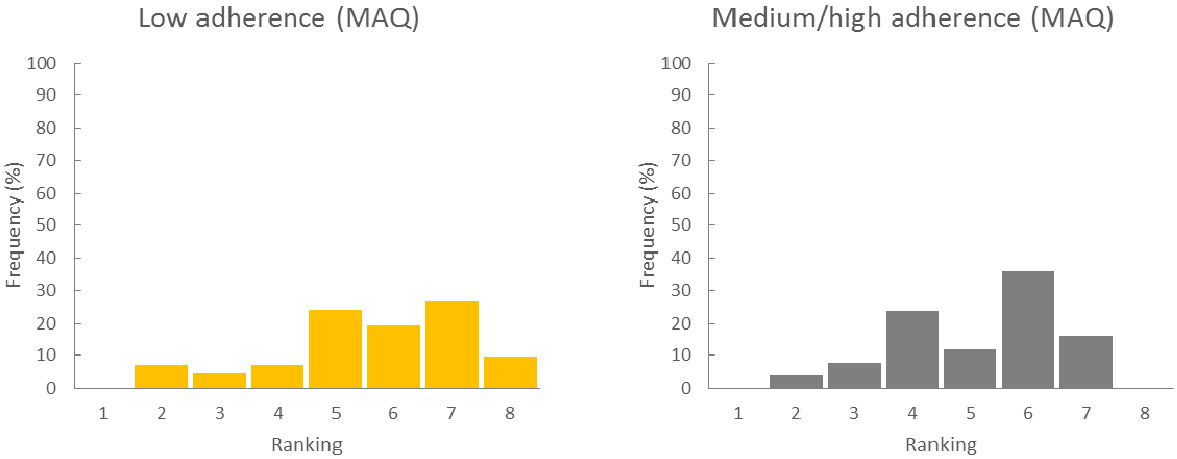


Figure 2.9 Frequency (%) of rankings of importance of 'volume' of protein substitutes in ET AwPKU with low (left) and medium/high adherence (right) (1 = most important, 8 = least important)

Again, accuracy of prediction of group membership was higher for the low (80.5%) compared to the medium/high adherence group (52%; see Table 2.5). Aftertaste ($F(1,64)=5.30$, $p=.03$, Wilk's $\Lambda=.92$) and convenience ($F(1,64)=5.48$, $p=.02$, Wilk's $\Lambda=.92$) of the protein substitutes were found to be significant predictors of group membership (see also Appendix E).

Table 2.5 Actual and predicted adherence group membership in Model 3

	Actual	Predicted group membership (n (%))	
Adherence group	**membership (n)**	**Low**	**Medium/high**
Low	41	33 (80.5)	8 (19.5)
Medium/high	25	12 (48)	13 (52)

A final discriminant function analysis was performed using the predictors, education, aftertaste and convenience from the three initial models. Additionally, 'having been off-diet in the past' was included as a predictor in line with the hypothesis stated in Section 2.1.2. Consistent with the other models, this model was able to predict membership of the low adherence group better than membership of the medium/high adherence group (82.9% vs. 60%; see Table 2.6). Aftertaste ($F(1,64)=5.30$, $p=.03$, Wilk's $\Lambda=.92$), convenience ($F(1,64)=5.48$, $p=.02$, Wilk's $\Lambda=.92$), and *'having been off-diet in the past'* ($F(1,64)=5.14$, $p=.03$, Wilk's $\Lambda=.93$) were all significant predictors of group membership (see also Appendix E). In addition, highest completed level of education was a marginally significant predictor of group membership ($F(1,64)=3.28$, $p=.08$, Wilk's $\Lambda=.95$). This final model, despite being significant, explained only 19% of the variance between the low and medium/high adherence groups (Wilk's $\Lambda=.81$; $X^2(1)=13.05$, $p=.01$).

Table 2.6 Actual and predicted adherence group membership in the final discriminant function analysis model

	Actual	Predicted group membership (n (%))	
Adherence group	**membership (n)**	**Low**	**Medium/high**
Low	41	34 (82.9)	7 (17.1)
Medium/high	25	10 (40)	15 (60)

2.5 Discussion

2.5.1 Summary of results

The aim of this study was to assess adherence to dietary management of ET AwPKU, in particular, adherence to protein substitutes, and to explore predictors of adherence. It is clear from responses to the survey that dietary practices vary widely amongst ET AwPKU. The majority of ET AwPKU who completed the survey reported that they were following a low-protein diet and taking protein substitutes. However, adherence to the protein substitutes was low in nearly two-thirds of the patients. Furthermore, of the respondents who were following a low-protein diet at the time of completion of the survey, approximately a third had been off-diet in the past, and this was a significant predictor of adherence to protein substitutes, confirming the hypothesis stated in Section 2.1.2. As discussed in Section 2.1.2, it is likely that these patients developed a taste for more palatable foods whilst they were off-diet, which in turn affected their acceptance of protein substitutes. In relation to this, aftertaste was found to be a significant predictor of protein substitute adherence. Another predictor of adherence to protein substitutes identified in the current research, was convenience. These results are in line with results of a recent survey of AwPKU, which showed that palatability and ease of use were important factors in the consumption of protein substitutes (Cazzorla et al., 2018). It has been previously shown that ready to drink (RTD) protein substitutes are often preferred over other formulations (e.g. powders, pills) as they are more convenient and have been associated with reduced self-consciousness and overall improved adherence in adolescents and adults with PKU (Gokmen-Ozel et al., 2009; MacDonald et al., 2006).

Interestingly, despite the restrictive nature of the diet frequently being identified as a barrier to adherence, individual dietary allowance (i.e. amount of daily natural protein prescribed) did not predict adherence as measured by the MMAS-8. A similar finding was reported by Olsson et al. (2007), who found no association between differences in metabolic control and severity of PKU. Patients with a stricter diet might be more cautious than those with a higher allowance (Olsson et al., 2007). Individual characteristics like age and gender were not significant predictors of protein substitute adherence in this sample of ET AwPKU. In contrast, a recent systematic review on

demographic and psychosocial influences on adherence in children and adolescents with PKU, found age to be the most reproducible association with adherence (as measured by metabolic control), with adherence decreasing with increasing age (Medford, Hare, & Wittkowski, 2017). Moreover, patient sex had the next most reproducible association with adherence (Medford, Hare, & Wittkowski, 2017).

2.5.2 Limitations and recommendations for future research

The final predictive model from the current study explained only 19% of the variance between groups of ET AwPKU with low or medium/high adherence to protein substitutes. This illustrates that predicting and, therefore, managing adherence to dietary management of PKU is complex and involves many factors over and above those measured in this survey. Other internal (e.g. behavioural) and external (e.g. social) factors, not measured here, contribute to difficulties with adherence and more research is needed to fully understand the predictors of continuous adherence to the dietary management of PKU and how these many change as AwPKU age.

One limitation of the current research is that it is entirely based on self-report measures and as such some responses may have been influenced by social desirability or a restricted range of response options. However, as outlined in section 2.1.3, most methods used to assess adherence in relation to the dietary management of PKU rely on self-report and even objective measures of metabolic control have been shown to be subject to social desirability bias and may underestimate typical Phe levels (Bilginsoy et al., 2005; Weglage et al., 1992). Instead of directly querying protein substitute intake, adherence to protein substitutes was assessed using the MMAS-8. MMAS-8 is a validated measure of treatment adherence and an advantage of this measure may be that it is less susceptible to socially desirable answers (Morisky et al., 2008). However, MMAS-8 is not a direct measure of protein substitute adherence and its correlation with actual protein substitute intake is unknown. Furthermore, the current research focused on adherence to protein substitutes, and even though this is a major component of the dietary management of PKU, it is important to get a comprehensive overview of the dietary practices of PKU patients. Consultants and dietitians assess and, where necessary, adjust the dietary recommendations for individual patients using a

combination of methods described previously (see Section 2.1.3), looking not only at metabolic control, but also at intake of nutrients from both the diet and protein substitutes (usually via 24-hour dietary recall) and patients' (micro)nutrient status. To get a better understanding of the effects of differing dietary practices of individual patients on several aspects of their health (e.g. cognitive functioning), future (psychological) research should assess and report measures other than metabolic control that could be relevant to specific outcomes (e.g. vitamin B12 intake/status in relation to cognitive functioning).

Finally, the ET AwPKU who participated in this research are likely to be a self-selected sample who are more engaged with their diet (85% reported to be on-diet) and protein substitutes (96% reported taking protein substitutes). Hence, the sample was likely to be unrepresentative of all ET AwPKU and the present research was not able to examine factors associated with being "off-diet" or taking no protein substitutes at all.

2.5.3 Implications and conclusion

Despite explaining an arguably low proportion of the variance between the adherence groups, results were in line with previous research and demonstrate that attributes of protein substitutes, especially aftertaste and convenience, contribute to dietary adherence in ET AwPKU (Bilginsoy et al., 2005; Cazzorla et al., 2018; Hoeks et al., 2009; MacDonald, 2000; MacDonald et al., 2006, 2010; Prince et al., 1997). Further improvements in the taste, aftertaste, texture and convenience of available protein substitutes might therefore improve adherence.

A relatively new development in the dietary management of PKU is the development of protein substitutes that are based on a mixture of casein glycomacropeptide (CGMP, sometimes referred to as GMP) and free AA (FAA). CGMP-based protein substitutes were not available in the UK at the start of this PhD research. CGMP is a 64-AA peptide derived from cheese whey which is the only known natural protein that is low in Phe, making it an appropriate source of protein for individuals with PKU (Ney et al., 2009; Strisciuglio & Concolino, 2014). CGMP is a bland, neutral tasting product, the functional properties of which mean it can be easily incorporated into a number of both sweet and savoury foods. Research with CGMP-based protein substitutes has demonstrated that

these substitutes are highly acceptable by AwPKU and were rated as more acceptable than AA-based protein substitutes, not only in terms of taste but also in terms of appearance and odour (Lim, van Calcar, Nelson, Gleason, & Ney, 2007; Ney et al., 2009, 2016; Proserpio et al., 2018). Because of their bland / neutral taste CGMP-based protein substitutes may help improve adherence to the dietary management of ET AwPKU, especially for those who have previously been off-diet. This possibility for potential of CGMP is explored further in Chapter 6.

Chapter 3 Systematic review of cognitive functioning in early treated adults with PKU (ET AwPKU)

3.1 Introduction

As introduced in Chapter 1 (see Section 1.1), when left untreated, PKU can cause severe and irreversible neurological impairments (Blau, Bélanger-Quintana, et al., 2010). However, these neurological impairments can be prevented via early dietary management (see Section 1.4.2.2). Nonetheless, early-treated individuals with PKU still demonstrate deficits in cognitive function, particularly in EF, attention and processing speed. As a result of increasing difficulties with adherence to the dietary management of PKU as patients grow older (see Section 1.4.3), the majority of ET AwPKU that have participated in research studies have discontinued their low-protein diet and protein substitutes at some point in their lives. Thus, very few people with PKU will truly be early and continuously treated (ECT), and the impact of such breaks in treatment on cognitive function is unknown.

There is some debate on the specific neuropsychological mechanism(s) responsible for the observed cognitive deficits in PKU (see section 1.2), but the general belief is that these deficits are related to patients' Phe levels at several stages throughout life (e.g. concurrent Phe levels, lifetime Phe levels, variation in Phe levels) (Jahja, Huijbregts, de Sonneville, van der Meere, & van Spronsen, 2014). In addition to uncertainties about the exact mechanism underlying suboptimal cognitive functioning, it is unclear whether observed deficits in EF are the consequence of reduced speed of processing or whether impairments in speed of processing are the consequence of deficits of EF (Romani, MacDonald, De Felice, & Palermo, 2018). Furthermore, the majority of research in ET AwPKU has focused on measures of EF, attention and processing speed, whereas other domains have received less attention.

This chapter provides a systematic review of cognitive functioning across different cognitive domains in ET AwPKU. It aims to provide a clear overview of cognitive functioning in ET AwPKU by addressing the following questions: (1)

Which cognitive domains are affected in ET AwPKU; (2) How are cognitive outcomes across different domains related to concurrent and lifetime Phe levels in ET AwPKU; (3) are there any differences in cognitive performance between ECT AwPKU and ET AwPKU who have ever discontinued their low-protein diet and/or protein substitutes?

3.2 Methods

This systematic review followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) 2009 checklist and is registered in PROSPERO. The registration number is CRD42016043706.

3.2.1 Search Strategy and Search Terms

Searches of electronic databases were carried out on 31 July 2017. This search was updated on 2 March 2018 and again on 18 June 2018. Databases searched were Ovid MEDLINE(R), PsycINFO, Web of Science, Cochrane, Scopus, Embase, ScienceDirect, and PubMed 1953 to June 2018. The following search terms were used: (‘phenylketonuria’ OR ‘PKU’) AND (‘cogniti*’ OR ‘memory’ OR ‘attention’ OR ‘visual-spatial’ OR ‘visuo-spatial’ OR ‘recall’ OR ‘recognition’ OR ‘problem solving’ OR ‘reaction time’ OR ‘vigilance’ OR ‘executive function*’ OR ‘reasoning’ OR ‘psychomotor’ OR ‘motor’ OR ‘processing’ OR ‘planning’ OR ‘verbal fluency’ OR ‘inhibit*’). Furthermore, the reference lists of existing reviews and identified articles were examined individually to supplement the electronic search. A total of 10803 citations were screened against inclusion and exclusion criteria.

3.2.2 Inclusion and Exclusion Criteria

This review was limited to articles published in peer-reviewed journals in English, Dutch or German. Case reports, abstracts and conference proceedings were not included. Papers were included or excluded in this review using the following criteria.

3.2.2.1 Participants

Studies of ET AwPKU aged 18 years and over of either gender were included. As treatment guidelines vary worldwide, age at the start of treatment for the ET AwPKU sample of each paper was included in the data extraction, where available. Animal studies were excluded. Studies where results of ET AwPKU were not reported separately (e.g. papers reporting combined outcomes of ET adolescent and adult PKU patients) were excluded from this review.

3.2.2.2 Intervention

Papers reporting on a sample of ET AwPKU patients who had been treated with the conventional low-protein diet with Phe-free protein substitutes, or protein substitutes low in Phe, were included. Studies reporting on cognitive outcomes in ET AwPKU as a result of (an acute) manipulation of Phe-levels or additional supplementation with Tyr, or vitamins and minerals were excluded. Finally, as this systematic review aims to give a clear overview of the efficacy of early treatment on cognitive outcomes in adulthood, interventions with new treatments such as Sapropterin dihydrochloride (Kuvan®) and Pegvaliase (Palynziq™), which were not available when the ET AwPKU commenced their treatment, were excluded.

3.2.2.3 Control(s)

Research including a healthy control group or a comparator group (e.g. diabetic patients, autistic patients) was included. Papers without a specific control group (e.g. comparison to standardized or normative data) were also included.

3.2.2.4 Outcome measures

Studies including any objective measure of cognitive performance were included. Metabolic outcomes (e.g. concurrent Phe levels) were not a requirement for inclusion but were considered where available.

3.2.3 Design

Observational studies (i.e. cross-sectional, cohort, case-control and longitudinal studies) were included in this systematic review.

3.2.4 Study Selection Process

The literature search yielded a total of 10803 citations. Following removal of 6287 duplicates, a total of 4516 citations were retrieved for possible inclusion in the review. The titles and abstracts of these citations were screened by one reviewer (DH) to remove obviously irrelevant reports (n=4371), resulting in retention of 145 papers. Another reviewer (CC) independently screened, at random, 5% of the titles and abstracts to establish agreement about the inclusion and exclusion of studies. The inter-rater agreement was 95%, and any disagreements during this process were resolved by discussion, and a consensus decision was reached. The full-text versions of the remaining 145 articles were retrieved and examined for eligibility based on the inclusion criteria, and authors were contacted to clarify any missing information. Inter-rater agreement was 100%. As a result of the screening process, a further 123 articles were excluded. A total of 16 studies reported in the remaining 22 articles were included in the review (see Figure 3.1).

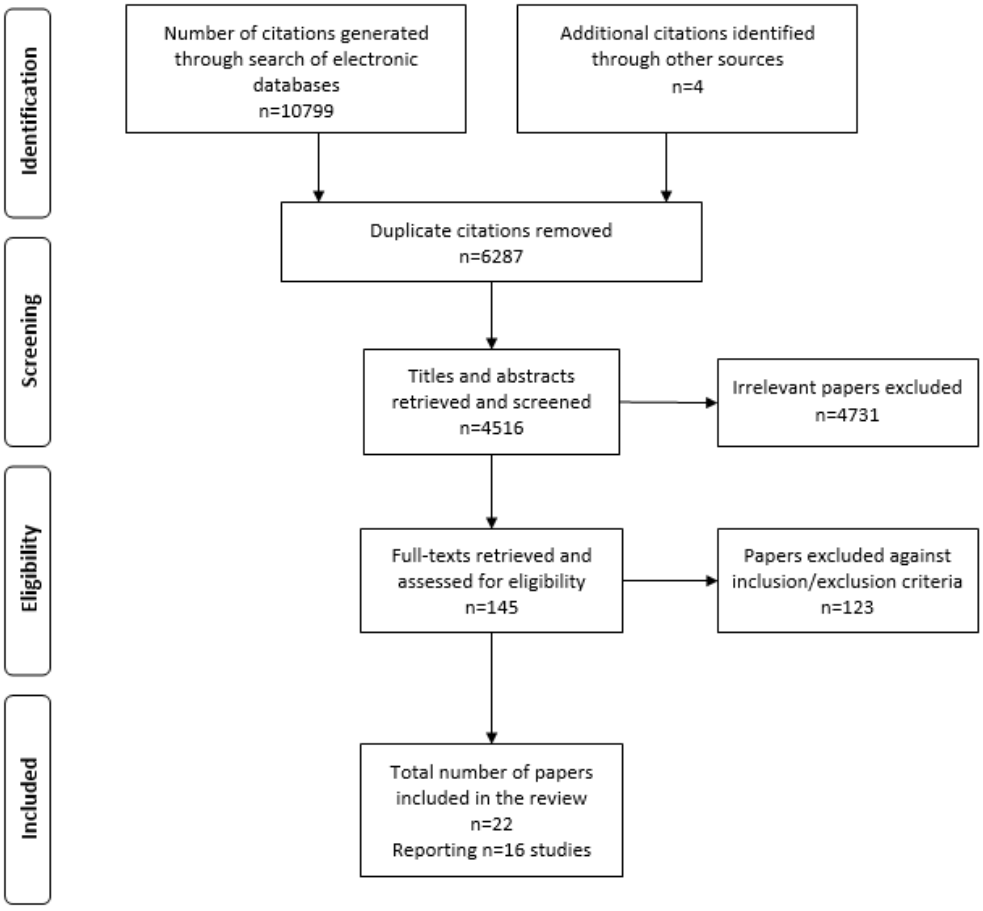


Figure 3.1 Flow diagram of the study selection process for the systematic review of cognitive functioning in early treated adults with PKU (ET AwPKU)

3.2.5 Quality assessment

The quality of all included papers was assessed using the 'quality assessment tool for reviewing studies with diverse design' (QATSDD) (Sirriyeh, Lawton, Gardner, & Armitage, 2012). Two reviewers (DH and CC) independently awarded each research paper quality scores by assessing each QATSDD criterion (for example 'Description of procedure for data collection') on a 4-point scale from 0 to 3 (0=the criterion is not at all described, 1= described to some extent, 2= moderately described and 3= described in full). The sum of scores of all relevant QATSDD criteria reflects the overall quality of each paper. The scores, expressed as a percentage of the maximum possible score of 42, are included in the data extraction table (Table 3.1).

Quality ratings ranged from 35.7% to 59.5% of the maximum score and overall average quality was rated at 48.3%. Papers scored particularly low with respect to reporting of statistics: there was no clear evidence of sample size considered in terms of analysis, justification for analytical method selected or assessment of reliability of the analytical process across publications. Publications scored particularly high on the following criteria: explicit theoretical framework, statement of aims/objectives, and description of procedure for data collection. Finally, whilst most papers had strong discussions in terms of interpretation and implications of the data, they lacked a critical discussion of the strengths and weaknesses of the studies reported.

3.2.6 Data extraction

The Cochrane data extraction form was modified for the purposes of this review. Data were extracted into the standardised form by one researcher (DH), and authors were contacted when insufficient information was provided in the published paper. Half (50%) of these articles were then double data extracted by another researcher (CC). Any disagreements were resolved by discussion, and a consensus decision was reached.

3.3 Results

3.3.1 Selected studies

Twenty-two articles reporting on outcomes from sixteen observational studies assessing cognitive functioning in ET AwPKU were included in this review.

Fourteen studies included healthy controls, often matched for gender and age, and (less often) IQ and socio-economic status. The two remaining studies compared the performance of ET AwPKU on cognitive tasks to either standardized (Brumm et al., 2004) or normative data (Bik-Multanowski, Pietrzyk, & Mozrzymas, 2011).

Four studies reported on a group of in ET AwPKU who had discontinued their diet (Burgard et al., 1997; Channon, Goodman, Zlotowitz, Mockler, & Lee, 2007; Dawson et al., 2011; Moyle, Fox, Bynevelt, et al., 2007), three of these also included ET AwPKU who were on-diet but reported results for on- and off-diet patients separately (Burgard et al.,

1997; Channon et al., 2007; Dawson et al., 2011). Furthermore, four studies described their sample of AwPKU as early and continuously treated (ECT) (Channon, German, Cassina, & Lee, 2004; Channon et al., 2007; Channon, Mockler, & Lee, 2005; Jahja et al., 2016; Jahja, Huijbregts, et al., 2017; Jahja, van Spronsen, et al., 2017; Liemburg et al., 2015; Moyle, Fox, Bynevelt, Arthur, & Burnett, 2006). However, the upper range of Phe levels at the time of testing of all ECT AwPKU samples exceeded upper target treatment levels. All other research included a mixed sample of both on-diet ET AwPKU and ET AwPKU who were either off-diet or following a relaxed diet in their study samples.

Seven publications compared effects of high versus low Phe levels (Bartus et al., 2018; Bik-Multanowski et al., 2011; Brumm et al., 2004; De Felice, Romani, Geberhiwot, MacDonald, & Palermo, 2018; Jahja, Huijbregts, et al., 2017; Nardecchia et al., 2015; Romani et al., 2017). However, all of these studies used different cut-off Phe levels for their high and low Phe groups: Bik-Multanowski et al. (2011) compared cognitive performance of ET AwPKU with concurrent levels of ≤720 µmol/L and >720 µmol/L; Brumm et al. (2004) used cut-off Phe levels of <1000 µmol/L and >1000 µmol/L at the time of testing; Jahja, Huijbregts et al. (2017) compared effects of concurrent, childhood, adolescent and lifetime Phe by comparing low and high Phe groups according to the most frequently used upper target treatment level during childhood, 360 µmol/L (low: <360 µmol/L, high: ≥360 µmol/L); Bartus et al. (2018), de Felice et al. (2018) and Nardecchia et al. (2015) compared cognitive functioning of patients with Phe levels below and above 600 µmol/L, a frequently used upper target treatment level during adolescence and adulthood (van Wegberg et al., 2017); additionally, Bartus et al. (2018) compared cognitive task performance of ET AwPKU with average childhood (0-12 years) Phe below and above 360µmo/L; and, finally, Romani et al. (2017) divided their sample into two equally large subgroups based on their adulthood Phe levels (low: <650 µmol/L, high: >950 µmol/L), noting that their ET AwPKU group with good metabolic control (low Phe group) had adulthood Phe levels close to current treatment guidelines in the UK (<700 µmol/L (Cockburn et al., 1993)).

The majority of publications (18 reporting results of 14 different studies) looked at correlations between cognitive performance and Phe levels during various periods and at various points throughout life.

Finally, three studies reported on a longer-term follow-up study of ET AwPKU (Jahja, van Spronsen, et al., 2017; Nardecchia et al., 2015; Weglage et al., 2013). Two of these compared cognitive outcomes during childhood with cognitive outcomes in the same sample in adulthood (Jahja, van Spronsen, et al., 2017; Nardecchia et al., 2015). The third followed ET AwPKU over a 5-year period (Weglage et al., 2013).

Included studies, with details of the cognitive tasks and metabolic measures utilised, as well as the reported results are summarised in Table 3.1. Table 3.2 summarises impairments observed in outcome measures of cognitive functioning, and Table 3.3 provides reported correlations between Phe and Tyr levels across the life-span and outcome measures cognitive function. Finally, Table 3.4 provides an overview of different tasks used across different cognitive domains in the studies included in this review. It shows the frequency of use of each of the tasks across all included studies, as well as their sensitivity in ET AwPKU.

Table 3.1 Summary of studies included in the systematic review reported in this chapter

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Bartus et al. (2018)	35.7	Hungary	Cross-sectional	46 ET AwPKU median age 29.5 yrs (range 19-39) *20 "on-diet"* (Phe <600µmol/L) 11F, 9M median age 30.5 yrs (range 19-39) *26 "loose diet"* (Phe >600 µmol/L) 10F, 16M median age 29.5 yrs (range 19-39) 31 controls 11 F, 20M median age 23 yrs (range 25-28)	Treatment initiated immediately upon diagnosis through neonatal screening.	**Cambridge Neuropsychological Test Automated Battery (CANTAB):** <u>Problem solving/strategy & working memory (WM)</u>: Stockings of Cambridge (SOC) & Spatial Working Memory (SWM) <u>Processing speed & motor skills</u>: Motor Screening Test (MOT)	Concurrent Phe & Tyr Lifetime Phe (n=33) Average Phe for 0-12 yrs of age (n=33)	Controls outperformed the ET AwPKU on the MOT (accuracy, p<.01), SOC (subsequent think time & accuracy, p<.001) & SWM (accuracy & strategy, p<.001). The same differences in performance were found between the on-diet ET AwPKU group & controls (p<.01). No significant differences in task performance were observed between on-diet ET AwPKU & those on a "loose diet".	ET AwPKU with better metabolic control during childhood (average Phe <360µmol/L for 0–12 yrs (n=10)) outperformed those with poorer metabolic control (average Phe >360µmol/L for 0–12 yrs (n=23)) on measures of accuracy & strategy on SWM (p<.001). No correlations between any of the cognitive outcome measures & concurrent Phe & Tyr, or lifetime Phe were reported.

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Bik-Multanowski et al. (2011)	38.1	Poland	Cross-sectional	49 ET AwPKU >16 yo divided in 2 groups based on concurrent Phe: Phe ≤0.72 mmol/L: n = 22; 11F, 11M mean age 19.5 yrs mean IQ 88.7 (range 74-105) Phe>0.72 mmol/L: n = 27; 12F, 15M mean age 19.7 yrs mean IQ 85.4 (range 74-105)	Diagnosis: early infancy Diet onset (majority): 2nd/3rd mth of life Phe ≤0.72 mmol/L mean age 49 days (range 15-101) Phe >0.72 mmol/L:: mean age 40 days (range 20-69)	Speed of response, response inhibition, sustained attention, working memory capacity (**CANTAB**): - Simple Reaction Time (SRT) - Choice Reaction Time (CRT) - Stop Signal Task (SST) - Rapid Visual Information Processing (RVP) - Spatial Span (SSP) - SWM Age-corrected normative data available for RVP, SSP & SWM.	Concurrent Phe Blood Phe in first 10 yrs of life (mean & range)	Non-adherent (Phe>0.72 mmol/L) group performed significantly worse than adherent (Phe≤0.72 mmol/L) group on RVP (p=.011), SSP (p=.034), SWM (p<.001) & SST (p=.024). Adherent ET AwPKUs performed worse than age-corrected normative data on selected tests (RVP, SSP, SWM).	N/A No significant differences observed in blood Phe in the first 10 yrs of life between groups.
Brumm et al. (2004)	50.0	USA	Cross-sectional	24 ET AwPKU *13 classical PKU* *5 moderate PKU* *1 mild PKU* 11F, 13M mean age 29 yrs (SD 2.7; range 21-32) mean FSIQ 104.6 (SD 13.3; range 75-126) mean VIQ 102.2 (SD 13.2; range 75-128) mean PIQ 106.7 (SD 14.2; range 77-142) mean duration of treatment 15.3 yrs (SD 10.3; range 4.8-31) Group split based on concurrent Phe levels: 1) High-Phe (n=13): >1000µmol/L	Diet onset: by 15th day of life Some discontinued at age 5/6, some resumed & some did not Mean time on-diet: 15.3 yrs (SD 10.3; range 4.8-31)	Attention: California Verbal Learning Test (CVLT), Continuous Performance Test (CPT), Trail Making Test part A (TMT-A), Digit Span Forward (WAIS-R) Executive Function (EF): CVLT, CPT, Controlled Oral Word Association Test (COWAT), Stroop, TMT part B (TMT-B), Digit Span Back (WAIS-R), Wisconsin Card Sorting Test (WCST) Learning & memory (verbal/visual): CVLT, Rey Osterrieth Complex Figure Test (ROCFT) - delayed recall Language functioning: Boston Naming Test (BNT),	Concurrent Phe Interim blood Phe childhood (6-month medians at 5.5-6 & 9.5-10 yrs)	Authors reported % of ET AwPKU with cognitive deficit, as well as median and 95% CI. ET AwPKU had a cognitive deficit in a certain task/domain if their score was >1SD below the standardized sample mean (= score <16th or > 84th centile for error scores). An overall deficit in the ET AwPKU sample was reported when median or 95% CI did not contain 50, or >32% of the ET AwPKU exhibited a deficit. Impairments on several, but not all, attention measures (deficits: CVLT List 1 Trial 1, Stroop) & EF (deficits: CVLT perservative errors, CPT commission errors, COWAT, Digit Span Back & WCST categories). Group deficits = all tasks of verbal & visual memory & learning, tasks of verbal fluency (COWAT & Animal Naming) & one semantic processing measures (BNT).	Performance on the language tasks was correlated with concurrent Phe & both childhood Phe measures (p<.05). CVLT List A Trial 1 was the only measure of attention correlated with any of the metabolic measures (Phe 5.5-6 yrs; p<.05). Several correlations between metabolic measures & tasks of EF (apart from CPT) were observed, most frequently with childhood Phe 9.5-10 yrs (p<.05). Some measures of verbal (CVLT) & visual (ROCFT) memory & learning were associated with median Phe at 5.5-6 yrs of age (p<.05). Visual perception tasks block

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
				2) Low-Phe (n=11): <1000µmol/L		Animal Naming Test, COWAT, Peabody Picture Vocabulary Test-Revised (PPVT-R), Vocabulary (WAIS-R), Similarities (WAIS-R) <u>Visual-perceptual skills</u>: ROCFT, Block Design (WAIS-R), Picture Completion (WAIS-R), Picture Arrangement (WAIS-R) <u>Psychomotor speed & fine motor coordination</u>: Grooved Pegboard, Digit Symbol (WAIS-R)		ET AwPKU performed below expectation on one measure of visual-perceptual skill (ROCF-figure copy). No group deficit on psychomotor speed & coordination. ET AwPKU with high concurrent Phe performed significantly worse than those with low Phe on several language tasks (COWAT, Animal Naming Test, BNT and PPVT-R; p<.05).	design & picture completion were correlated with childhood Phe levels (p<.05). ** all observed correlations were negative (indicating worse performance with higher Phe levels)* *** Pearson's correlations were of moderate strength (range 0.4-0.6); for specific correlations, see Brumm et al.(2004)*

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Burgard et al. (1997)	47.6	France & Germany	Cross-sectional	22 (French) ET AwPKU, off-diet 8 adults: 3F, 5M *6 classical PKU* *2 mild PKU* aged 17-25 yrs (mean 19.5, SD 1.4) mean IQ 108 (SD 14.1) 23 (German) ET AwPKU, on-diet 8 adults: 3F, 5M *7 classical PKU* *1 mild PKU* aged 17-25 yrs (mean 19.7, SD 1.1) mean IQ 108 (SD 10.0) 21 healthy controls 8 adults: 3F, 5M aged 17-25 yrs (mean 20.2, SD 2.4) mean IQ 107 (SD 10.1)	<u>French cohort:</u> Before the 3rd mth of life <u>German cohort</u>: mean age of 16 days (range 4-45 days) - reference Burgard et al. (1996)	**Sonneville Visual Attention Tasks (SVAT):** <u>Visuomotor RT</u>: Finger Motor Speed Exercise (FSME) <u>Sustained attention</u>: Dot Pattern Exercise (DPE) <u>Visual stimulus scanning/ search</u>: Letter Pattern Exercise (LPE)	Concurrent Phe IDC (mean of all yearly median Phe blood levels) age 1-6, 7-10 & 11-14	No significant differences between groups on FMSE & LPE performance. Reaction times (RT) increased significantly by load (for all groups). On-diet ET AwPKU performed worse on DPE than controls ($p<.05$). Stability of performance of both ET AwPKU groups was worse than that of controls ($p<.05$), but on-diet ET AwPKU had a better stability than off-diet ET AwPKU.	Results indicate influence of concurrent Phe levels on neuropsychological test results. Pattern of results suggests outcomes will be the same whenever Phe levels increase after the period of strict treatment during childhood.

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Channon et al. (2004)	47.6	UK	Cross-sectional	20 ECT AwPKU: 8F, 12M *18 classical PKU* *2 moderate PKU* aged 18-33 yrs (mean 24.60, SD 4.62) VIQ range 88-128 (mean 108.55, SD 9.47) PIQ range 88-129 (mean 112.30, SD 9.68) 20 healthy controls: 8F, 12M aged 19-34 yrs (mean 24.00, SD 3.96) VIQ range 81-126 (mean 107.20, SD 10.84) PIQ range 86-129 (mean 114.65, SD 10.43)	Within 1st mth of life	Attentional control: Telephone Search Test & Telephone Search Test with Counting (subtests of TEA) WM: Self-Ordered Pointing Test (SOPT) Word generation: Letter Fluency Test Multitasking: Six Elements Test Inhibition: Hayling Sentence Completion Test (Part B) Rule finding: Brixton Test Memory & learning: Rey Auditory Verbal Learning test (RAVLT), ROCFT	Concurrent Phe Lifetime Phe Recent Phe (=mean Phe level yr preceding testing)	ECT AwPKU produced significantly less correct responses on tasks of attention & working memory (TEA p=.009; SOPT p=.0001) & generated fewer words (p=.013) than controls.	Performance on TEA was negatively correlated with Phe levels at age 25-28 (r=-.59, p=.042). Performance on SOPT was negatively correlated with concurrent Phe (r=-.49, p=.032), Phe levels at age 21-24 (r=-.63, p=.021) & age 25-28 (r=-.77, p=.005); but positively correlated with recent Phe (r=.67, p=.002) **authors used a significance level of 0.05* ***negative correlation on these measures indicates poorer performance with higher Phe levels*
Channon et al. (2005)	52.4	UK	Cross-sectional	25 ECT AwPKU: 12F, 13M *24 classical PKU* *1 moderate PKU* aged 18-33 yrs (mean 26.68, SD 4.92) mean FSIQ 107.04 (SD 12.01) 25 healthy controls: 12F, 13M aged 18-33 yrs (mean 26.48, SD 5.45) mean FSIQ 107.28 (SD 10.35)	Within 1st mth of life	Attention & WM: n-back Inhibition: Flanker(FL) Object alternation learning Perceptual judgement: Birmingham object recognition battery	Concurrent Phe Lifetime Phe Recent Phe (=mean Phe level yr preceding testing)	Overall speed of performance of ECT AwPKU was significantly slower than controls on n-back (p=.013) & *marginally* significantly slower on flanker (p=.04)*. No significant between group differences in performance on object alternation learning or the perceptual judgement task observed. **authors used a criterion of .025*	Speed on 0-back was positively correlated with Phe levels at age 5-8 (r=.56, p<.01). **authors used a significance level of 0.01*

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Channon et al. (2007)	57.1	UK	Cross-sectional	25 ECT AwPKU *see Channon et al. (2005)* 25 ET AwPKU who discontinued their diets in adolescence *23 classical PKU* *2 atypical PKU* 8F, 17M aged 18-38 yrs (mean 27.48, SD 4.55) mean FSIQ 101.48 (SD 14.60) 45 healthy controls 19F, 26M aged 20-47 yrs (mean 28.76, SD 7.46) mean FSIQ 106.98 (SD 8.9)	Within 1st mth of life	Attention & WM: n-back Inhibition: flanker Object alternation learning Perceptual judgement: Birmingham object recognition battery	Lifetime Phe: annual median Phe levels used to calculate means for each 4-yr period from birth Concurrent Phe (most recent level) Recent Phe (mean level for the yr preceding testing)	N-back: Off-diet ET AwPKU less accurate than on-diet ECT AwPKU across conditions (p=.007); neither off-diet nor on-diet ET AwPKU groups differed from the control group. Both ET AwPKU groups significantly slower than controls across conditions (p=<.01); the two PKU groups did not differ significantly. Flanker: Off-diet ET AwPKU slower than controls (p=.001) & on-diet ECT AwPKU (p=.05) across conditions; the on-diet group did not differ significantly from controls. No significant differences in accuracy were observed. No significant between group differences in performance on object alternation learning or the perceptual judgement task observed.	Off-diet: Speed on 0-back was negatively correlated with Phe at age 1–4 yrs (r=-.58, p<.01). Speed on 2-back was negatively correlated with Phe at age 13–16 yrs (r=-.54, p<.01). Speed on 0-back was positively correlated with concurrent Phe (r=0.55, p<.01). On-diet: Speed on 0-back was positively correlated with Phe levels at age 5-8 (r=.55, p<.01). **authors used a significance level of 0.01*
Dawson et al. (2011)	50.0	UK	Cross-sectional	110 ET AwPKU Treated at least until adolescence: *56 off-diet:* 31F, 25M mean age 32.8 yrs (SD 5.7) *21 on-diet:* 19F, 2M mean age 29.4 yrs (SD 6.6) *33 on Pre-conception/ maternal (PC/Mat) diet:* mean age 32.3 yrs (SD 5.2) 110 age & gender matched controls	Unknown	Processing speed: reciprocal median latency (saccadometry)	Concurrent Phe Target levels (umol/L): Off-diet: N/A On-diet: <800 PC/Mat: 100-300	Significant difference in reciprocal median latency between off-diet ET AwPKU & controls (p=.02). No significant differences between on-diet ET AwPKU (p=.82) or ET AwPKU on PC/Mat diets (p=.85) & controls were found. Off-diet ET AwPKU had longer median latencies than on-diet ET AwPKU (p=.04) or those on PC/Mat diet (p=.01). Median latency improved significantly after onset of PC/Mat diet (p=.04).	Reciprocal median latency was correlated with concurrent Phe (r^2=.05, p=.02) - persisted after adjustment for age.

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
de Felice et al. (2018)	54.8	UK	Cross-sectional	38 ET AwPKU *31 on-diet* *7 unrestricted diet* mean age 27.3 yrs (SD 8.1) 25F, 12M mean FIQ 103.9 (SD 13.9) mean VIQ 101.4 (SD 13.8) mean PIQ 105.3 (SD 14.4) 39 healthy controls mean age 27.8 yrs (SD 8.4) 29F, 10M mean FIQ 113.9 (SD 9.7) mean VIQ 112.2 (SD 9.2) mean PIQ 112.4 (SD 10.9)	<u>Diagnosis:</u> 5-7 days after birth	<u>Narrative production</u>: Recalling the Cinderella story Blocked cyclic naming task <u>Prosody</u>: Nonemotional-, Emotional-, & Conflicting Prosody tests <u>Pragmatics</u>: Metaphor Picture test, Appreciation of Humour test, Comprehension of Inferred Mearning test <u>Lexical inhibition & strategic planning</u>: Hayling Sentence Completion Test	Concurrent Phe Lifetime Phe (across lifetime & 3 age bands): 1) childhood: 0–10 yrs old 2) adolescence: 11–16 yrs old 3) adulthood: 17 yrs - time of testing Phe level + Phe fluctuation	Compared to controls, ET AwPKU produced significant fewer correct information units (CIU; %) on the narrative production task (p=.01), but did not differ in speech errors (%), speech rate, mean length of utterance (MUL) or main concepts (%). No significant differences between groups on the Blocked Cyclic Naming task in terms of speed, accuracy or semantic interference. No significant between group differences in errors on any of the prosody tasks. ET AwPKU were significantly slower on tasks related to metaphor interpretation (p<.05) & comprehension of inferred meaning (p<.01), but not appreciation of humour (p=.15); no significant difference from controls in terms of accuracy on these tasks. No significant differences in performance (speed & accuracy) observed on part 1 of the Hayling Sentence Completion Task but ET AwPKU were significantly slower (p<.05) & made significantly more errors (p<.05) than controls on part 2.	No correlations between performance on complex language tasks & any of the metabolic measures were found.
Jahja et al. (2016)	50.0	the Nether-lands	Cross-sectional	95 ECT AwPKU *56 adults (>18 yrs)* 31F, 25M mean age 28.9 yrs (SD 6.5; range 18.7-42.8) 95 healthy controls *53 adults (>18 yrs)* 39F, 14M mean age 26.0 yrs (SD 5.8; range 18.1-40.8)	Treatment initiated immediately upon diagnosis through neonatal screening	<u>Social-cognitive abilities</u>: **Amsterdam Neurological Tasks (ANT)**: Face Recognition (FR) & Identification of Facial Emotions (IFE) tasks <u>Pen & paper (P&P) tasks:</u> Faux-Pas Recognition Test (FPT) & Reading the Mind in the Eyes (RME) task	Concurrent Phe Lifetime Phe Lifetime Phe: 0-7y, 8-12y 13-17y >18y	ECT AwPKU performed worse than controls on both the ANT (p<.05, η^2p=.040) and P&P tasks (p<.01, η^2p=.079). When including age as covariate, differences for the ANT tasks were no longer significant. When IQ was taken into account, no significant differences between ECT AwPKU & controls were observed.	No significant associations between social-cognitive abilities & any of the metabolic measures were observed.

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Jahja et al. (2017)	59.5	the Nether-lands	Cross-sectional	57 ECT AwPKU 24F, 33M mean age 27.7 yrs (SD 6.0; range 18.7- 40.0) 12 BH4 users 57 healthy controls 38F, 19M 4 relatives & 6 friends/ acquaintances of PKUs	Treatment initiated immediately upon diagnosis through neonatal screening	**ANT:** <u>WM</u>: Visuo-Spatial Sequencing (VSS), Memory Search 2-Dimensions (MS2D) Feature Integration (FI) <u>Inhibitory control & sustained attention</u>: Sustained Attention Dots (SAD)	Concurrent Phe <u>Childhood Phe:</u> mean of all half-yr median levels between 0-12 yrs of age <u>Lifetime Phe:</u> mean of all half-yr median levels from birth-day of testing	Lower accuracy on tasks with higher WM load were found across all groups ($p<.05, \eta^2p=.038$) ECT AwPKU were less accurate on the VSS task ($p<.01$, $\eta^2p=.060$), but not the MS2D & FI tasks. ECT AwPKU were significantly slower on all WM tasks ($p=.041$, $\eta^2p=0.186$) & showed a greater decline in RT when WM load increased ($p<.05$, $\eta^2p=.055$). ECT AwPKU were less accurate ($p<.01$, $\eta^2p=0.078$) & slower ($p=.034$ $\eta^2p=.038$) on the inhibitory control and sustained attention task (SAD). All participants made significantly more errors towards the end of the task & ECT AwPKU (not controls) showed a significant decrease in speed as the task progressed ($p<.05$, $\eta^2p=.043$). **Excluding BH4 users from analysis did not alter the results.*	Concurrent & lifetime Phe were significantly correlated ($r=.57$, $p<.001$). Concurrent Phe was significantly, positively correlated to speed in MS2D ($r=.33$, $p=.006$; high WM load condition), FI (low WM load: $r=.26$, $p=.024$; high WM load: $r=.26$, $p=.026$) & SAD ($r=.36$, $p=.003$ & $r=.41$, $p=.001$ at the end of the task). Lifetime Phe was significantly, positively correlated to number of errors (accuracy) on FI ($r=.24$, $p=.039$) & a trend was observed for childhood Phe ($r=.22$, $p=.051$). ECT AwPKU with childhood Phe ≥360µmol/L were less accurate than controls (VSS: $p=.23$, FI: $p=.020$) & ECT AwPKU with childhood Phe <360µmol/L (FI: $p=.02$) when WM load was high. ECT AwPKU with lifetime Phe ≥360µmol/L were less accurate on tasks of WM (VSS: $p=.007$) & inhibition & sustained attention (SAD: $p=.003$), as well as slower on a WM task (MS2D, high WM load: $p=.002$), when compared to controls. ECT AwPKU with concurrent Phe ≥360µmol/L were significantly slower than controls on tasks of WM (MS2D, high WM load: $p=.004$) & inhibition & sustained attention (SAD, end of task: $p=.009$).

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Jahja et al. (2017)	54.8	the Nether-lands	Longitudinal For: T1 see Huijbregts et al. (2002a, 2002b,2003) T2 approx. 15 yrs later)	21 ECT AwPKU 15F, 6M mean age 25.8 yrs (SD 2.3; range 21.0-30.5) 5 BH4 users Split into groups based on childhood Phe (IDC1) & Phe from age 13 (low: <360µmol/L & high: ≥360µmol/L): 1) low-low (n=3) 2) low-high (n=11) 3)high-high (n=7)	Treatment initiated immediately upon diagnosis through neonatal screening	**ANT:** Inhibitory control: Flanker (FL) Set Shifting Visual (SSV) Cognitive flexibility: SSV Executive motor control: Pursuit (PU)	Concurrent Phe mean of all half-yr median levels between 0-12; between 13-17 & ≥18 yrs of age IDC1: mean of all half-yr median levels from birth-day of testing (T1) IDC2/Lifetime Phe: mean of all half-yr median levels from birth-day of testing (T2) IDC difference score: IDC2-IDC1	Low-high ECT AwPKU were significantly faster than high-high ECT AwPKU on SSV (cognitive flexibility; p=.013, η^2p=.349) & had significantly more stable executive motor control (p=.031, η^2p=.259). All ECT AwPKU were significantly more accurate (p=.033, η^2p=.255) & more stable (p=.050, η^2p=.219) in executive motor control at T2 than T1. **Only the low-high (mean Phe <360µmol/L when 0-12 yrs; mean Phe ≥360µmol/L from age 13 onwards) & high-high (mean Phe ≥360µmol/L in childhood & onwards) groups were included in the analyses (n=18).*	FL: number of errors (accuracy) at T2 significantly, positively correlated Phe age 13-17 (r=.578, p=.004), IDC difference score (r=.533, p=.008; higher increase in Phe between T1 & T2 associated with poorer task performance) & IDC2 (r=.436, p=.027). SSV (cognitive flexibility): number of errors (accuracy) at T2 significantly, positively correlated with Phe aged 13-17 (r=.426, p=.039); speed at T2 significantly, positively associated with IDC1 (r=.446, p=.028) & Phe aged 0-12 (r=.429, p=.033). PU: mean (accuracy) & SD (stability) deviation at T2 significantly, positively related to IDC1 (r=.490, p=.012 & r=.550, p=.005, respectively) & Phe aged 0-12 (r=.404, p=.035 & r=.515, p=.008, respectively).
Liemburg et al. (2015)	50.0	the Nether-lands	Cross-sectional	55 ECT AwPKU 30F, 25M mean age 28.3 yrs (SD 6.2; range 18.7-40.0)	Treatment initiated immediately upon diagnosis through neonatal screening	**ANT:** Inhibitory control: SSV & SAD Cognitive flexibility: SSV WM: FI	Phe at diagnosis Concurrent Phe & Tyr Lifetime Phe	ECT AwPKU showed mainly problems with inhibitory control (SAD) & cognitive flexibility (SSV) compared to the healthy population (30-40% of the ECT AwPKU had a T-score ≥60; (1 SD above the mean); ANT z-scores were converted to T-scores).	N/A

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Moyle et al. (2006)	40.5	Australia	Cross-sectional	9 ECT AwPKU: 8F, 1M median age 26 yrs (IQR 25-30) 9 controls 8F, 1M median age 25 yrs (IQR 23-28) matched for education level	Unknown	Inhibition & processing speed: Go-Nogo	N/A	No significant differences observed between groups. Mean level of performance of ET AwPKU was in the predicted direction: more omissions & false alarms, & slower reaction times.	N/A
Moyle et al. (2007)	40.5	Australia	Cross-sectional	12 ET AwPKU unrestricted/ relaxed diet at time of testing 10F, 2M mean age 28.5 yrs (SE 3.3) 12 controls mean age 29.2 yrs (SE 3.2) matched for gender & yrs of education	From birth (discontinued during adolescence)	Verbal & visuo-spatial abilities, working memory, processing speed: Wechsler Adult Intelligence Scale-III (WAIS-III) Immediate & delayed visual & auditory memory: Wechsler Memory Scale-III (WMS-III) EF: TMT (set Switching), COWAT (phonemic & semantic fluency)	Lifetime Phe Most recent Phe	WAIS-III: ET AwPKU scored significant lower on POI ($p<.05$, $d=1.07$) & PSI ($p<.01$, $d=1.27$) than controls & significantly lower on PSI ($p=.045$) when compared to expected results from normative population. Compared to controls, ET AwPKU were significantly slower on TMT-A ($p<.05$, $d=1.02$) but not TMT-B. No significant differences observed between ET AwPKU & controls or normative data on the WMS-III. Observed between group differences on the COWAT were not significant.	No significant correlations between lifetime Phe & any of the cognitive measures were observed. Concurrent Phe levels were significantly correlated with POI scores ($r=-.60$, $p<.05$), all WMS-III scores (apart from WM; $r = -.74$ to $-.64$, $p<.05$) & the TMT-B ($r=.68$, $p<.05$). **All observed correlations indicate poorer performance with higher Phe levels.*

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Nardecchia (2015)	42.9	Italy	Longitudinal For: T1 see Leuzzi et al. (2004). T2 approx. 14 yrs later	14 ET AwPKU *9 with classical PKU* *5 with mild PKU* 12F, 2M aged 22.2-27.7 yrs (mean 24.97, SD 1.57) IQ 81-117 14 healthy controls 12F, 2M aged 21.0-28.2 yrs (mean 23.73, SD 2.59)	Diet onset: 15-61 (mean 33.14, SD 12.70) days after birth	EF: WCST (set-shifting), Elithorn's Perceptual Maze Test (EMPT; spatial planning, visuo-spatial & sustained attention), ROCFT - with copy & from memory (sustained attention, planning, visual organization of complex data, visual memory), Tower of London (ToL; planning & organization)	Concurrent Phe life-long IDC (IDC0-2): mean of all yearly medians from the beginning of the diet T2 IDC1-2: IDC during the interval between T1 & T2) IDC0-1: IDC from the beginning of the diet to T1 PHE04: IDC from the beginning of the diet to the fourth yr of life (index of early dietary control quality)	T2: Compared to controls, ET AwPKU performed significantly worse on EPMT (p=.0015) & the number of trials (p=.0255) & learning to learn (p=.0026) subtests of the WCST. ET AwPKU had significantly better performance on the ROCFT (with copy, p=.0142) & the number of trials subtest of WCST (p=.0072), & had a better global EF1 index (sum of scores from EPMT, ToL & ROCFT; p=.0219), compared to T1.	IDC1-2 significantly affected EF2 (sum of differential scores on WCST subtests; r=−.3177, p=.01) & ToL (r=−.0514, p=.016). IDC0-2 significantly affected EF2 (r=−.5410, p=.026). **All observed correlations indicate poorer performance with higher Phe levels.*

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Palermo et al. (2017)	50.0	UK	Cross-sectional	37 ET AwPKU *classical PKU* 24F, 13M mean age 27.5 yrs (SD 7.3) mean FSIQ 103.9 (SD 14.3) *7 unrestricted diet* *30 low-Phe diet* 30 healthy controls 20F, 10M mean age 27.6 yrs (SD 7.4) mean FSIQ 113.8 (SD 10.9)	Diagnosis: 5-7 days after birth	Visuo-spatial attention: simple detection, CRT (CANTAB), detection with distractors, opposite detection with distractors, feature search, conjoined search Visual memory & learning: delayed matching to sample, paired associates visual learning, RAVLT Visuomotor coordination: grooved pegboard, digit symbol Complex EF: TMT, Tower of Hanoi (TOH), WCST-64 card version Inhibition: Stroop interference, semantic interference WM: digit span, nonword repetition, Corsi block tapping Sustained attention: RVP (CANTAB) Verbal fluency: letter & semantic fluency Orthographic language: word & nonword reading, spelling, phoneme deletion, spoonerisms Spoken language: picture naming, colour naming (Stroop), similarities (WASI), vocabulary (WASI)	Concurrent Phe Lifetime Phe (across lifetime & 3 age bands): 1) childhood: 0–10 yrs old 2) adolescence: 11–16 yrs old 3) adulthood: 17 yrs - time of testing Phe level + Phe fluctuation	Overall, 17 ET AwPKU (46%) had a significantly higher rate of impaired measures than controls; 9 ET AwPKU (24%) showed a clear impairment and ET 14 AwPKU (38%) showed a completely normal profile. Impairments in most (except easiest) visuospatial attention tasks (slower on CRT, feature search, & conjoined search; p<.01), the sustained attention task (p=.03) & both visuomotor coordination tasks (p≤.01) were observed in the ET AwPKU group. ET AwPKU performed significantly worse on tasks of complex EF (WCST, ToH, but not TMT; p.05) & working memory (p≤.05), but not inhibition. Impairments in speed in word (p=.02) & nonword reading (p<.01) & semantic (but not letter) fluency (p=.01). ET AwPKU performed worse on the vocabulary (p<.01) & similarities (p=.01) subtests of the WASI; could be due to the reasoning component involved in these tests. No impairments in memory & learning were observed.	ET AwPKU with impaired (n=9) versus normal (n=14) performance did not have significantly different Phe levels during childhood, but differed significantly for metabolic control during adolescence (p=.04) & adulthood (p=.003), and at the time of testing (concurrent Phe; p=.01). They also differed in terms of Phe variability (average SD per yr) during adolescence (p=.002) and throughout life (p=.02).

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Pietz et al. (1998)	47.6	Germany	Cross-sectional	57 ET AwPKU *classical PKU* 33F, 24M aged 17-33 yrs (mean 23.6, SD 3.4) 40 healthy controls 24F, 16 aged 17-33 yrs (mean 23.0, SD not reported)	Diet onset: 4-90 days after birth	Fine motor abilities: **Motorische Leistungsserie (MLS)**: Steadiness (static tremor), Line Following, Short & Long Pins, Tapping, Pursuit Rotor (two sets) Visual-attention: Videotracking (slow & fast)	Phe0-12: mean of all half-yr medians for first 12 yrs of life Phe12-ADULT: mean of all half-yr medians from 12 yrs of age up to adulthood Phe0-ADULT: mean of all half-yr medians Concurrent Phe	A tremor was observed in 28% ET AwPKU (vs. 15% controls). MLS: factor analysis of all control data resulted in 4 MLS indices: hand-wrist steadiness (steadiness & line following), finger-hand dexterity (long pins & short pins), hand-wrist speed (aiming & tapping), & visuomotor ability (two pursuit rotor sets). ET AwPKU performed significantly worse on MLS indices of steadiness ($p<.01$), dexterity ($p<.01$) & speed ($p<.001$) as well as the videotracking task ($p<.001$).	No significant differences in metabolic control (all measures) between ET AwPKU with (n=16) & without (n=41) tremor were observed. No significant correlations between any of the MLS indices & measures of metabolic control or concurrent Phe were found. Performance on the videotracking task was significantly correlated with Phe0-12 ($r=-.34$, $p<.05$) & concurrent Phe ($r=-.37$, $p<.01$).
Ris et al. (1994)	40.5	USA	Cross-sectional	25 ET AwPKU *classical PKU* 12F, 13M aged 18+ yrs (mean 22) 15 unaffected siblings 9F, 6M Aged 18+ (mean 23)	Diet onset: <30 days of age (mean 16) Majority discontinued diet by/during adolescence; n=10 continuously treated	Mental flexibility & complex problem solving: WCST-Perseverative Responses (WCST-PR) Visuoconstructual ability: ROCFT Attention, organized visual search and information processing: Attention Diagnostic Method (ADM)	Concurrent Phe	ET AwPKU performed significantly worse than controls on ADM ($p=.04$) & ROCF ($p=.02$) tasks. Observed differences in performance on WCST-PR were not significant. No significant differences in performance observed between ET AwPKU & controls in 10 ET AwPKU-unaffected sibling pairs.	Concurrent Phe was significantly correlated to WCST-PR performance ($r=.59$, $p<.01$), with higher levels correlating to worse performance on this task.

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Romani et al. (2017)	54.8	UK	Cross-sectional	37 ET AwPKU 24F, 13M *classical PKU* mean age 27.5 yrs (SD 7.3) mean FSIQ 103.9 (SD 14.3) *7 unrestricted diet* *30 low-Phe diet* Split into 2 groups: 1) lower-Phe: (n=14) adult Phe <650 µmol/L 2) higher-Phe: (n=14) adult Phe >950 µmol/L 30 healthy controls 20F,10M mean age 27.6 yrs (SD 7.4) mean FSIQ 113.8 (SD 10.9) matched for educational status	<u>Diagnosis:</u> 5-7 days after birth	*See Palermo et al. (2017)*	Concurrent Phe Lifetime Phe across lifetime & 3 age bands: 1) childhood: 0–10 yrs old 2) adolescence: 11–16 yrs old 3) adulthood: 17 yrs–time of testing Phe level + Phe fluctuation	Compared to controls, ET AwPKU were significantly impaired in most cognitive domains except inhibitory control, & memory & learning (*see Palermo et al., 2017*). The lower-Phe group (adult Phe <650 µmol/L) outperformed the higher-Phe group (adult Phe >950 µmol/L) on all domains except inhibitory control & performance was significantly different for visuospatial attention ($p<.05$), visuomotor coordination ($p<.05$), & visual ($p=.01$) & verbal ($p<.05$) memory & learning. Overall, the lower-Phe group performed very close to the controls even if significant differences remained across most domains.	No significant correlations between metabolic measures & inhibitory control, working memory & orthographic language. Spoken language correlated with Phe fluctuation, not average Phe levels. For all other cognitive domains, a great majority of correlations were significant across different metabolic measures. Observed correlations were stronger for accuracy than speed measures. Associations with Phe levels diminish with age in the case of visuospatial attention. In contrast, for tasks tapping memory & learning, visuomotor coordination & sustained attention, correlations with current Phe are generally very high & higher than with measures taken at previous times. ** all observed correlations indicated worse performance with higher Phe levels* *** Pearson's correlations were of moderate to high strength (range 0.4-0.7); for specific correlations, see Romani et al. (2017)*

Author (year)	QAS (%)	Country	Design/ Intervention	Sample	Diagnosis/ Treatment initiated/ discontinued	Cognitive measures	Metabolic measures	Reported findings - Cognitive functioning	Relation to metabolic measures
Romani et al. (2017)	59.5	UK	Cross-sectional	37 ET AwPKU 24F, 13M *classical PKU* mean age 27.5 yrs (SD 7.3) mean FSIQ 103.9 (SD 14.3) *7 unrestricted diet* *30 low-Phe diet* 30 healthy controls 20F, 10M mean age 27.6 yrs (SD 7.4) mean FSIQ 113.8 (SD 10.9) matched for educational status	<u>Diagnosis:</u> 5-7 days after birth	*See Palermo et al. (2017)*	Concurrent Phe Lifetime Phe (across lifetime & 3 age bands: 1) childhood: 0–10 yrs old; 2) adolescence: 11–16 yrs old; 3): adulthood: 17 yrs–time of testing) Phe level + Phe fluctuation	Compared to controls, ET AwPKU were significantly impaired in most cognitive domains except inhibitory control, & memory & learning (see Palermo et al., 2017). ET AwPKU showed deficits in tasks tapping EFs & reduced speed across tasks. ET AwPKU do not seem to suffer from a generalized speed deficit. The observed patterns are consistent with a speed deficit in visuo-spatial attention, but not in lexical access. Slower responses across domains may be linked to a slower/more careful executive mechanism that decides when enough evidence is available to return an answer. A more conservative decision mechanism may wait for more evidence before returning an answer &/or hesitate/re-check the answer before returning it.	N/A
Weglage et al. (2013)	38.1	Germany	Longitudinal	57 ET AwPKU 37F, 20M *classical PKU* aged 19-41 yrs (mean 31, SD 5.9) 46 healthy controls 24F, 22M aged 20-45 yrs (mean 34.2, SD 10.5) matched for SES	<u>Diet onset:</u> within first 10 weeks of life (mean 7.8, SD 2.1)	<u>Information processing</u>: Zahlen-Verbindungs-Test (ZVT) <u>Sustained attention</u>: Test d2 All repeated at a five-yr-follow-up	Yearly blood Phe medians for 32 yrs & median blood Phe in adolescence (age 10-21 in intervals of 3 yrs)	No significant differences between ET AwPKU & controls were reported. When comparing younger & older ET AwPKU & controls, older (age >32 yrs) ET AwPKU showed significant slowing in information processing without making more errors. Performance for AwPKU compared to controls remained constant over the 5 yr period.	Performance on d2 as well as speed on ZVT were significantly correlated with adolescent median blood Phe at all intervals. ** all observed correlations were negative (indicating worse performance with higher Phe levels)* *** Pearson's correlations were of moderate strength (range 0.4-0.6); for specific correlations, see Weglage et al. (2013)*

Key Table 3.1: ADM: Attention Diagnostic Method; ANT: Amsterdam Neurological Tasks; AwPKU: adults with Phenylketonuria; BH4: tetrahydrobiopterin; BNT: Boston Naming Test; CANTAB: Cambridge Neuropsychological Test Automated Battery; COWAT: Controlled Oral Word Association Test; CPT: Continuous Performance Test; CRT: Choice Reaction Time; CVLT: California Verbal Learning Test; DPE: Dot Pattern Exercise; ECT: Early and continuously treated; EF: Executive Function; EMPT: Elithorn's Perceptual Maze; ET: Early treated; F: female; FI: Feature Integration; FIQ: full scale Intelligent Quotient; FL: Flanker; FPT: Faux-Pas Recognition Test; FR: Face Recognition; FSME: Finger Motor Speed Exercise; IDC: Index of dietary control; IFE: Identification of Facial Emotions; IQ: Intelligent Quotient; IQR: Interquartile range; LPE: Letter Pattern Exercise; M: male; MLS: Motorische Leistungsserie; MOT: Motor Screening Test MS2D: Memory Search 2-Dimensions; mth: month; P&P: Pen & Paper; Phe: Phenylalanine; PIQ: performance Intelligent Quotient; PKU: Phenylketonuria; PPVT-R: Peabody Picture Vocabulary Test-Revised; PSI: Processing Speed Index; PU: Pursuit; RAVLT: Rey Auditory Verbal Learning test; RME: Reading the Mind in the Eyes; RT: Reaction time; RVP: Rapid Visual Information Processing; ROCFT: Rey Osterrieth Complex Figure Test; SAD: Sustained Attention Dots; SD: Standard deviation; SES: socio-economic status; SOPT: Self-Ordered Pointing Test; SRT: Simple Reaction Time; SSP: Spatial Span; SSV: Set Shifting Visual ; SVAT: Sonneville Visual Attention Tasks; SWM: Spatial Working Memory; SOC: Stockings of Cambridge; SST: Stop Signal Task; TEA: Test of Everyday Attention; TOH: Tower of Hanoi; TOL: Tower of London; TMT (-A/B): Trail Making Test (-Part A/B); VIQ: verbal Intelligent Quotient; VSS: Visuo-Spatial Sequencing; WAIS-R/III: Wechsler Adult Intelligence Scale- Revised/ 3rd edition; WCST: Wisconsin Card Sorting Test; WCST(-PR): Wisconsin Card SortingTest (-Perseverative Responses); WM: Working memory; WMS(-III): Wechsler Memory Scale(-3rd edition); yrs: years; ZVT: Zahlen-Verbindungs-Test

Table 3.2 Overview of impairments reported in outcome measures of cognitive functioning in ET AwPKU across studies

	DISCONTINUED TREATMENT ("OFF-DIET")				MIXED SAMPLE (ON-DIET, RELAXED DIET, OFF-DIET)									"ON-DIET" (*=CONTINUOUSLY TREATED)							TOTAL
Reference [#]	**[1]**	**[2]**	**[3]**	**[4]**	**[5]**	**[6]**	**[7]**	**[8]**	**[9]**	**[10,11,12]**	**[13]**	**[14]**	**[15]**	**[1]**	**[16]**	**[17]**	**[3]**	**[18,19,20]**	**[21]**	**[22]**	
N (ET AwPKU)	*24*	*25*	*56*	*12*	*46*	*49*	*24*	*38*	*14*	*37*	*57*	*25*	*57*	*23*	*20**	*25**	*21*	*55-57**	*21**	*9**	
Attention & processing speed																					
Attentional capacity	0/1	**2/2**[e]				0/1	**3/4**			**4/10**[d]	**1/1**			0/1	**1/1**	**1/2**[d]					*11/20*[k]
Sustained attention	**1/1**					**1/1**	0/1			**1/1**			**1/1**[b]	**1/1**	**1/1**			**2/2**			*7/8*[k]
Processing speed			**1/1**[a]	**2/2**	0/2	0/1	0/2			0/1		**1/1**	**1/1**[b]				0/1			0/1	*5/12*[k]
Executive functions																					
Complex EF		0/1		**1/2**	**3/3**		**1/6**		**3/13**	**3/6**		0/1			0/2	0/1			**1/1**[f]		*12/35*[k]
Inhibitory control		**1/2**[d]				**1/1**[j]	**1/2**			0/3						0/2		**2/2**	0/4	0/1	*5/15*[k]
Working memory	0/1	**2/2**[e]		0/2	**1/1**	**2/2**	**2/2**			**2/3**				0/1	**1/1**	**1/2**[d]		**4/6**[g]			*14/20*[k]
Verbal fluency				0/2			**2/2**			**1/2**[h]					**1/1**						*4/8*
Language																					
Basic language skills: semantic processing		0/1		0/1			**1/2**	0/9		**1/5**[d]						0/1					*2/18*[k]
Complex language skills							0/2	**7/21**		**3/7**					0/1						*10/31*
Memory & learning				0/1																	
Immediate recall: verbal or visual				0/3			**2/2**[c]			0/3											*2/8*
Delayed recall: verbal or visual				0/2			**3/3**		0/1	0/3					0/2						*3/11*
Recognition: verbal or visual				0/1			**1/1**[c]			0/1					0/2						*1/5*
Motor skills					**1/1**		0/2			**2/2**	**3/4**								**1/1**[f]		*7/10*
Social-cognitive abilities																		**2/2**[j]			*2/2*
Visual-spatial abilities							**1/4**		0/1			**1/1**			0/1						*2/7*

Notes Table 3.2: [a] only for off-diet ET AwPKU; [b] only for older (>32) ET AwPKU; [c] Verbal memory/learning only; [d] significant differences observed in RT, not accuracy; [e] accuracy: different to controls, RT only different from on-diet ET AwPKU; [f] high-high PKU group worse performance than low-high PKU group (no controls included in analysis); [g] differences mainly observed in RT (only 1 measure of accuracy sign. different); [h] impairments in semantic, not letter fluency; [i] 1/2 disappeared when including age as covariate; no impairments observed when including IQ as a covariate; [j] only difference between <720 and >720 umol/L (no normative data available); [k] where off-diet and on-diet ET AwPKU reported separately, the total represents sum of outcome measures per paper, not row.

References Table 3.2: [1] Burgard et al. (1997); [2] Channon et al. (2007); [3] Dawson et al. (2011); [4] Moyle et al. (2007); [5] Bartus et al. (2018); [6] Bik-Multanowski et al. (2011); [7] Brumm et al. (2004); [8] de Felice et al. (2018); [9] Nardecchia et al. (2015); [10] Palermo et al. (2017); [11] Romani et al. (2017); [12] Romani et al. (2018); [13] Pietz et al. (1998); [14] Ris et al. (1994); [15] Weglage et al. (2013); [16] Channon et al. (2004); [17] Channon et al. (2005); [18] Jahja et al. (2017a); [19] Jahja et al. (2016); [20] Liemburg et al. (2015); [21] Jahja et al. (2017b); [22] Moyle et al. (2006).

Table 3.3 Overview of reported associations between metabolic control and measures of cognitive functioning in ET AwPKU

ATTENTION AND PROCESSING SPEED	**Attentional Capacity**			**Sustained attention**			**Processing speed**
	Overall	Accuracy	Speed	Overall	Accuracy	Speed	Overall
Concurrent Phe	**1/7**[a]	0/1	**1/1**[a]	**1/3**	0/1	**1/1**	**2/7**[b]
Concurrent Tyr							0/2
Recent Phe	0/1	0/1	0/1	0/1			
CHILDHOOD Phe							
0-10 years							
Average	**1/1**			0/1			
Variation	**1/1**			0/1			
Average + variation	0/1			0/1			
0-12 years	1/2				0/1	0/1	
0-4/1-4 years	0/1	0/1	**1/1**	0/1			
5.5-6 years (median)	**1/4**			0/1			0/2
5-8 years	0/1	0/1	**1/1**[a]	0/1			
9.5-10 years (median)	0/4			0/1			0/2
9-12/10-12 years	0/1	0/1	0/1	**1/2**			**1/1**
ADOLESCENT Phe							
11-16 years							
Average	0/1			0/1			
Variation	0/1			0/1			
Average + variation	0/1			0/1			
12 years – adulthood	0/1						
13-15/13-16/13-17 years	0/1	0/1	0/1	**1/2**			**1/1**
16-18 years	0/1	0/1	0/1	**1/2**			**1/1**

ATTENTION AND PROCESSING SPEED	Attentional Capacity			Sustained attention			Processing speed
	Overall	Accuracy	Speed	Overall	Accuracy	Speed	Overall
ADULT Phe							
17+ years							
Average	**1/1**			**1/1**			
Variation	0/1			**1/1**			
Average + variation	**1/1**			**1/1**			
17-20/19-21 years	0/2	0/2	0/2	**1/3**			**1/1**
21-24 years	0/1	0/1	0/1	0/1			
25-28 years	**1/1**	0/1	0/1	**1/1**			
29-32 years		0/1	0/1				
LIFETIME Phe							
Lifetime Phe					0/1	0/1	0/3
Average	**1/1**			0/1			
Variation	0/1			**1/1**			
Average + variation	**1/1**			**1/1**			
0 years – adulthood	0/1						

Table 3.3 (continued) - Overview of reported associations between metabolic control and measures of cognitive functioning in ET AwPKU

EXECUTIVE FUNCTIONS (EF)	**Complex executive functions**			**Inhibition**			**Working memory**		
	Overall	Accuracy	Speed	Overall	Accuracy	Speed	Overall	Accuracy	Speed
Concurrent Phe	**2/7**[a]	0/2	0/2	0/1	0/1	**1/3**	**1/5**	0/9	**3/7**
Concurrent Tyr	0/1	0/1	0/1					0/1	
Recent Phe						0/1	**1/1**[a]		
CHILDHOOD Phe									
0-10 years									
Average	0/1			0/1			0/1		
Variation	**1/1**			0/1			0/1		
Average + variation	0/1			0/1			0/1		
0-12 years		0/1	**1/1**		0/2	**1/2**		0/6	0/5
0-4/1-4 years	0/3					0/1	0/1	0/2	0/2
5.5-6 years (median)	0/1						**1/1**		
5-8 years						0/1	0/1	0/2	0/2
9.5-10 years (median)	0/1						0/1		
9-12/10-12 years						0/1	0/1	0/2	0/2
ADOLESCENT Phe									
11-16 years									
Average	**1/1**			0/1			0/1		
Variation	**1/1**			0/1			0/1		
Average + variation	**1/1**			0/1			0/1		
13-15/13-16/13-17 years		**1/1**	0/1		**1/1**	0/2	0/1	0/2	**1/2**
16-18 years						0/1			

EXECUTIVE FUNCTIONS (EF)	Complex executive functions			Inhibition			Working memory		
	Overall	Accuracy	Speed	Overall	Accuracy	Speed	Overall	Accuracy	Speed
ADULT Phe									
17+/18+ years		0/1	0/1		0/1	0/1			
Average	0/1			0/1			0/1		
Variation	0/1			0/1			0/1		
Average + variation	**1/1**			0/1			0/1		
17-20/19-21 years						0/1	0/1	0/2	0/2
21-24 years						0/1	**1/1**	0/2	0/2
25-28 years						0/1	**1/1**	0/2	0/2
29-32 years						0/1		0/2	0/2
LIFETIME Phe									
Lifetime Phe	0/1	0/1	0/1		0/1	0/1	0/2	**1/8**	0/5
Average	0/1			0/1					
Variation	**1/1**			0/1					
Average + variation	**1/1**			0/1					
MULTIPLE TESTING (Phe)									
IDC1		0/1	**1/1**		0/1	0/1			
IDC2		0/1	0/1	**1/1**		0/1			
IDC difference (IDC2-IDC1)		0/1	0/1		1/1	0/1			
IDC0-1 (8-13 years)	0/3								
IDC1-2	2/3								
IDC0-2 (22-27 years)	1/3								

Table 3.3 (continued) - Overview of reported associations between metabolic control and measures of cognitive functioning in ET AwPKU

EF + LANGUAGE	Verbal fluency (EF)	Basic language skills: semantic processing	Complex language skills	Orthographic language	Spoken language
Concurrent Phe	**1/5**	**2/11**	**1/24**	0/1	0/1
Recent Phe	0/1				
CHILDHOOD Phe					
0-10 years					
Average		0/9	0/21	0/1	0/1
Variation		0/9	0/21	0/1	**1/1**
Average + variation				0/1	0/1
0-12 years					
0-4/1-4 years	0/1				
5.5-6 years (median)	0/1	**2/2**	**1/2**		**4/5**
5-8 years	0/1				
9.5-10 years (median)	**1/2**	**2/2**	**1/2**		**4/5**
9-12/10-12 years	0/1				
ADOLESCENT Phe					
11-16 years					
Average		0/9	0/21	0/1	0/1
Variation		0/9	0/21	0/1	**1/1**
Average + variation				0/1	**1/1**
13-15/13-16 /13-17 years	0/1				
16-18 years	0/1				

EF + LANGUAGE	Verbal fluency (EF)	Basic language skills: semantic processing	Complex language skills	Orthographic language	Spoken language
	Overall	Overall	Overall	Overall	Overall
ADULT Phe					
17+/18+ years					
Average		0/9	0/21	0/1	0/1
Variation		0/9	0/21	0/1	**1/1**
Average + variation				0/1	**1/1**
17-20/19-21 years	0/1				
21-24 years	0/1				
25-28 years	0/1				
LIFETIME Phe					
Lifetime Phe	0/1		0/1	0/1	0/1
Average		0/9	0/21	0/1	**1/1**
Variation		0/9	0/21	0/1	**1/1**
Average + variation				0/1	0/1

Table 3.3 (continued) - Overview of reported associations between metabolic control and measures of cognitive functioning in ET AwPKU

MEMORY AND LEARNING (M&L)	**GM[c]**	**Immediate recall (verbal/visual)**			**Delayed recall (verbal/visual)**			**Recognition (verbal/visual)**			**Verbal M&L**	**Visual M&L**
		Overall	Verbal	Visual	Overall	Verbal	Visual	Overall	Verbal	Visual	Overall	Overall
Concurrent Phe	**1/1**	**1/1**	**1/2**	**1/2**		0/3	**1/2**		**1/2**		**1/1**	**1/1**
CHILDHOOD Phe												
0-10 years												
Average											**1/1**	**1/1**
Variation											0/1	**1/1**
Average + variation											0/1	0/1
0-4/1-4 years				0/1								
5.5-6 years (median)			**1/1**			0/2	**1/1**		**1/1**			
9.5-10 years (median)			0/1			0/2	0/1		0/1			
ADOLESCENT Phe												
11-16 years												
Average											0/1	**1/1**
Variation											**1/1**	**1/1**
Average + variation											**1/1**	**1/1**
ADULT Phe												
17+/18+ years												
Average											**1/1**	**1/1**
Variation											0/1	**1/1**
Average + variation											**1/1**	**1/1**
LIFETIME Phe												
Lifetime Phe		0/1	0/1	0/1		0/1	0/1		0/1			
Average											0/1	**1/1**
Variation											**1/1**	**1/1**
Average + variation											**1/1**	**1/1**

MEMORY AND LEARNING (M&L)	GM[c]	Immediate recall (verbal/visual)			Delayed recall (verbal/visual)			Recognition (verbal/visual)			Verbal M&L	Visual M&L
		Overall	Verbal	Visual	Overall	Verbal	Visual	Overall	Verbal	Visual	Overall	Overall
MULTIPLE TESTING (Phe)												
IDC0-1 (8-13 years)				0/1								
IDC1-2				0/1								
IDC0-2 (22-27 years)				0/1								

Table 3.4 Overview of tasks used to assess cognitive functioning in ET AwPKU across cognitive domains

Cognitive domain	Task[a] (reference [#])	Sensitivity[b]
Attention and processing speed		
Attentional capacity	California Verbal Learning Test (CVLT) – List A trial 1 [1]	1/1
	Choice Reaction Time [2,3]	1/2[c]
	Conjoined Search [2]	1/1[c]
	Detection with Distractors [2]	0/1
	Digit Span Forward (WAIS-R) [1]	0/1
	Feature Search [2]	1/1[c]
	n-back (0-back and 1-back trials) [4]	1/1[c]
	Stroop colour [1,2]	2/2[c]
	Stroop word [1]	0/1
	Telephone Search Test (TEA) [5]	1/1
	Video tracking [6]	1/1
		9/13
Sustained attention	Continuous Performance Test (CPT) – omission errors [1]	0/1
	Rapid Visual Processing [2,3]	2/2
	Sustained Attention Dots (ANT) [7]	2/2
	Dot Pattern Exercise (SVAT) [8]	
	Telephone Search Test with Counting (TEA) [5]	1/1
	Test d2 [9]	1/1[d]
		6/7
Processing speed	CPT – response rate [1]	0/1
	Motor Screening Test – latency [10]	0/1
	Saccadic Latency [11]	1/1[e]

Cognitive domain	Task[a] (reference [#])	Sensitivity[b]
	Simple Detection [2]	0/3
	Simple Reaction Time (CANTAB) [3]	
	Finger Motor Speed Exercise (SVAT) [8]	
	Stockings of Cambridge – initial thinking time [10]	0/1
	Trail Making Test-A [1,9]	2/3
	Attention Diagnostic Method [12]	
	WAIS-III (Processing Speed Index) [13]	1/1[e]
		4/11
Executive functions		
Complex executive functions	Brixton Test [5]	0/1
	Elithorn Perceptual Maze Test [14]	1/1
	Object alternation learning [4]	0/1
	Set Shifting Visual (ANT) [15]	1/1[f]
	Six Elements Test [5]	0/1
	Spatial Working Memory – Strategy [10]	1/1
	Stockings of Cambridge [10]	1/1
	Tower of Hanoi [2]	1/2
	Tower of London [14]	
	Trail Making Test (TMT) B-A [1, 2, 13]	0/3
	WAIS-III (Perceptual Organisation Index) [13]	1/1[e]
	Wisconsin Card Sorting Test (WCST) [1, 2, 12, 14]	3/4
		9/17
Inhibitory control	CPT [1]	1/1
	Flanker [4, 15]	1/2[c]
	Go-nogo [16]	0/1
	Set Shifting Visual (ANT) [15]	0/1

Cognitive domain	Task[a] (reference [#])	Sensitivity[b]
	Stop Signal Task [3]	1/1
	Stroop (interference) [1, 2]	0/2
	Sustained Attention Dots (ANT) [7]	1/1
		4/9
Working memory	Corsi Block Tapping Test [2]	0/1
	CVLT – perseverative error [1]	1/1
	Digit Span – backward [1, 2]	2/2
	Feature Integration (ANT) [7]	1/1
	Letter Pattern Exercise (SVAT) [8]	0/1
	Memory Search 2 Dimensional (ANT) [7]	1/1
	n-back (2-back trials) [4]	1/1[c]
	Non-word Repetition [2]	1/1
	Self-Ordered Pointing test [5]	1/1
	Spatial Span [3]	1/1
	Spatial Working Memory [3, 10]	2/2
	Visuo-Spatial Sequencing (ANT) [7]	1/1
	WAIS-III (Working Memory Index) [13]	0/1
		12/15
Verbal fluency	Animal naming [1, 2]	2/2
	Controlled Oral Word Association Test [1, 13]	1/2
	Letter fluency [2, 5]	1/2
		4/6
Language		
Basic language skills: semantic processing	Boston Naming Test [1] Picture naming [2]	1/2
	Blocked cyclic naming [17]	0/1

Cognitive domain	Task[a] (reference [#])	Sensitivity[b]
	Emotional Prosody Discrimination test [17]	0/1
	Hayling Sentence Completion Test – Part A [17]	0/1
	Narrative production: Recalling the Cinderella story [17]	0/1
	Non-emotional Prosody Discrimination test [17]	0/1
	Peabody Picture Vocabulary Test – Revised (PPVT-R) [1]	0/1
	Word reading [2]	1/1[c]
	Word spelling [2]	0/1
		2/10
Complex language skills	Appreciation of Humour test [17]	0/1
	Blocked cyclic naming – semantic Inference [17]	0/1
	Comprehension of Inferred Meaning test [17]	1/1[c]
	Conflicting prosody – attend to prosody test [17]	0/1
	Hayling Sentence Completion Test – Part B [5, 17]	1/2
	Metaphor Picture test [17]	1/1[c]
	Naming: semantic inference [2]	0/1
	Narrative production: Recalling the Cinderella story [17]	1/1
	Non-word reading [2]	1/1[c]
	Non-word spelling [2]	0/1
	Phoneme deletion [2]	0/1
	Similarities (WAIS-R/WASI) [1,2]	1/2
	Spoonerisms [2]	0/1

Cognitive domain	Task[a] (reference [#])	Sensitivity[b]
	Vocabulary (WAIS-R/WASI) [1, 2]	0/2
	WAIS-III (Verbal Comprehension Index) [13]	0/1
	Perceptual judgement task [4]	0/1
		6/19
Memory and learning		
Immediate recall: verbal / visual	CVLT – List A trial 5 and trials 1-5 [1]	1/1
	Paired Associates Verbal Learning – trial 1-5 [2]	0/1
	Paired Associates Visual Learning [2]	0/1
	Rey Auditory Verbal Learning Test (RAVLT) – Trial A1-A5 [2]	0/1
	ROCFT – Immediate recall [14]	0/1
	WMS-III [13]	0/1
		1/6
Delayed recall: verbal / visual	CVLT – Sort delayed recall and Long delayed recall [1]	1/1
	Paired Associates Verbal Learning – delayed [2]	0/1
	RAVLT – Delayed recall [2, 5] and retention [2]	0/2
	ROCFT – Delayed recall [1, 5]	0/2
	WMS-III [13]	0/1
		1/7
Recognition: verbal / visual	CVLT – Recognition memory [1]	0/1
	Delayed Matching to a Sample [2]	0/1
	RAVLT – Recognition [5]	0/1
	ROCFT – Recognition [5]	0/1
	WMS-III [13]	0/1
		0/5

Cognitive domain	Task[a] (reference [#])	Sensitivity[b]
Motor skills		
	Digit Symbol (Substitution) Task [1, 2]	1/2
	Grooved Pegboard [1, 2]	1/2
	Motorische Leistungsserie [6]	1/1
	Motor Screening Test – errors [10]	1/1
	Pursuit (ANT) [15]	1/1[f]
		5/6
Social-cognitive abilities		
	Face Recognition (ANT) [18]	1/1[g]
	Faux-Pas Recognition Test [18]	1/1[h]
	Identification of Facial Emotions (ANT) [18]	1/1[g]
	Reading the Mind in the Eyes Task [18]	1/1[h]
		4/4
Visual-spatial abilities		
	Block design (WAIS-R) [1]	0/1
	Picture arrangement (WAIS-R) [1]	0/1
	Picture completion (WAIS-R) [1]	0/1
	ROCFT – with copy/initial copy [1, 5, 12, 14]	2/4
		2/7

Notes Table 3.4: [a] number of observed impairments in task performance in ET AwPKU / frequency of us; b (–sub measure) if task relates to multiple cognitive domains); [c] compared to controls, ET AwPKU differed in speed (slower), not accuracy; [d] only for older (>32) ET AwPKU; [e] only for off-diet ET AwPKU; [f] high-high ET AwPKU group worse performance than low-high PKU group (no controls included in analysis); [g] effect disappeared after adding age as a covariate; [h] effect disappeared after including IQ as a covariate

References Table 3.4: [1] Brumm et al. (2004); [2] Palermo et al. (2017); [3] Bik-Multanowski et al. (2011); [4] Channon et al. (2007); [5] Channon et al. (2004); [6] Pietz et al. (1998); [7] Jahja et al. (2017a); [8] Burgard et al. (1997); [9] Weglage et al. (2013); [10] Bartus et al. (2018); [11] Dawson et al. (2011); [12] Ris et al. (1994); [13] Moyle et al. (2007); [14] Nardecchia et al. (2015); [15] Jahja et al. (2017b); [16] Moyle et al. (2006); [17] de Felice et al. (2018); [18] Jahja et al. (2016).

3.3.2 Cognitive outcomes in ET AwPKU: overview of reported results

The following section provides an overview of cognitive outcomes in ET AwPKU. Where possible, outcomes in adulthood are compared with outcomes in the same sample during childhood (Jahja, van Spronsen, et al., 2017; Nardecchia et al., 2015).

As can be seen in Tables 3.1 and 3.4, a large number of different cognitive tasks were used, spanning various cognitive domains. Furthermore, some discrepancy between papers exists with regards to the domains that cognitive tasks are ascribed to. For the purpose of this review, cognitive outcomes are categorised according to their cognitive domains. There are many different conceptualisations regarding how different cognitive tasks associate with one another and with particular cognitive domains. The framework used for the current review was adapted from a commonly used approach to understanding and measuring cognitive domains (Lezak, Howieson, Bigler, & Tranel, 2012). For a description of cognitive domains, subdomains and examples of tests reflecting each domain as applied to the studies reported in this review, see Galioto et al. (2016). Note, however, that Galioto et al. (2016) describe verbal fluency as a function of language, whereas this review follows Lezak et al. (2012)'s original framework, classifying it as an EF. Table 3.1 summarises cognitive outcomes as reported in the papers included in this review. In Tables 3.2-3.4, outcomes have been re-categorised in line with the framework used here.

3.3.2.1 Attention and processing speed

3.3.2.1.1 Attentional capacity

Healthy controls outperformed ET AwPKU on the majority of measures of attentional capacity used across several studies included in this review (Brumm et al., 2004; Channon et al., 2004, 2007; Palermo et al., 2017). However, it was found that ET AwPKU were often slower, but not less accurate, than controls (Channon et al., 2007; Palermo et al., 2017). Furthermore, Channon et al. (2007) observed differences in accuracy between off- and on-diet ET AwPKU, with the off-diet group making more errors compared to the on-diet group. Using an aggregate score for performance on attention tasks included in their study, Romani et al. (2017) reported that the ET AwPKU with low adult Phe levels significantly outperformed the high-Phe group. Bik-Multanowski et al.

(2011) and Brumm et al. (2004) found no differences in performance ET AwPKU with high compared to low concurrent Phe levels.

The relationship between performance on tasks reflecting attentional capacity and measures of metabolic control was assessed in seven studies. Only two of these reported a relationship between concurrent Phe and measures of attentional capacity (Channon et al., 2007; Pietz et al., 1998). However, the observed correlations were not in the expected direction, suggesting that attentional capacity was better with higher concurrent levels of Phe. Several papers reported significant correlations with metabolic control during childhood (Brumm et al., 2004; Channon et al., 2007; Romani et al., 2017), adulthood (Channon et al., 2004; Romani et al., 2018) as well as throughout life (Romani et al., 2017), with the majority (n=10/11, see Table 3.3) suggesting lower Phe levels were associated with better task performance. However, no correlations between adolescent Phe levels and attentional capacity were reported. Furthermore, the correlations observed by Channon et al. (2007) were limited to measures of speed, with no correlations for accuracy.

3.3.2.1.2 Sustained attention

Compared to healthy controls, ET AwPKU have consistently been found to show impairment on measures of sustained attention (Burgard et al., 1997; Channon et al., 2004; Jahja, Huijbregts, et al., 2017; Palermo et al., 2017; Weglage et al., 2013). In one study, however, this impairment was only observed in older (>32 years old) ET AwPKU (Weglage et al., 2013). Brumm et al. (2004) reported no group deficit on a continuous performance task (CPT) when comparing number of omission errors of ET AwPKU with normative data, but did find that ET AwPKU with high concurrent Phe performed significantly worse than those with low concurrent Phe. This is in line with results reported by Bik-Multanowski et al. (2011) and Romani et al. (2017), although observed differences in performance of the low and high Phe groups in the latter study failed to reach significance.

Observed associations between measures of metabolic control and sustained attention in ET AwPKU are somewhat inconsistent but suggest childhood Phe levels are not related to sustained attention in ET AwPKU, whereas significant negative correlations with adult Phe have been found. Inconsistent results have been reported for concurrent,

adolescent and lifetime Phe levels Jahja, Huijbregts et al. (2017) and Romani et al. (2017) reported significant correlations between concurrent Phe and measures of sustained attention, whereas Brumm et al. (2004) did not. Romani et al. (2017) also reported a significant association between sustained attention and metabolic control during adolescence. However, this was not observed by Weglage et al. (2013). Finally, Romani et al. (2017) found a significant correlation between an aggregated score of measures of sustained attention and lifetime Phe, whereas Jahja, Huijbregts et al. (2017) reported no significant associations between the two.

3.3.2.1.3 Processing speed

It has been suggested that observed cognitive deficits in ET AwPKU could be due to a deficit in information processing in these patients. It is not uncommon for ET AwPKU to be slower, but not less accurate on various measures spanning different cognitive domains. Romani et al. (2018) investigated processing speed in ET AwPKU. Their results suggest that ET AwPKU do not suffer from an overarching deficit in speed of processing, but rather that reduced speed of performance on tasks across multiple cognitive domains could be the result of slower or more cautious executive decision-making processes (Romani et al., 2018).

In line with their findings, performance of ET AwPKU on 'pure' processing speed outcome measures, such as simple reaction time, was not generally impaired in the studies included in this review. Compared to controls, ET AwPKU demonstrated slower reaction times on approximately half of the processing speed measures reported in studies included in this review (Dawson et al., 2011; Moyle, Fox, Bynevelt, et al., 2007; Ris et al., 1994; Weglage et al., 2013). In two of these studies, these deficits were observed in a group of ET AwPKU who had discontinued dietary treatment (Dawson et al., 2011; Moyle, Fox, Bynevelt, et al., 2007). In another study, the impairment in information processing was only found for older (>32 years) ET AwPKU (Weglage et al., 2013). However, four of the studies included in this review reported no impairments in performance on measures of processing speed in either on or off-diet ET AwPKU (Bartus et al., 2018; Brumm et al., 2004; Burgard et al., 1997; Palermo et al., 2017). When comparing groups of ET AwPKU with different levels of metabolic control, Brumm et al. (2004) reported that ET AwPKU with high concurrent Phe levels were significantly slower

than those with low concurrent Phe levels, whereas Bik-Multanowski et al. (2011) and Bartus et al. (2018) found no differences between patients with good versus poor concurrent and childhood (between 0-12 years) metabolic control.

Five studies investigated associations between simple measures of processing speed and measures of metabolic control. Brumm et al (2004) and Bartus et al. (2018) observed no correlations, whereas Weglage et al. (2013) reported negative correlations with Phe levels during childhood, adolescence and young adulthood. Furthermore, two studies reported a relationship between speed of processing and concurrent Phe levels, but the direction was inconsistent: one study reported a negative relationship (Dawson et al., 2011) while the other reported a positive relationship (Ris et al., 1994). Significant correlations were generally more frequently observed with measures of speed compared with measures of accuracy.

3.3.2.2 Executive functions

3.3.2.2.1 Complex executive functions

Although reasoning and planning, flexibility (set-shifting/switching), organisation, monitoring and rule finding are separate executive functions (EF), several of the cognitive tasks used in the studies reported here concurrently engage more than one EF and are often reported as measures of complex EF, higher order EF, or “multi-tasking”. Reported findings across studies suggest a contrast between performance on tasks that require different levels of planning/reasoning and flexibility, with deficits in ET AwPKU being more pronounced in tasks requiring more planning/reasoning and flexibility. For example, deficits in performance on the Wisconsin Card Sorting Test (WCST) were reported by Brumm et al. (2004), Nardecchia et al. (2015) and Palermo et al. (2017), but not by Ris et al. (1994). Furthermore, Bartus et al. (2018) reported that controls outperformed ET AwPKU on measures of problem solving (Stockings of Cambridge of the Cambridge Neuropsychological Test Automated Battery (CANTAB)) and strategy (Spatial Working Memory (CANTAB)), whereas Channon et al. (2004) and Nardecchia et al. (2015) did not observe any deficits in performance on the Brixton task or Elithorn Perceptual Maze Test respectively. Some of the reported impairments in complex EF were only observed for ET AwPKU with poor metabolic control throughout childhood

(Bartus et al., 2018; Jahja, van Spronsen, et al., 2017) or off-diet ET AwPKU (Moyle, Fox, Bynevelt, et al., 2007). However, although ET AwPKU with lower concurrent Phe-levels showed better performance on complex EF tasks, none of the studies reported significant differences between ET AwPKU with good versus poor concurrent metabolic control (Bartus et al., 2018; Brumm et al., 2004; Romani et al., 2017).

Relationships with metabolic control throughout life and complex EF were observed, but better metabolic control during adolescence seems to be the strongest indicator of better complex EF during adulthood (Nardecchia et al., 2015; Romani et al., 2017). Reported correlations between concurrent Phe and complex EF were not in the expected direction, suggesting ET AwPKU with higher concurrent levels of Phe performed better on complex EF tasks than those with better metabolic control at the time of testing (Moyle, Fox, Bynevelt, et al., 2007).

3.3.2.2.2 Inhibitory control

The majority of the studies that included measures of inhibitory control did not reveal any significant impairments in inhibition in ET AwPKU compared to controls (Jahja, Huijbregts, et al., 2017; Jahja, van Spronsen, et al., 2017; Moyle et al., 2006; Palermo et al., 2017), although the PKU group tended to be slower, not less accurate, than the control group in one of the studies included in this review (Channon et al., 2007). The PKU-COBESO study was the only study to report ET AwPKU were both significantly less accurate and slower compared to controls (Jahja, Huijbregts, et al., 2017). Moyle et al. (2006) observed a similar trend in a smaller sample of ET AwPKU but failed to find any significant differences. Based on normative data available for measures included in their study, Brumm et al. (2004) reported that ET AwPKU performed below expectation (see Table 1 (Additional file 1)) on several (CPT, Digit Span backwards and WCST), but not all (Stroop, Trail Making Task part B), measures of inhibitory control. However, they observed no significant differences in performance between ET AwPKU with good and poor concurrent metabolic control on any of the tasks. Similarly, a recent study found no significant differences in task performance between ET AwPKU with low and high concurrent Phe levels (Romani et al., 2017). In contrast, Bik-Multanowski et al. (2011) reported significant differences in performance on the CANTAB Stop-Signal Task

between ET AwPKU with good and poor metabolic control, with the ET AwPKU with poor metabolic control showing worse performance.

After splitting their ET AwPKU sample into high and low Phe groups, Jahja, Huijbregts et al. (2017) reported that, compared to controls, only ET AwPKU with high lifetime Phe levels were slower and less accurate on an inhibitory control task. Furthermore, their results showed that concurrent Phe was positively associated with reaction times, but no correlations between childhood, adolescent, adult or lifetime Phe levels and accuracy or speed were found. Romani et al. (2017) observed no correlations between measures of inhibition and any of the measures of metabolic control included in their research.

3.3.2.2.3 Working memory

Studies investigating performance of ET AwPKU on working memory or short-term memory tasks showed contradictory findings (Bartus et al., 2018; Bik-Multanowski et al., 2011; Brumm et al., 2004; Burgard et al., 1997; Channon et al., 2004, 2007; Jahja, Huijbregts, et al., 2017; Moyle, Fox, Bynevelt, et al., 2007; Palermo et al., 2017).

In terms of accuracy, the majority of studies reported that ET AwPKU made significantly more errors compared to controls or normative data (Bartus et al., 2018; Bik-Multanowski et al., 2011; Brumm et al., 2004; Channon et al., 2004; Jahja, van Spronsen, et al., 2017; Palermo et al., 2017). In contrast, the remaining three studies, two of which included off-diet ET AwPKU, did not find significant differences in accuracy on working memory tasks between ET AwPKU and healthy controls (Burgard et al., 1997; Channon et al., 2007; Moyle, Fox, Bynevelt, et al., 2007). Even though they did not observe any differences between ET AwPKU and controls, Channon et al. (2007) reported that off-diet ET AwPKU were significantly less accurate on the n-back task than on-diet ET AwPKU. Again, roughly half of the papers reporting measures of speed found that both on-diet and off-diet ET AwPKU were significantly slower than healthy controls (Channon et al., 2007; Jahja, Huijbregts, et al., 2017). Jahja, Huijbregts et al. (2017) observed a significantly greater decline in speed with increasing working memory load on two of their measures, whereas other studies did not (Channon et al., 2007; Moyle, Fox, Bynevelt, et al., 2007).

When exploring relationships between working memory and metabolic control, Channon et al. (2004) reported poor performance on tasks of working memory was related to high concurrent and average recent (year preceding testing) Phe levels as well as elevated Phe levels between the ages of 21 and 28 years. In another study, despite not showing any significant deficits in working memory in on-diet and off-diet ET AwPKU, speed on the 2-back task was found to be related to Phe levels between the ages of 13-16 years (Channon et al., 2007). In the PKU-COBESO study (Jahja, Huijbregts, et al., 2017), ET AwPKU were divided into low- and high-Phe groups based on concurrent as well as average childhood, adolescence, adult and lifetime Phe levels. In line with findings of Bik-Multanowski et al. (2011), results showed that higher concurrent Phe levels resulted in slower speed on two of the three working memory tasks used in this study (Feature Integration (FI) and Memory Search 2-Dimensional (MS2D) of the Amsterdam Neurological Tasks (ANT) battery). Additionally, lifetime Phe levels were positively related to the number of errors made on tasks with a high working memory load. Furthermore, analyses revealed that ET AwPKU with high average childhood Phe levels were significantly less accurate than controls on two of three working memory tasks (Visuo-Spatial Sequencing (VSS) and FI). They were also significantly less accurate on the FI task compared to ET AwPKU with low childhood Phe levels. Finally, ET AwPKU with high childhood and lifetime Phe levels were found to be significantly slower than controls on the MS2D task. Romani et al. (2017) did not find any significant relationships between performance on tasks of working memory and concurrent Phe or averages and variations of childhood, adolescent, adult and lifetime Phe levels, but reported that the group with low concurrent Phe levels outperformed the high-Phe group. Bartus et al. (2018) did not find any significant differences in accuracy on the SWM (CANTAB) task between on-diet ET AwPKU and those on a “loose” diet, but did show that ET AwPKU with better metabolic control during childhood made less errors than those with poorer control. There does not seem to be a clear association between measures of working memory and measures of metabolic control: the majority of studies observed no relationships, with the exception of some correlations found with concurrent Phe and Phe at different stages of life (see Table 3) (Brumm et al., 2004; Channon et al., 2004, 2007; Jahja, Huijbregts, et al., 2017).

3.3.2.2.4 Verbal fluency

Verbal fluency refers to the ability to orally produce words that either fit into a specific category (category or semantic fluency) or start with a specific letter (letter or phonemic fluency). It has been suggested that language processing is the critical component of verbal fluency (Whiteside et al., 2016). However, because verbal fluency tasks involve a planned, systematic search of the lexicon, they are often regarded as measures of EF (Lezak et al., 2012). Four studies included in this review assessed verbal fluency in ET AwPKU (Brumm et al., 2004; Channon et al., 2004; Moyle, Fox, Bynevelt, et al., 2007; Palermo et al., 2017). Letter fluency was reported to be impaired by Brumm et al. (2004) and Channon et al. (2004), but not Palermo et al. (2017). Palermo et al. (2017) did, however, find deficits in category fluency, as did Brumm et al. (2004). In contrast, Moyle et al. (2007) found no deficits in either category or letter fluency in a small sample of off-diet ET AwPKU. There was no clear evidence for associations between metabolic control and verbal fluency abilities in ET AwPKU.

3.3.2.3 Language (semantic processing)

Measures of semantic processing assess comprehension of language as well as speed of retrieval of information (Galioto & Spitznagel, 2016; Lezak et al., 2012). Examples of semantic processing tasks include expressive and receptive vocabulary, expressive naming (spoken language), as well as measures of spelling and reading (orthographic language). Five studies assessed language processing in ET AwPKU. In contrast to Brumm et al. (2004), Palermo et al. (2017) and De Felice et al. (2018) found no deficits on a basic picture naming task. In line with this, apart from a reduction in speed of word reading (Palermo et al., 2017), no issues in basic language skills, including receptive vocabulary, as well as measures of prosody, reading and spelling without inference were observed in ET AwPKU (Brumm et al., 2004; De Felice et al., 2018; Palermo et al., 2017). Performance of ET AwPKU on complex language tasks, requiring EF such as planning, inhibition and reasoning, has been inconsistent (see Table 4). Most studies reported no deficits (Brumm et al., 2004; Channon et al., 2007; Moyle, Fox, Bynevelt, et al., 2007), but impaired performance has been observed on several, but not all, complex language tasks included in two studies (De Felice et al., 2018; Palermo et al., 2017). When

measures of accuracy and speed have been reported separately, it appears that ET AwPKU are slower but not less accurate on tasks that suggest impaired complex language processing (De Felice et al., 2018; Palermo et al., 2017).

With respect to the impact of Phe, Romani et al. (2017) found significant correlations between a composite measure of the spoken language tasks used in their study (picture and colour naming and both WASI verbal subtests) and fluctuations in Phe as well as overall metabolic control, but not average Phe levels, across the lifespan. No correlations were found between any metabolic measures and performance on tasks assessing orthographic language. Furthermore, ET AwPKU with better metabolic control during adulthood performed better on all language tests, but these differences were only significant for measures of spoken language. However, no significant differences in composite measures of spoken or orthographic language were observed between groups of ET AwPKU with high versus low concurrent Phe levels (Romani et al., 2017). Brumm et al. (2004) reported that performance on spoken language tasks (expressive naming, expressive vocabulary and receptive vocabulary, but not the similarities subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R)) was better in ET AwPKU with better metabolic control at the time of testing and that performance on these measures was negatively correlated with blood Phe levels across the lifespan. De Felice et al. (2018) found no associations between measures of metabolic control and any of the language processing measures administered and, moreover, reported no significant differences in performance between ET AwPKU with low versus high average Phe levels.

3.3.2.4 Memory and Learning

The majority of studies assessing verbal and visual immediate recall, delayed recall, or recognition memory in both on-diet and off-diet ET AwPKU did not report any impairments in ET AwPKU (Channon et al., 2004; Moyle, Fox, Arthur, et al., 2007; Nardecchia et al., 2015; Palermo et al., 2017). However, Romani et al. (2017) reported that despite not finding any significant differences between ET AwPKU and controls on individual tasks of memory and learning, ET AwPKU seemed to perform marginally worse across tasks when the scores were aggregated (Romani et al., 2017). Furthermore, they reported that their lower-Phe group outperformed their higher-Phe

group. In contrast, Brumm et al. (2004) reported cognitive impairments in immediate, short-term and long-term verbal and visual delayed recall, but did not report any significant differences in memory task performance between ET AwPKU with high or low concurrent Phe levels. In their study, immediate and delayed verbal and visual recall were found to be negatively correlated with median Phe levels between the ages of 5.5 and 6 years (Brumm et al., 2004). Romani et al. (2017) reported negative correlations between aggregated memory and learning scores and concurrent Phe as well as average Phe and variation of Phe levels across the lifespan. Other studies found no correlations between performance on memory tasks and any of the metabolic measures included (Channon et al., 2004; Moyle, Fox, Arthur, et al., 2007; Nardecchia et al., 2015).

3.3.2.5 Motor skills

Results of assessments of motor skills are mixed but suggest an impairment in ET AwPKU (Bartus et al., 2018; Brumm et al., 2004; Jahja, van Spronsen, et al., 2017; Palermo et al., 2017; Pietz et al., 1998). Using a battery of 7 tests to assess fine motor abilities, Pietz et al. (1998) reported deficits in steadiness (tremor), dexterity and speed, but not visuomotor abilities. None of the observed deficits appeared to correlate significantly with any of their indices of metabolic control. Jahja, Huijbregts et al. (2017) found that ET AwPKU with low average Phe levels during childhood were better at a motor task that involved continuous monitoring of task performance (following a randomly moving target) than those who had high average childhood Phe levels. They reported significant correlations between task performance and childhood Phe levels (Jahja, van Spronsen, et al., 2017). Palermo et al. (2017) also observed significant deficits in ET AwPKU on two tasks (Digit Symbol Substitution Task (DSST) and Grooved Pegboard) assessing visuomotor coordination and, using a composite measure, reported that ET AwPKU with low concurrent Phe levels outperformed those with high levels at the time of testing. Furthermore, they reported significant correlations between a composite score of both tasks and concurrent Phe, childhood Phe variation and average levels, and adolescent, adult and lifelong Phe variation as well as overall metabolic control (Romani et al., 2017). Using the same two tasks, Brumm et al. (2004) did not find any deficits in ET AwPKU but did report that ET AwPKU with low Phe levels at the time of testing outperformed those with high concurrent Phe levels on the DSST. Finally, Bartus et al. (2018) reported

significant differences in accuracy on the CANTAB Motor Screening Test (MOT) between ET AwPKU and controls, with controls outperforming the ET AwPKU, but did not report any differences between ET AwPKU with good versus poor metabolic control during childhood (0-12 years) or at the time of testing. Both Brumm et al. (2004) and Bartus et al. (2018) did not find any associations between visuomotor coordination and any of the metabolic outcomes included in their studies.

3.3.2.6 Social-cognitive abilities

"Social cognition involves all mental processes that underlie social interactions and comprises the ability to perceive, to interpret and to respond appropriately to social cues" (Jahja et al., 2016, p.356). Examples of social-cognitive abilities include the ability to recognise faces and identify emotions (Jahja et al., 2016). Only one study to date has assessed social-cognitive abilities in ET AwPKU (Jahja et al., 2016). ET AwPKU performed worse than controls on all four tasks included in the research. When controlling for age, impairments in ET AwPKU were only observed on two of the tasks. When IQ was taken into account, no significant differences between ET AwPKU and controls were reported. No significant associations between social-cognitive outcomes and concurrent or lifetime measures of metabolic control were found.

3.3.2.7 Visual-spatial abilities

Measures of visual-spatial abilities reflect planning, reasoning, memory and motor skills. Using the 'with copy' subtest of the Rey Österrieth Complex Figure Test (ROCFT), two studies reported impairments in a mixed sample of on- and off-diet ET AwPKU (Brumm et al., 2004; Ris et al., 1994), whereas two other studies did not (Channon et al., 2004; Nardecchia et al., 2015). Furthermore, Brumm et al. (2004) found no impairments on visual-spatial subtests of the WAIS-R (Block Design, Picture Arrangement and Picture Completion) and no difference in performance between ET AwPKU with high versus low concurrent Phe levels on any of the visual-spatial measures included in their study. They did, however, observe negative correlations between performance on two of the WAIS-R subtests (Block Design and Picture Completion) and median Phe levels between the ages of 5.5-6 and 9.5-10 years. Other studies did not observe any associations between

visual-spatial abilities and measures of metabolic control (Nardecchia et al., 2015; Ris et al., 1994).

3.3.3 Cognitive outcomes in early treated adults with PKU (ET AwPKU): long-term follow-up

Two studies included in this review were long-term follow-up studies of a cohort of ET AwPKU who participated in research during their childhood: Nardecchia et al. (2015) assessed cognitive functioning of 14 ET AwPKU previously examined by Leuzzi et al. (2004). Jahja, van Spronsen, et al., (2017) tested 21 of 69 ET AwPKU (48 of the original sample were lost to follow-up (69%)) who had been involved in previous research (Huijbregts et al., 2003; Huijbregts, de Sonneville, Licht, Sergeant, & van Spronsen, 2002; Huijbregts, de Sonneville, van Spronsen, Licht, & Sergeant, 2002). Both follow-up studies were conducted approximately 14 years after the original research and found that cognitive performance across a range of tests, mainly assessing EF, either remained stable or improved (Jahja, van Spronsen, et al., 2017; Nardecchia et al., 2015). Nardecchia et al. (2015) noted that differences in neuropsychological outcome between ET PKU and controls had become smaller at T2, but had not disappeared entirely. Furthermore, as expected, Phe levels increased with age and results suggest that ET AwPKU who had low childhood Phe and those who had better metabolic control during adolescence had better cognitive outcomes in adulthood (Jahja, van Spronsen, et al., 2017; Nardecchia et al., 2015).

3.4 Discussion

3.4.1 Summary of findings

3.4.1.1 Cognitive functioning

Cognitive performance of ET AwPKU varied across the different studies and cognitive domains included in this review. In general, impairments in cognitive functioning across domains tended to be observed more on measures of speed than accuracy. ET AwPKU were slower when compared to healthy controls or normative data. However, these

speed deficits were rarely observed in tasks of 'pure' processing speed (e.g. simple reaction time), apart from in off-diet ET AwPKU (Dawson et al., 2011; Moyle, Fox, Bynevelt, et al., 2007). As suggested by Romani et al. (2018), these findings could indicate that ET AwPKU may not suffer from a processing speed deficit *per se*. Reductions in speed of performance across multiple cognitive domains are more likely to be the result of speed-accuracy trade-offs due to slower or more cautious executive decision-making processes.

Compared to healthy controls and normative data, impairments in cognitive performance of ET AwPKU have been most consistently found on tasks of sustained attention, working memory and motor skills. Furthermore, there is some evidence for deficits in performance on tasks of attentional capacity, verbal fluency, complex language skills, complex EF and inhibitory control. For both complex EF and working memory tasks, deficits appear to be more pronounced on tasks which have a higher cognitive load, i.e. requiring more planning/reasoning and flexibility or working memory, respectively. Performance on tasks of simple processing speed, memory, visual-spatial abilities, and simple language processing does not seem to be impaired in ET AwPKU. Social-cognitive abilities were reported to be affected in ET AwPKU, but these abilities were only assessed in one of the fifteen studies included in this review. Finally, Jahja, van Spronsen et al. (2017), Nardecha et al. (2015) and Weglage et al. (2013) reported that overall cognitive performance remained stable or improved over extended periods, despite an observed increase in Phe. This could be due to adequate adherence to treatment after childhood.

3.4.1.2 Impact of metabolic control on cognitive performance

3.4.1.2.1 Good versus poor metabolic control

Several papers included in this review explored differences in cognitive performance between groups with high versus low levels of Phe at the time of testing, often using different criteria to discriminate the high- and low-Phe groups. Some, but not all, of these studies reported that ET AwPKU with low concurrent Phe levels outperformed ET AwPKU with high concurrent Phe on tasks of selective attention, memory and learning, and semantic language skills. The majority of studies observed a similar pattern for

performance on sustained attention tasks as well as motor skills. No differences in performance on visual-spatial measures or measures of complex EF were observed between groups of ET AwPKU with high and low Phe levels at the time of testing. Results from a few studies suggest that ET AwPKU with high Phe levels at the time of testing may have worse inhibitory control than those with low concurrent Phe levels. Finally, some studies suggest that ET AwPKU with high concurrent Phe and those with high childhood-Phe levels are more at risk of developing impairments in working memory compared to ET AwPKU with low concurrent or childhood Phe, respectively.

3.4.1.2.2 Associations with metabolic control throughout life

Associations between Phe levels and memory and learning, as well as motor skills, were observed across the lifespan. The relationship appears more robust for visual delayed and recognition memory than measures of verbal memory. Language skills appear to be moderately correlated with childhood Phe levels, which might reflect the fact that language skills are developed during childhood (Berman, 2004). In contrast, sustained attention, complex EF, inhibition and working memory were most frequently reported to be correlated with lifetime Phe and Phe later in life (concurrent Phe and Phe during adolescence and adulthood). A possible explanation for this is that these cognitive functions, supported by the prefrontal cortex, are affected by decreased levels of dopamine resulting from poor metabolic control (Boot et al., 2018; Smith, Klim, Mallozzi, & Hanley, 1996). Limited associations were observed between verbal fluency and concurrent and childhood Phe levels and no associations between Phe and social-cognitive abilities and visual-perceptual abilities were found. Furthermore, limited evidence suggests fluctuations in Phe levels throughout life affect cognitive performance of ET AwPKU. Finally, in studies reporting relationships with Phe for outcome measures of speed and accuracy separately, significant correlations were generally more frequently observed with measures of speed compared to measures of accuracy. Speed-specific associations were predominantly observed with Phe earlier in life (childhood and adolescent Phe). As suggested by Romani et al. (2017), speed deficits might be modulated by structural myelin damage caused by suboptimal Phe control early in life.

The vast majority of reported correlations were of moderate strength (see Table 1 (Additional file 1)) and in the expected direction, such that cognitive performance worsened with an increase in Phe.

3.4.2 Limitations/ Methodological issues

Several factors may have contributed to inconsistent findings across studies in ET AwPKU.

3.4.2.1 Sample

Samples of ET AwPKU are highly heterogeneous: patients are likely to have different PAH-genotypes and will have had varying degrees of dietary adherence throughout life and at the time of testing, leading to inter and intra-individual variability in Phe-levels. Furthermore, some studies included mixed samples of on-diet and off-diet ET AwPKU in the same analysis, whereas others split samples based on their dietary management status. However, no studies clearly defined what was meant by 'off-diet', and it is unclear whether the ET AwPKU included followed an omnivorous diet, vegan or vegetarian diet or whether they were still (unconsciously) limiting their protein intake. ET AwPKU doing the latter might suffer from nutritional deficiencies (Hoeks et al., 2009) that could affect cognitive functioning (e.g. vitamin B12 (Brenton & Pietz, 2000; Vogel, Dali-Youcef, Kaltenbach, & Andrès, 2009)) alongside raised Phe. Moreover, although some authors stated that their sample of ET AwPKU were continuously treated, they report concurrent Phe-levels outside of target treatment ranges, suggesting that at least some of their sample were not adherent to dietary recommendations at the time of testing. Therefore, the question remains whether observed cognitive deficits are present in ECT AwPKU. Future research would benefit from the inclusion of additional nutritional measures to better characterise the sample of ET AwPKU and explore the impact of potential nutritional deficiencies on cognitive outcomes. Moreover, to better evaluate the efficacy of current treatments, research should focus on homogeneous samples, or, where this is not possible, include an analysis of carefully characterised subgroups (e.g. on-diet and off-diet).

The inconsistent findings in ET AwPKU in the studies included in this review may be due to issues of sample size. Because PKU is a rare disorder, it is difficult to recruit and retain large samples. Generally, studies of PKU tend to consist of small single centre studies, with a limited number of PKU patients living within study catchment areas. Studies on cognitive performance in ET AwPKU often include a relatively small (<50 AwPKU) number of participants (Bilder et al., 2016) and are likely to be underpowered. For example, Moyle et al. (2007) observed no impairments in cognitive functioning in 12 ET AwPKU who discontinued their treatment during adolescence, whereas Palermo et al. (2017) and Jahja, Huijbregts et al. (2017) reported several deficits in cognitive functioning in relatively well controlled ET AwPKU (n=37 and n=57, respectively). Research in PKU may benefit from more national and international multi-centre collaborations, in order to increase sample size to achieve sufficient power, and address the need to recruit more homogeneous samples.

Furthermore, ET AwPKU who participate in research are likely to be a self-selected sample who are more engaged with their dietary management which could positively bias findings. Deficits in cognitive functioning are likely to be more prevalent and more severe in those who are less adherent to their dietary management, but these patients are likely to be underrepresented in the literature. To illustrate, in the PKU COBESO study, only 21 of the original 68 ET PKU patients took part in the long-term follow-up study (Jahja, van Spronsen, et al., 2017). Authors reported that at initial testing, approximately 14 years earlier, this subsample did not differ from controls on any of the cognitive measures, whereas the sample as a whole showed signs of cognitive impairments on several measures. Furthermore, the patients who were lost to follow-up had higher Phe levels at the time of initial testing. This suggests that those patients who were retained for a second test demonstrated better adherence to their dietary management than the ET AwPKU who were lost to follow-up. The percentage of participants who were lost to follow-up in this research (69% of the original sample) is similar to the percentage of AwPKU who were estimated to not access regular clinical therapy in the United States in 2013 (>70%) (Berry et al., 2013), suggesting little is known about cognitive functioning in the majority of ET AwPKU. To our knowledge, only a few studies have assessed cognitive performance in a group ET AwPKU who discontinued their diet (Burgard et al., 1997; Dawson et al., 2011; Moyle, Fox, Bynevelt, et al., 2007).

3.4.2.2 Cognitive performance testing

As is apparent from Table 4, the studies included in this review used a wide variety of cognitive tests spanning a range of cognitive domains and differing in sensitivity. Besides sample size affecting the power of a study to detect any cognitive deficits, cognitive tests differ in sensitivity. This makes it difficult to compare outcome measures from different studies and draw coherent conclusions. Furthermore, a number of the tests employed in the studies do not necessarily test just one cognitive domain, but rather recruit multiple cognitive functions simultaneously. This can lead to discrepancies in the interpretation of results. For example, the Stroop word and colour subtests are regarded as language skills by Palermo et al. (2017) whereas others have reported Stroop to be a measure of attention (Brumm et al., 2004). Additionally, because they require planning a systematic search of the lexicon, tests of verbal fluency are often believed to reflect EF (Henry & Crawford, 2005b, 2005a). However, as these tests tap into the lexicon, one could also argue that performance primarily reflects language skills (Whiteside et al., 2016). In line with the framework used in this review (Lezak et al., 2012), the majority (3/4) of studies that included tests of verbal fluency classified these as a measure of EF. In addition to discrepancies in the interpretation of cognitive test performance, there are also discrepancies in the manner of reporting cognitive outcomes. Most papers report outcomes of speed and accuracy separately, where possible. However, Romani et al. (2017) used aggregated scores of cognitive performance on tests attributed to a cognitive domain to explore the association with metabolic control. Limitations of the use of aggregated scores, even if well-constructed, are potential differences in reliability and sensitivity of the individual measures in relation to the construct (i.e. cognitive domain) that is being measured (Riordan, 2017). Finally, only five of the twenty-two publications included in this review reported effect sizes for their statistical test outcomes (Jahja et al., 2016; Jahja, Huijbregts, et al., 2017; Jahja, van Spronsen, et al., 2017; Moyle et al., 2006; Moyle, Fox, Bynevelt, et al., 2007). Effect sizes are crucial for the interpretation of observed differences between groups. Even though p-values indicate whether or not a significant difference exists, they provide no information about the magnitude of the difference (Sullivan & Feinn, 2012). Moyle et al. (2007) reported large effect sizes for observed deficits in cognitive performance in off-diet ET AwPKU. In contrast, reported deficits in cognitive functioning of ECT AwPKU in the PKU-

COBESO study were small (Jahja, Huijbregts, et al., 2017). However, observed improvements in motor performance between T1 and T2 had medium to large effect sizes (Jahja, van Spronsen, et al., 2017). Furthermore, Jahja, Huijbregts et al. (2017) reported large effect sizes for differences in cognitive performance between ECT AwPKU with good versus poor metabolic control during childhood. Significant differences in performance on cognitive tasks between ET AwPKU and controls or normative or standardized data should be interpreted with caution, especially when no effect sizes have been reported. There is a need for greater homogeneity amongst measurement tools and the analysis and reporting of these in research in PKU.

3.4.2.3 Metabolic outcomes

Levels of metabolic control (i.e. Phe levels) at the time of testing varied both between and within study samples. A major contributor to such differences is the variation in guidelines for the management of PKU between countries and sometimes even between clinics within the same country (Aguiar et al., 2015; Ahring et al., 2009). Furthermore, because guidelines have changed throughout the life of the ET AwPKU included in the research (e.g. diet for life is relatively recent advice and was probably introduced after some ET AwPKU included in the studies reviewed had already ceased the diet), time of diagnosis, onset of treatment, and metabolic control throughout life are also likely to have varied amongst participants. Moreover, it has been shown that different methods for the analysis of dried blood spots (DBS) as well as differences in the size of the bloodspots that are measured could lead to significantly different results (George & Moat, 2016; Stroup et al., 2016), and oversaturation or undersaturation of the filter paper could lead to inaccurate results (George & Moat, 2016). Research has also suggested that individuals with PKU often change adherence to their dietary management in the days leading up to a blood test, suggesting measured levels of Phe may underrepresent typical Phe levels (Bilginsoy et al., 2005; Weglage et al., 1992). The large variance in Phe-data reported and limitations of measures of metabolic control, combined with relatively small sample sizes, reduces the likelihood that observed correlations are reliable. As a result of the variability in metabolic control between participants, several studies created subgroups of ET AwPKU with high or low Phe levels using different cut-off criteria. In addition to using different cut-off criteria to create

subgroups for analysis, studies also differed in how they reported measures of metabolic control throughout life. Again, these discrepancies in reporting make it difficult to compare study outcomes and obtain a clear picture of how metabolic control throughout life influences cognition in ET AwPKU. Only a few of the studies included in this review explored the relationship between cognitive performance and Phe variation throughout life (De Felice et al., 2018; Romani et al., 2017, 2018) and these found correlations across cognitive domains. Moreover, only one of the studies included measures of Phe:Tyr ratio but did not explore the relationship between this outcome and cognitive performance (Bartus et al., 2018). Limited research on the association between Phe:Tyr and EF in PKU suggests that high lifetime ratios rather than average Phe levels were associated with observed deficits in EF (Luciana, Sullivan, & Nelson, 2001; Sharman, Sullivan, Young, & McGill, 2009). It should be noted that Tyr levels obtained via DBS could be inaccurate if patients contaminate the filter paper by not washing their hands prior to blood sampling. Future research should include assessment of Phe fluctuations and Phe:Tyr ratio throughout life to enable a better understanding of the impact of metabolic control throughout life on outcomes in adulthood. However, due to limitations in measurements of metabolic control described previously, any observed associations should be interpreted with caution.

3.4.3 Conclusions

Results from the studies included in this systematic review suggest that, despite early treatment, ET AwPKU have (subtle) deficits in sustained attention, working memory, and motor skills compared to healthy controls. Long-term cognitive outcomes of ECT AwPKU remain unclear. Furthermore, several associations between cognitive performance and metabolic control throughout life were observed. However, these findings were inconsistent and therefore, it is difficult to determine the long-term effects of poor metabolic control at different stages in life on cognitive function in AwPKU.

To gain a better understanding of cognitive functioning and cognitive deficits in ET AwPKU the study presented in the following chapter aimed to assess cognitive function in a large sample of ET AwPKU compared to healthy controls. Recognising the variety of cognitive tests used and the limited sample sizes of the studies presented in this

systematic review, the study presented in Chapter 4 employed online cognitive tests, in different languages, to maximize potential sample size.

Chapter 4 Remote assessment of cognitive performance of ET AwPKU relative to healthy controls (Study 2)

4.1 Introduction

As discussed in Chapter 3, cognitive deficits related to the frontal brain regions such as EF and attention have been well documented in ET AwPKU (Bilder et al., 2016; Christ, Huijbregts, de Sonneville, & White, 2010). Yet, only a limited amount of research in ET AwPKU has focused on performance in other cognitive domains such as language, motor control, memory and learning. Brumm et al. (2004) reported deficits in immediate, short-term and long-term verbal and visual memory in a mixed sample of on- and off-diet ET AwPKU, compared to standardised data. However, the majority of research on memory and learning has not revealed any specific impairments in these domains in both on- and off-diet ET AwPKU (Channon, German, & Lee, 2004; Moyle, Fox, Bynevelt, Arthur, & Burnett, 2007; Nardecchia et al., 2015; Romani, Palermo, Macdonald, Limback, & Hall, 2017). Nevertheless, several studies in rodents (both with and without PKU) as well as a volumetric magnetic resonance (MR) study in ET AwPKU suggest that PKU patients could have deficits related to the hippocampus, a brain structure known for its involvement in memory and learning (Bakker, Kirwan, Miller, & Stark, 2008; Yassa & Stark, 2011).

4.1.1 Hippocampal functioning in PKU

4.1.1.1 Evidence from animal studies

Studies in rodents suggest that (high levels of) Phe depresses glutamatergic synaptic transmission in the hippocampus and cerebral cortex (Glushakov et al., 2002; Martynyuk et al., 2005). Elevated Phe levels have also been shown to lead to in vitro lipid oxidation and oxidative protein damage in these areas of the brain (Fernandes et al., 2010). Furthermore, Phe decreases acetylcholinesterase (AChE) activity and inhibits sodium-

potassium adenosine triphosphatase (Na^+K^+-ATPase) in these brain regions (Doulgeraki, Papadopoulou-Daifoti, & Tsakiris, 2002). These observed impairments in hippocampal synaptic transmission caused by elevated Phe could lead to a disturbance in the regulation of memory formation and learning (Glushakov et al., 2002). Moreover, research in PKU mouse models suggests an increased expression and density of glutamate receptors in the forebrain of these mice (Martynyuk et al., 2005), as well as impaired synaptic transmission in the CA3-CA1 regions of the hippocampus (Horling et al., 2015). AChE, Na^+K^+-ATPase and glutamate receptors are all believed to be involved in memory and learning (Doulgeraki et al., 2002; Martynyuk et al., 2005).

4.1.1.2 Evidence from human studies

In addition to the animal studies summarised above, a magnetic resonance (MR) volumetric study which compared ET AwPKU and healthy controls found the hippocampus to be one of the most affected brain structures in PKU in terms of volume loss (Pfaendner et al., 2005).

4.1.2 Role of the hippocampus in pattern separation: a test of episodic memory

Pattern separation, which is defined as *"a process whereby similar representations are stored in a distinct, non-overlapping fashion"* (Yassa & Stark, 2011, p. 515), places a significant demand on the memory functions of the hippocampus. Pattern separation is essential to episodic memory because it allows discrimination between closely similar situations. Without it, new experiences could interfere with previously stored information, affecting recognition memory (Yassa & Stark, 2011). Evidence from functional magnetic resonance imaging (fMRI) studies suggests that the dentate gyrus (DG) and CA3 sub-regions of the hippocampus play an important role in pattern separation (Bakker et al., 2008; Brickman et al., 2014; Yassa & Stark, 2011).

4.2 Study aims

As there is limited, predominantly animal based, evidence that 1) elevated levels of Phe in the brain of individuals with PKU could adversely affect the DG and CA3 regions of the hippocampus involved in pattern separation; and 2) the observed hippocampal volume loss in ET AwPKU could potentially result in impaired pattern separation in the DG (Schoenfeld, McCausland, Morris, Padmanaban, & Cameron, 2017), the current study aimed to assess cognitive performance on a task assessing a cognitive domain associated to the hippocampus (i.e. episodic memory) in ET AwPKU compared to healthy controls using a two-part pattern separation task. Filler tasks were also used to assess cognitive performance related to frontal brain regions (i.e. working memory and attention), which have previously been shown to be impaired in ET AwPKU (Bilder et al., 2016; Jahja et al., 2017; Palermo et al., 2017).

4.3 Methods

4.3.1 Design

The study presented in this chapter (Study 2) was cross-sectional: it employed a remote (online) assessment of hippocampal functioning (pattern separation, episodic memory) as well as attention and working memory in ET AwPKU and healthy controls.

4.3.2 Participants

English, Dutch and German speaking AwPKU (n=111), aged 18 years or over, were recruited worldwide via social media (e.g. Twitter, Facebook and blogs) and PKU patient associations. Late diagnosed individuals (i.e. AwPKU who were not diagnosed via new-born screening) were excluded from participation (n=1). Healthy controls, closely matched on age, gender and highest completed level of education, were recruited using social media and online participant databases. Participants were required to complete an online battery of cognitive tests (CogTrack^{TM}) on two separate occasions (T1 and T2; see below). Twenty-seven ET AwPKU and 28 healthy controls, who completed both test

sessions and complied with the testing protocol, were included in the analysis (see Figure 4.1).

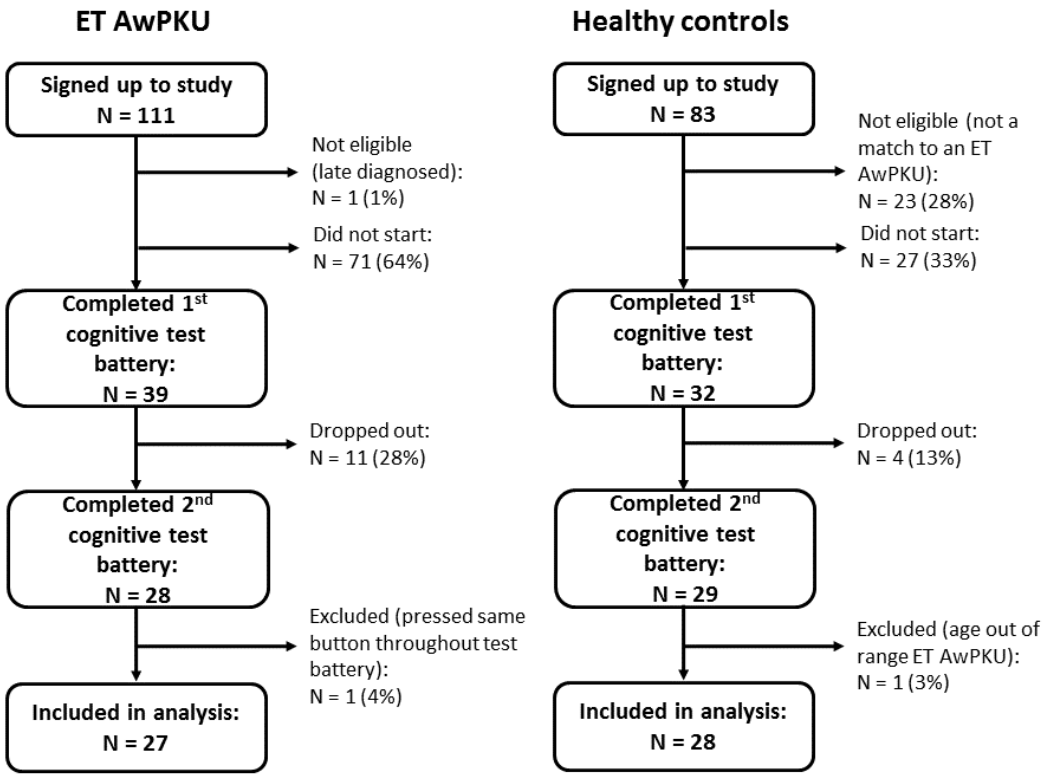


Figure 4.1 Flowchart depicting the recruitment and retention of participants in Study 2

4.3.3 Questionnaires

A short demographics survey (Appendix A, part 1), as well as a brief survey regarding the dietary habits and metabolic control of the ET AwPKU (see Appendix F) were used as self-report measures. The surveys were administered online using Qualtrics© software (Qualtrics, 2017).

4.3.4 Cognitive tests

Cognitive functioning of ET AwPKU and healthy controls was assessed remotely using the CogTrack™ system (see Table 4.1 for an overview of outcome measures). The CogTrack™ system is a validated and practical online cognitive test battery that can be used to assess everyday core aspects of cognitive functioning (Watson et al., 2018;

Wesnes, Brooker, Ballard, et al., 2017; Wesnes, Brooker, Watson, Bal, & Okello, 2017). To increase accessibility for patients and control participants across Europe, the CogTrack™ battery was translated from English into Dutch and German.

Participants were required to complete the cognitive tests on two separate occasions, at least one week apart. To avoid practise effects caused by repeating the same information at each test session (e.g. pictures used in the pattern separation task), parallel forms of the tests were used across the two test sessions (Goldberg, Harvey, Wesnes, Snyder, & Schneider, 2015). For each task, participants were instructed to keep their fingers lightly resting on the right (and left, where applicable) arrow key(s) on their keyboard and respond as quickly and accurately as possible.

Table 4.1 Overview of outcome measures of the CogTrack™ system

Task	Outcome measure
Pattern Separation	Overall accuracy (% correct)
	Overall speed (msec)
	Original stimuli – Accuracy (% correct)
	New stimuli – Accuracy (% correct)
	Original stimuli – Speed (msec)
	New stimuli - Speed (msec)
Simple Reaction Time (SRT)	Speed (msec)
Choice Reaction Time (CRT)	Accuracy (% correct)
	Speed (msec)
Digit Vigilance (DV)	Speed (msec)
	Accuracy (% correct)
	False alarms (n)
Spatial Working Memory (SWM)	Overall accuracy (% correct)
	Overall speed (msec)
	Original stimuli – Accuracy (% correct)
	New stimuli – Accuracy (% correct)
	Original stimuli – Speed (msec)
	New stimuli - Speed (msec)
Numeric Working Memory (NWM)	Overall accuracy (% correct)
	Overall speed (msec)
	Original stimuli – Accuracy (% correct)
	New stimuli – Accuracy (% correct)
	Original stimuli – Speed (msec)
	New stimuli - Speed (msec)

4.3.4.1 Episodic memory: Pattern separation

This task measured the ability to store and subsequently retrieve visual information. Participants were shown a series of 20 pictures of everyday objects and scenes, one by one, at a rate of 1 every 3 seconds (i.e. presentation phase). They were instructed to pay close attention to the detail of each picture and told that the pictures would be shown again later, mixed with very similar ones. No responses were made during the initial part of the task. Approximately 10 minutes later, after participants completed a set of filler tasks (described below), the 20 original pictures were presented mixed with 20 closely similar pictures (i.e. recognition phase; see Figure 4.2). Each picture had a closely similar paired picture, and the participants were instructed to press the right arrow key whenever an original picture was presented, or the left arrow key if it was a new one, and to guess if not sure. The order of presentation was randomised and counterbalanced, such that half of the original pictures were presented before their matched pair, and half after. The pictures remained on the screen until a response was recorded and the accuracy (% correct) and speed in milliseconds (msec) for correct responses were recorded. Issues in identifying closely similar pictures, but not original pictures (i.e. a significantly lower accuracy (% correct) on the trials showing the closely similar pictures compared to the trials showing the original pictures), suggests deficits in pattern separation.

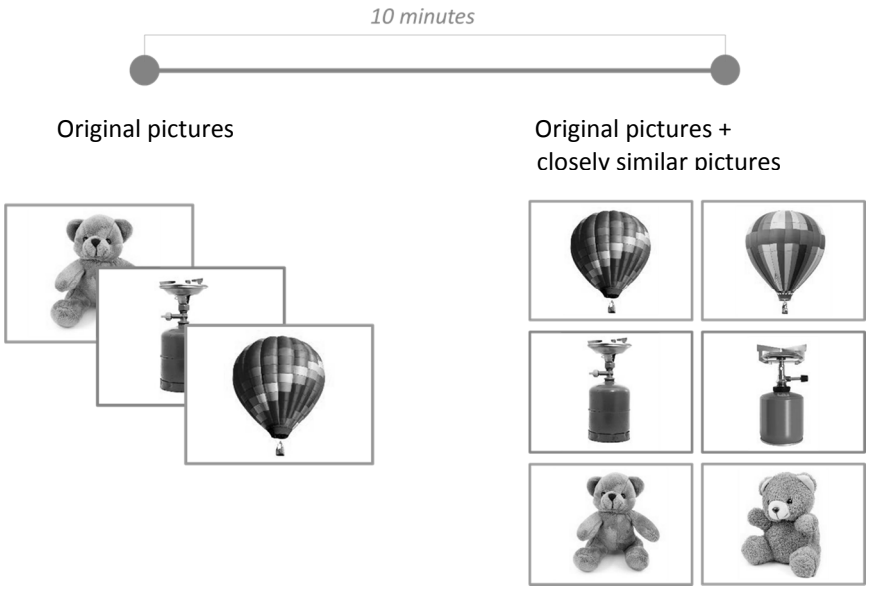


Figure 4.2 Example trials for both parts of the pattern separation task

4.3.4.2 Attention and processing speed

4.3.4.2.1 Simple Reaction Time (SRT)

This task was used to assess alertness and focussed attention. Participants were instructed to press the right arrow key on their keyboard as quickly as possible every time a right facing arrow appeared in the centre of the screen (see Figure 4.3). The right facing arrow was presented a total of 50 times at variable intervals of 1 to 3.5 seconds. Speed of responses was recorded in msec.

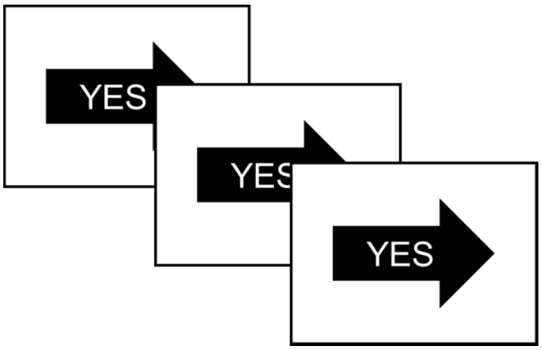


Figure 4.3 Trials on the SRT task

4.3.4.2.2 Choice Reaction Time (CRT)

In this task, either a right or left facing arrow appeared in the middle of the screen (see Figure 4.4), and participants had to press the corresponding key (i.e. right arrow key or left arrow key) as quickly as possible. There were 50 trials in which the right and left facing arrows appeared at random variable intervals, between 1 and 3.5 seconds. Accuracy (% correct) and speed (msec) for correct responses were recorded.

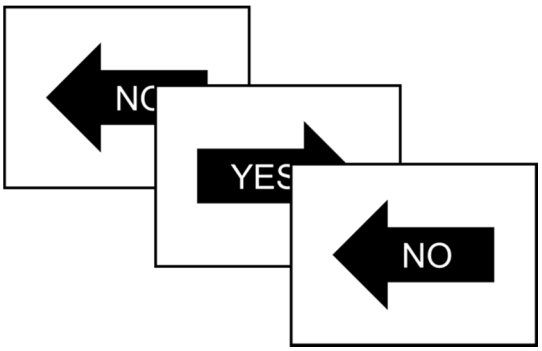


Figure 4.4 Example trials of the CRT task

4.3.4.2.3 Digit Vigilance (DV)

This task measured sustained and intensive attention; also known as vigilance. At the start of this task, a randomly selected target digit appeared on the right-hand side of the screen and remained there. Then, single digits appeared in the centre of the screen, and the participants had to press the right arrow key as quickly as possible every time a digit matched the target on the right (see Figure 4.5). The digits were presented in an unpredictable order, and there were 15 targets every minute. Accuracy (% correct), number of false alarms, and speed (msec) for correct responses were recorded. The high target rate required the participant to sustain their attention for the duration of the task. The continuous presence of the target digit removed any involvement of working memory from the task so that it was a pure measure of sustained attention.

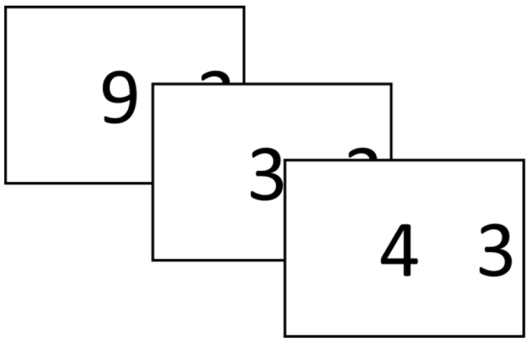


Figure 4.5 Example trials of the DV task

4.3.4.3 Working memory

4.3.4.3.1 Spatial Working Memory (SWM)

This task measured the ability to temporarily hold spatial information in working memory. Participants were initially presented with a 3x3 array of light bulbs, four of which were lit (see Figure 4.6 – left). The array remained on screen for 10 seconds, and the participants had to remember the positions of the four lit bulbs. Following this, 36 trials were shown in which the 3x3 array contained just a single bulb lit each time (see Figure 4.6 – right). On each occasion, the participants had to press the right arrow key if the bulb was lit initially, or the left arrow key if it was not. Over the 36 trials, each of the nine bulbs was lit on four occasions in a randomised order. Accuracy (% correct) and speed (msec) for correct responses were recorded.

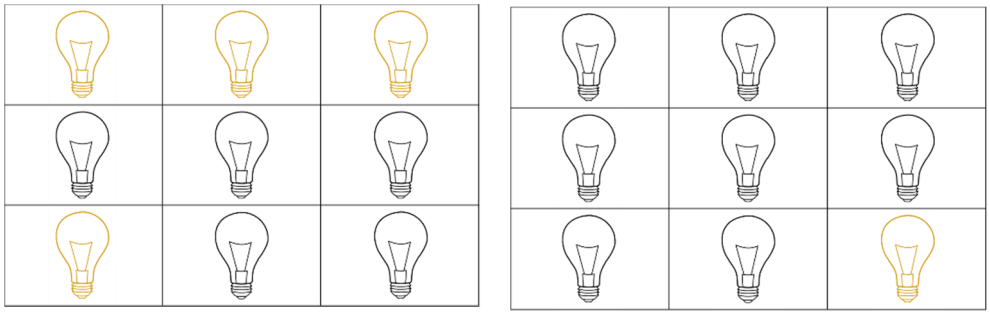


Figure 4.6 Example target array (left) and trial (right) of the SWM task

4.3.4.3.2 Numeric Working Memory (NWM)

This task measured the ability to hold five digits in working memory. A target series of 5 different digits (from 0 to 9) were presented in the centre of the screen at the rate of one digit every 1.2 seconds. Then a series of 30 digits were presented one at a time in the centre of the screen (see Figure 4.7), and the participants were instructed to press the right arrow key if the digit was one of the five presented initially, or the left arrow key if it was not. The stimulus remained on screen until a response was made. Half of the 30 stimuli required a right arrow key response and half a left arrow key response.

The order of presentation was randomised, and each of the initial five digits was presented three times. Accuracy (% correct) and speed (msec) for correct responses were recorded.

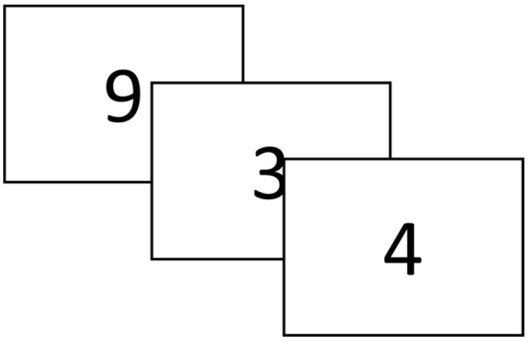


Figure 4.7 Example trials on the NWM task

4.3.5 Procedure

Upon reading the participant information (Appendix G), providing informed consent (Appendix H) and creating their individual participant IDs (Appendix D), ET AwPKU and healthy controls were required to complete a demographics survey (Appendix A, part 1). If eligible (i.e. 18 years or older and diagnosed via new-born screening), ET AwPKU were invited to complete their first online test session (T1). They were required to report their most recent measures of metabolic control (if known) prior to completing the cognitive test battery (see Appendix F). Healthy controls, if matched to one of the ET AwPKU, were provided with a direct link to the cognitive test battery. At least one week after completing the familiarisation session (T1), participants were invited to complete the survey (if applicable) and cognitive test battery a second time (T2). All participants started the cognitive test battery with the first part of the pattern separation task (i.e. presentation phase), followed by the SRT, CRT, DV, SWM and NWM tasks. Finally, participants were presented with the second part of the pattern separation task (i.e. recognition phase). On average, it took participants approximately 15 minutes to complete the test battery.

4.3.6 Ethical considerations

The study was approved by the University of Leeds' School of Psychology Research Ethics Committee (reference number: 17-0064) and undertaken in accordance with the ethical principles expressed in the Declaration of Helsinki (World Medical Association, 2013).

Participants were informed about the nature and aims of the study (Appendix G) and were required to provide informed consent (Appendix H) prior to completing the demographics survey (Appendix A, part 1). They were also required to create a unique participant ID (Appendix D) to ensure no personally identifiable information was recorded on the third party CogTrack™ system. Furthermore, the CogTrack™ system was secured with HTTPS, and cookies were not used to remember or track participants.

Participants were entered into a prize draw to win one of 50 Amazon vouchers (worth £12.50 or the equivalent value in local currency) upon completion of both test sessions.

4.3.7 Statistical analysis

Participant characteristics, obtained via the demographics surveys, were compared using independent t-tests or Chi-squared tests as appropriate. The first cognitive test session (T1) was used to familiarise participants with the tasks and ensure they understood the testing protocol (Goldberg, Harvey, Wesnes, Snyder, & Schneider, 2015; Wesnes et al., 2002). SPSS Version 22 was used to analyse data obtained at T2. Skewed reaction time data were log transformed before analysis. Where appropriate, data points beyond (+/-) 3 standard deviations (SD) from the mean were considered outliers and analysis was performed without these data points. Speed (msec) and accuracy (% correct and number of false alarms) on tasks of attention and processing speed were subject to a 2 (group; ET AwPKU vs healthy controls) x 2 (gender; male vs female) Mixed Model Analysis of Covariance (MANCOVA) with age and highest completed level of education included as covariates. Accuracy (%) and speed (msec) scores on the working memory and pattern separation tasks were subject to a 2 (group; ET AwPKU vs healthy controls) x 2 (gender; male vs female) x 2 (type of stimulus; original vs new) ANCOVA with age and highest completed level of education included as covariates. All main

effects and interactions were requested in the first model and the model fit, F values and significance of main effects and interactions examined. Non-significant interactions were removed, starting with highest order interactions, and the resulting model was compared to the previous model using the small-sample Akaike's Information Criterion (AICC; McQuarrie & Tsai, 1998). If an improvement in model fit was found (smaller AICC), other non-significant effects were removed and again the AICC criterion was used to evaluate the model fit. Models were chosen on the basis of 'best fit', and interaction terms that improved the fit were retained. Significant covariates and interactions in the final model were plotted to determine their relationship with reaction times or accuracy of task performance. In the case of significant main effects and interactions, post hoc tests were calculated using Bonferroni corrections.

4.4 Results

4.4.1 Participant characteristics

Table 4.2 provides an overview of charactaristics of participants who completed both test sessions and complied with the testing protocol.

Table 4.2 Participant characteristics: number, gender split, age and highest completed level of education for ET AwPKU and healthy controls (HC) included in analysis

	ET AwPKU	**HC**	**ET AwPKU vs. HC**
n (female)	27 (13)	28 (20)	X^2(1, N=55)=3.10, p=.08
Age (years)			t(53)= .17, p=.87, d=.04
Mean (SD)	31.81 (7.94)	31.43 (9.02)	
Range	20-50	19-53	
Educational level[1] (frequency)			X^2(4, N=55)=13.47, p=.009
1	0	0	
2	6	0	
3	3	10	
4	10	4	
5	7	9	
6	1	4	

Notes: [1] *Highest completed level of education ranging from 1 (primary education) to 6 (Doctoral degree)*

As can be seen in Table 4.2, gender and education were not represented equally across both study groups. Initially, healthy controls were matched to enrolled ET AwPKU on age, gender and achieved educational level. Differences in gender and highest completed level of education in final study sample are the result of unequal drop-out across matched ET AwPKU- healthy control-pairs.

4.4.2 Cognitive outcomes

4.4.2.1 Episodic memory: Pattern Separation

Table 4.3 provides an overview of mean and standard deviations of measures of accuracy (% correct) and speed (msec) on the pattern separation task for ET AwPKU and healthy controls. SPSS Linear Mixed Models for accuracy (% correct) and speed (msec) are displayed in Appendix I.

4.4.2.1.1 The effect of age on pattern separation

Pattern separation (the ability to discriminate between the closely similar pictures and the original pictures) has been shown to be impaired in non-demented older (age 50+ years) adults (Toner, Pirogovsky, Kirwan, & Gilbert, 2009; Wesnes, 2010), and this pattern of age-related decline (see Figure 4.8) has been shown on the test used in this study (Wesnes, 2010). Accuracy on the pattern separation task for both types of stimuli was plotted against age for both ET AwPKU (see Figure 4.9) and healthy controls (see Figure 4.10).

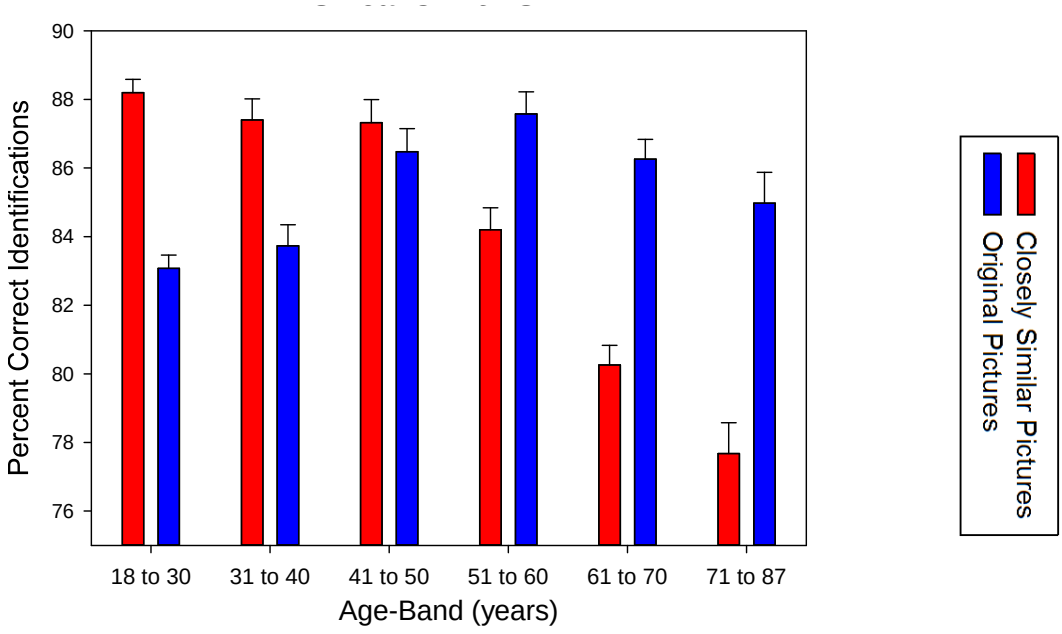


Figure 4.8 The relationship between age and accuracy (% correct) for original and closely similar pictures on the CDR Pattern Separation task in healthy volunteers (error bars represent SE) (from: Wesnes, 2010)

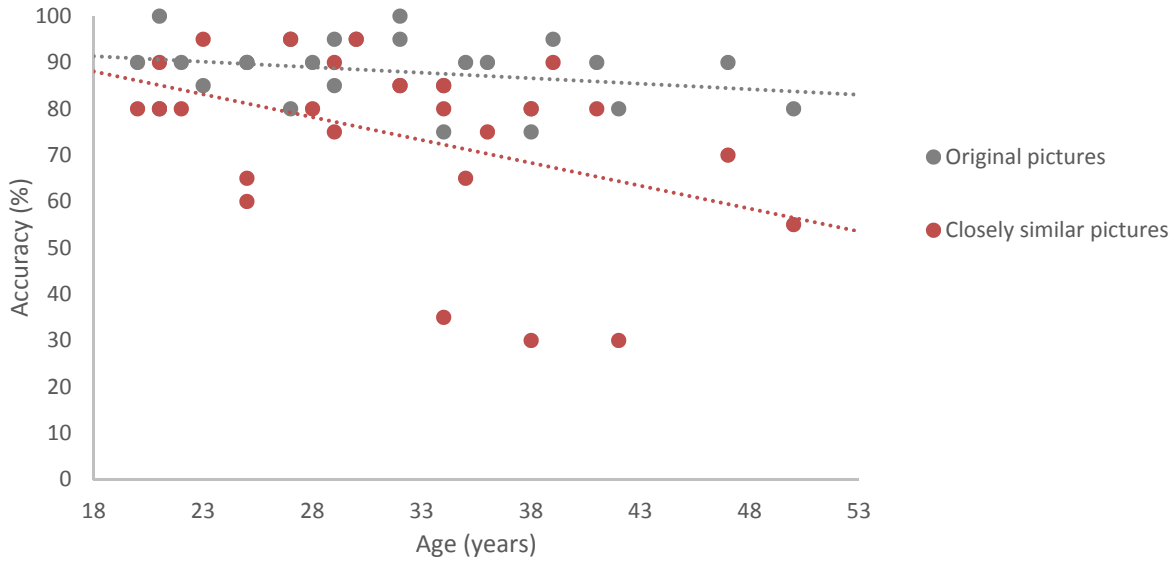


Figure 4.9 The relationship between age and accuracy (% correct) for original and closely similar pictures on the CogTrack™ Pattern Separation task for ET AwPKU

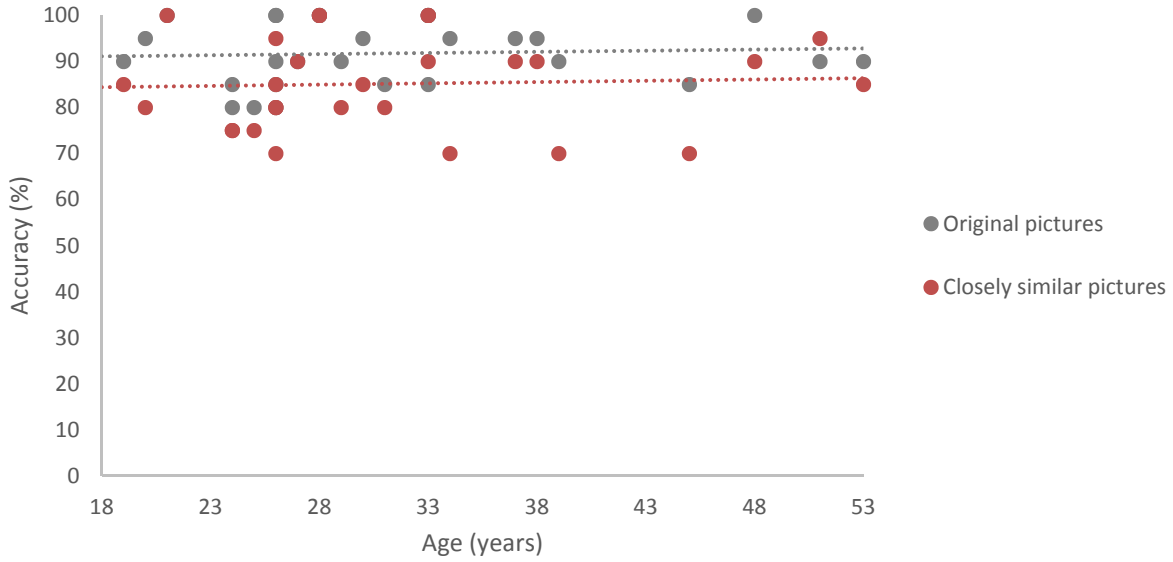


Figure 4.10 The relationship between age and accuracy (% correct) for original and closely similar pictures on the CogTrack™ Pattern Separation task for healthy controls (HC)

It is evident that a decline in accuracy of pattern separation occurs in ET AwPKU from about age 20 in this sample, which is not apparent in healthy controls.

4.4.2.1.2 Accuracy

Analysis revealed no main effect of group or type of stimulus. Age was a significant covariate ($F(1,95)=5.89$, $p=.02$, $\eta_p^2=.06$), such that older participants were less accurate than younger participants. There was a significant interaction between group and age ($F(1,95)=6.32$, $p=.01$, $\eta_p^2=.06$), which showed that, whereas accuracy for ET AwPKU decreased with increased age, this pattern was not observed in healthy controls (see Figure 4.11). No further significant main effects, covariates or interactions were observed.

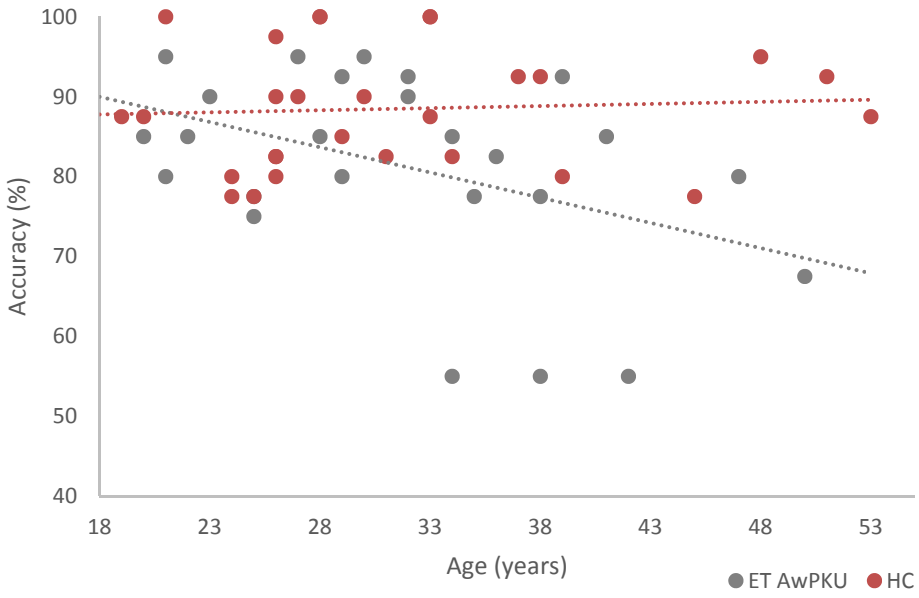


Figure 4.11 The relationship between age and accuracy (%) on the pattern separation task for ET AwPKU and healthy controls (HC)

Table 4.3 Mean (SD) accuracy (% correct) and speed (msec) of ET AwPKU and healthy controls (HC) on the pattern separation task at T2

	ET AwPKU			HC		
	Overall	**Female**	**Male**	**Overall**	**Female**	**Male**
Overall accuracy (% correct)	81.15 (15.71)	83.75 (10.56)	78.93 (18.97)	88.48 (9.19)	88.00 (9.18)	89.69 (9.39)
Overall speed (msec)	1345.40 (342.22)	1332.35 (381.63)	1357.51 (315.37)	1167.80 (223.24)	1162.63 (184.40)	1180.74 (315.70)
Original pictures						
Accuracy (% correct)	88.08 (7.08)	89.58 (5.82)	86.79 (7.99)	91.79 (6.83)	91.25 (7.05)	93.13 (6.51)
Speed (msec)	1308.41 (367.94)	1327.69 (438.81)	1290.51 (303.82)	1106.48 (181.39)	1221.00 (232.81)	1101.10 (199.38)
Closely similar pictures						
Accuracy (% correct)	74.23 (18.80)	77.92 (11.17)	71.07 (23.47)	85.18 (10.14)	84.75 (10.06)	86.25 (10.94)
Speed (msec)	1421.43 (432.28)	1346.29 (347.10)	1491.19 (501.63)	1234.93 (310.74)	1162.63 (184.40)	1269.73 (472.64)

4.4.2.2 Speed

There was a significant main effect of type of stimulus ($F(1,100)=4.53$, $p=.04$, $\eta_p^2=.04$). Overall, participants were faster at identifying the original pictures than the closely similar pictures. Furthermore, there was a main effect of group ($F(1,100)=9.98$, $p=.002$, $\eta_p^2=.09$), such that ET AwPKU were slower compared to healthy controls when data across both stimulus types was pooled (see

Table 4.3). Although age and education were not significant covariates, analysis revealed significant interactions between group and age ($F(1,100)=9.40$, $p=.003$, $\eta_p^2=.09$), group and education ($F(1,100)=14.63$, $p<.001$, $\eta_p^2=.13$), and group, age and education ($F(1,100)=7.20$, $p=.001$, $\eta_p^2=.13$). Younger ET AwPKU were slower than younger healthy controls but this group difference dissipated with increasing age, such that older ET AwPKU and healthy controls had similar speeds (see Figure 4.12).

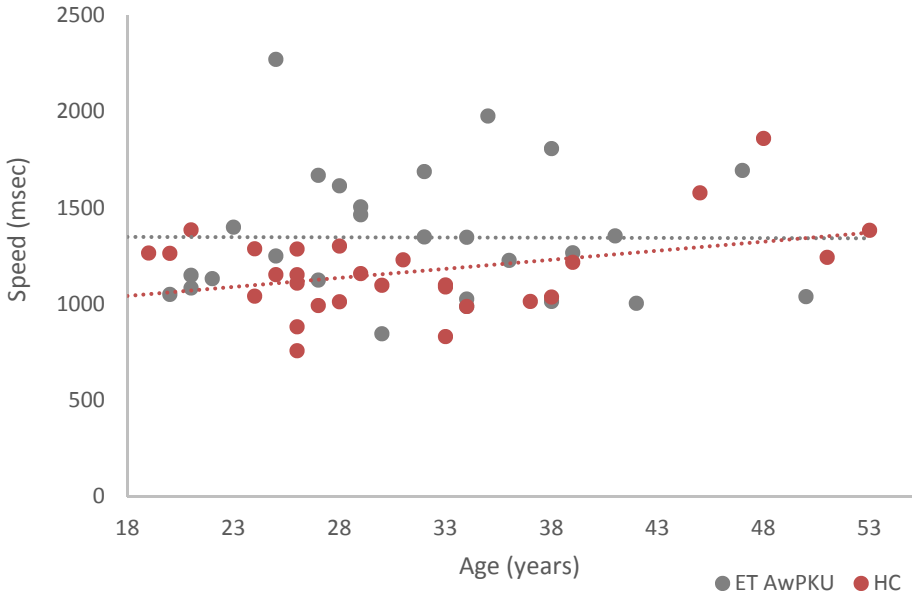
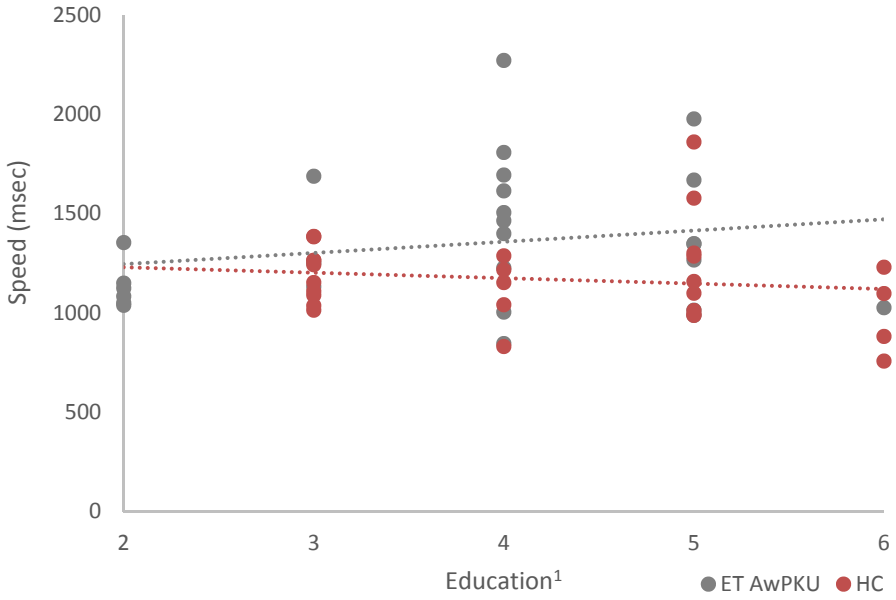


Figure 4.12 The relationship between age and speed of correct responses (msec) on the pattern separation task for ET AwPKU and healthy controls (HC)

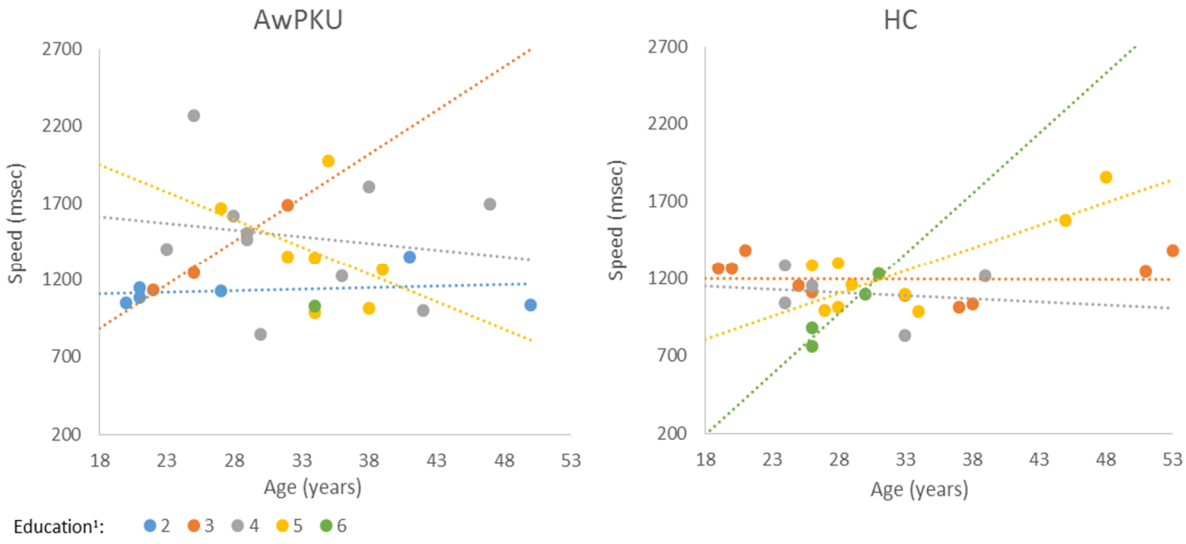
Furthermore, ET AwPKU and healthy controls with a low educational level had similar reaction times, but higher educated ET AwPKU tended to be slower than higher educated HC (see Figure 4.13). Figure 4.14 illustrates relationships between age and the speed of making correct responses (msec) at different levels of education across groups.

Most notably, older ET AwPKU with a higher level of education (level 5) were faster compared to younger ET AwPKU at the same level of education, whereas the opposite relationship was observed for healthy controls. Analysis revealed no further significant main effects or interactions.



[1] Highest completed level of education ranging from 1 (primary education) to 6 (Doctoral degree)

Figure 4.13 The relationship between education and speed of correct responses (msec) on the pattern separation task for ET AwPKU and healthy controls (HC)



[1] Highest completed level of education ranging from 1 (primary education) to 6 (Doctoral degree)

Figure 4.14 The relationship between age, education and speed of correct responses (msec) on the pattern separation task for ET AwPKU and healthy controls (HC)

4.4.2.3 Attention and processing speed

Means and standard deviations of accuracy (% correct responses and number of false alarms) and speed (msec) scores on measures of attention and processing speed for both groups are displayed in Table 4.4. SPSS Linear Mixed Models for accuracy (% correct and false alarms) and speed (msec) are displayed in Appendices I and J.

4.4.2.3.1 Simple Reaction Time (SRT)

There were no main effect of group on performance on the SRT task. Age was a significant covariate ($F(1,50)=21.22$, $p<.001$, $\eta_p^2=.30$), such that older participants were slower than younger participants. Furthermore, there was a significant main effect of gender ($F(1,50)=11.27$, $p=.002$, $\eta_p^2=.18$), such that female participants were slower than male participants. Analysis revealed no significant interactions between group, gender and age (education was not retained in the final analytic model for performance on the SRT task).

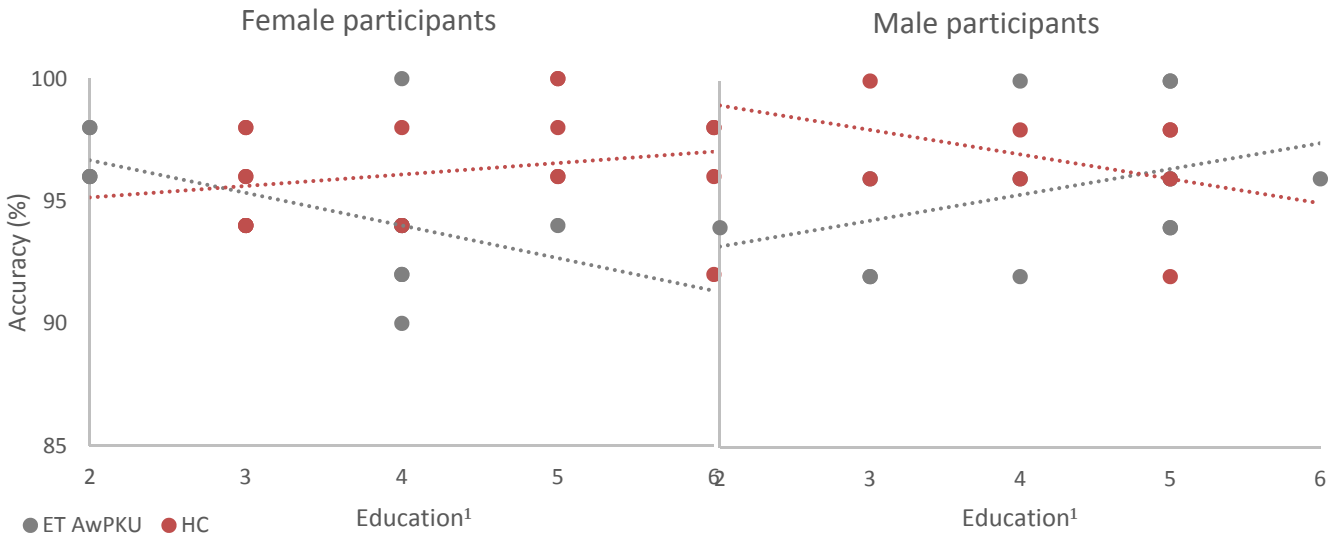
4.4.2.3.2 Choice Reaction Time (CRT)

4.4.2.3.2.1 Accuracy

Age and education were not significant covariates and no main effects of group or gender were observed for accuracy (% correct) on the CRT task. However, analysis revealed a significant group*gender ($F(1,45)=5.20$, $p=.03$, $\eta_p^2=.10$) interaction qualified by a significant group*gender*education ($F(1,45)=6.25$, $p=.02$, $\eta_p^2=.12$) interaction. Female ET AwPKU with higher educational levels were less accurate than those with a lower education whilst male ET AwPKU with higher educational levels were more accurate than those with a lower education, whereas the opposite relationships for each gender were observed in the healthy controls (see Figure 4.15). No further interactions were observed.

Table 4.4 Mean (SD) accuracy (% correct and false alarms) and speed (msec) of ET AwPKU and healthy controls (HC) on tasks of attention and processing speed at T2

	ET AwPKU			HC		
	Overall	**Female**	**Male**	**Overall**	**Female**	**Male**
Simple Reaction Time (SRT)						
Speed (msec)	342.89 (66.22)	369.55 (68.37)	318.14 (55.55)	328.81 (38.38)	334.45 (42.01)	314.69 (23.89)
Choice Reaction Time (CRT)						
Accuracy (% correct)	95.26 (2.78)	94.92 (2.78)	95.57 (2.85)	96.36 (2.25)	96.20 (2.24)	96.75 (2.38)
Speed (msec)	492.51 (87.05)	518.38 (92.76)	468.49 (76.96)	436.10 (56.35)	438.45 (59.48)	430.23 (50.90)
Digit Vigilance (DV)						
Accuracy (% correct)	97.78 (3.44)	97.78 (2.72)	97.78 (4.16)	98.97 (1.96)	99.45 (0.99)	97.78 (3.14)
False alarms (n)	1.36 (1.58)	1.27 (2.00)	1.43 (1.22)	0.89 (0.91)	0.70 (0.86)	1.38 (0.92)
Speed (msec)	466.93 (48.05)	475.63 (47.70)	458.86 (48.71)	446.62 (48.05)	450.22 (53.20)	437.63 (33.06)



[1] *Highest completed level of education ranging from 1 (primary education) to 6 (Doctoral degree)*

Figure 4.15 The relationship between group, education and accuracy (%) on the CRT task for male and female participants

4.4.2.3.2.2 Speed

There was a main effect of group on speed of performance on the CRT task ($F(1,45)=4.29$, $p=.04$, $\eta_p^2=.09$), such that ET AwPKU were significantly slower than healthy controls. Additionally, analysis revealed a significant group*age interaction ($F(1,45)=5.32$, $p=.03$, $\eta_p^2=.11$), where older participants were slower than younger participants in both groups, but there was a greater difference in reaction time in older ET AwPKU compared to older healthy controls (see Figure 4.16).

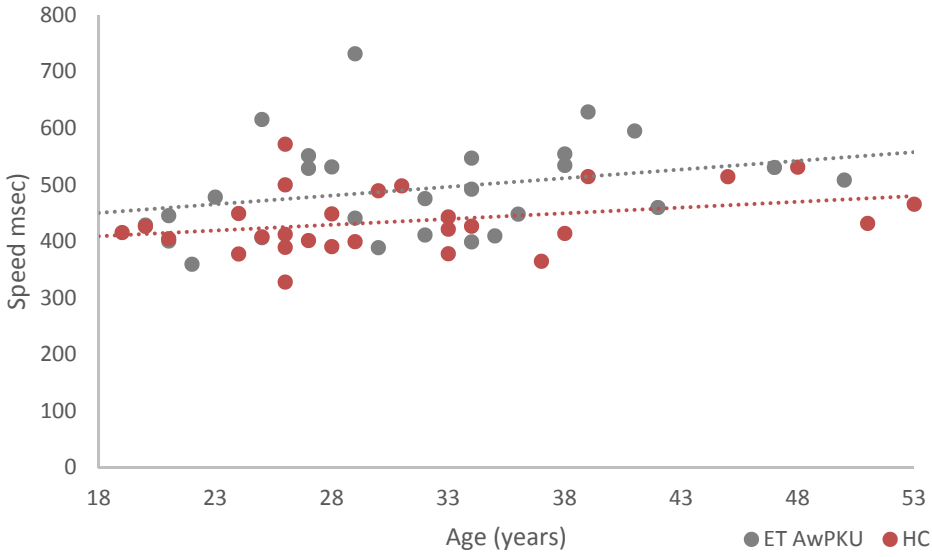
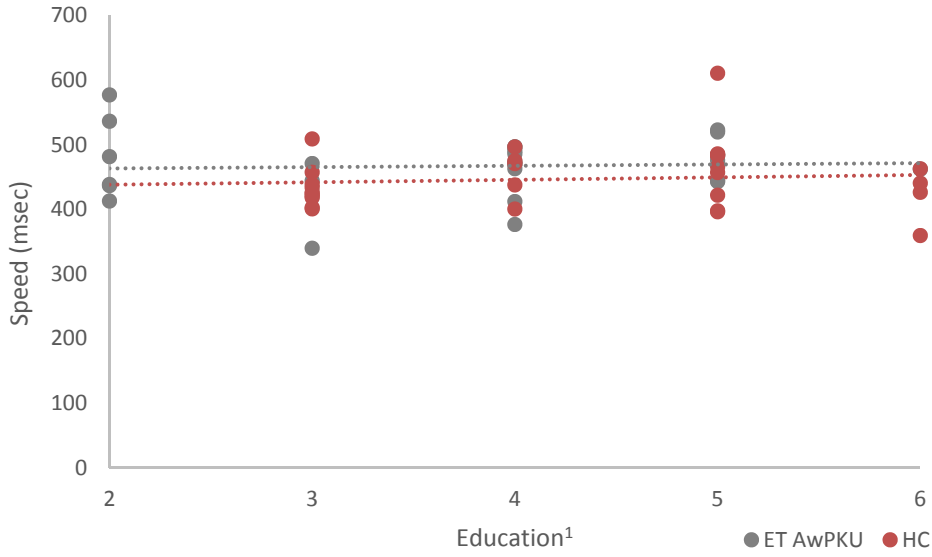


Figure 4.16 The relationship between age and speed of correct responses (msec) on the CRT task for ET AwPKU and healthy controls (HC)

Furthermore, there was a significant interaction between group and education ($F(1,45)=5.92$, $p=.02$, $\eta_p^2=.12$; see Figure 4.17). Additionally, there was a significant main effect of gender ($F(1,45)=4.47$, $p=.04$, $\eta_p^2=.09$), such that female participants were slower than male participants (see Table 4.4). No further significant main effects, covariates or interactions were observed.



[1] Highest completed level of education ranging from 1 (primary education) to 6 (Doctoral degree)

Figure 4.17 The relationship between education and speed of correct responses (msec) on the CRT task for ET AwPKU and healthy controls (HC)

4.4.2.3.3 Digit Vigilance (DV)

4.4.2.3.2.3 Accuracy

No significant main effects, covariates or interactions were observed for any of the accuracy measures (% correct and false alarms) of the DV task.

4.4.2.3.2.4 Speed

There was a main effect of group on speed on the DV task ($F(1,40)=6.13$, $p=.02$, $\eta_p^2=.13$), such that ET AwPKU were significantly slower than healthy controls. Moreover, a significant group*age interaction ($F(1,40)=9.14$, $p=.004$, $\eta_p^2=.19$) was observed, where older participants tended to be slower than younger participants in both groups, but there was a greater difference in reaction time between older and younger ET AwPKU than older and younger healthy controls (see Figure 4.18).

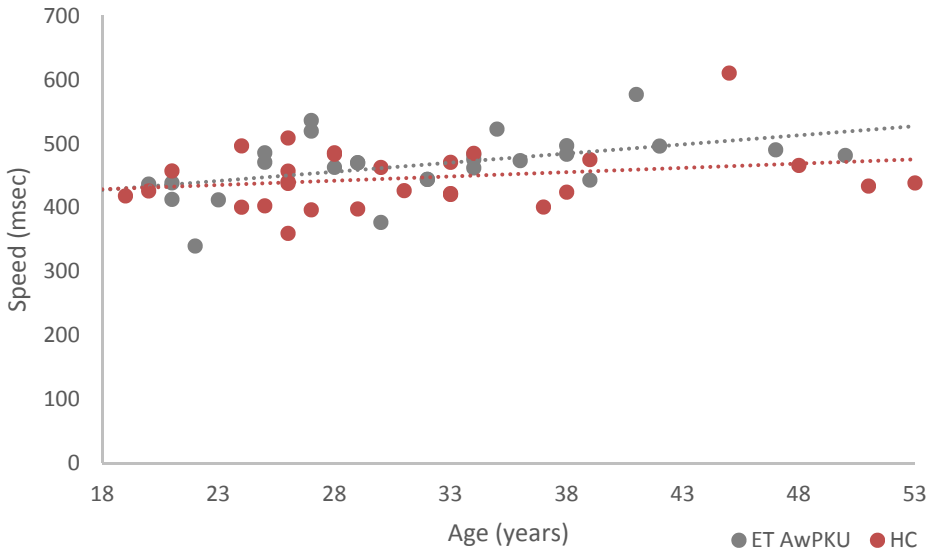
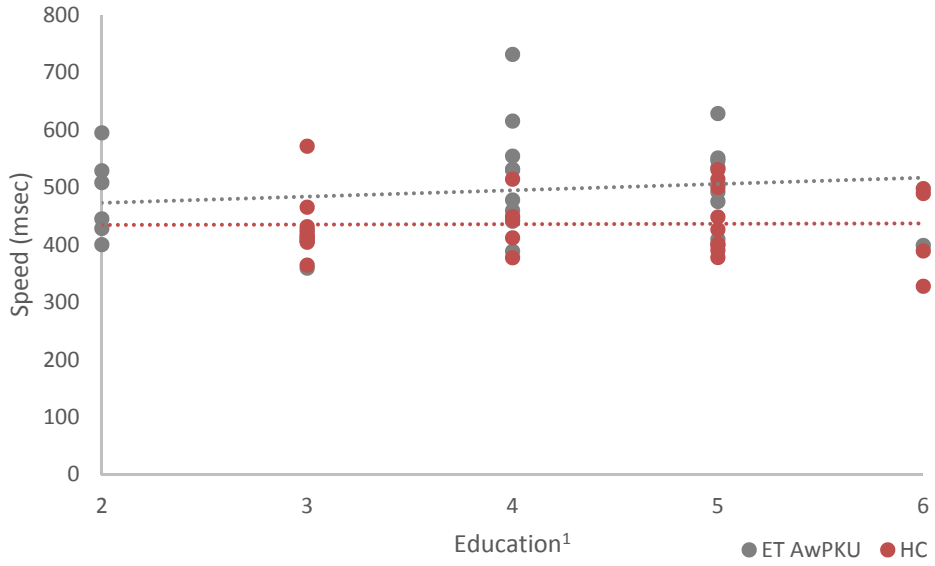


Figure 4.18 The relationship between age and speed of correct responses (msec) on the DV task for ET AwPKU and healthy controls (HC)

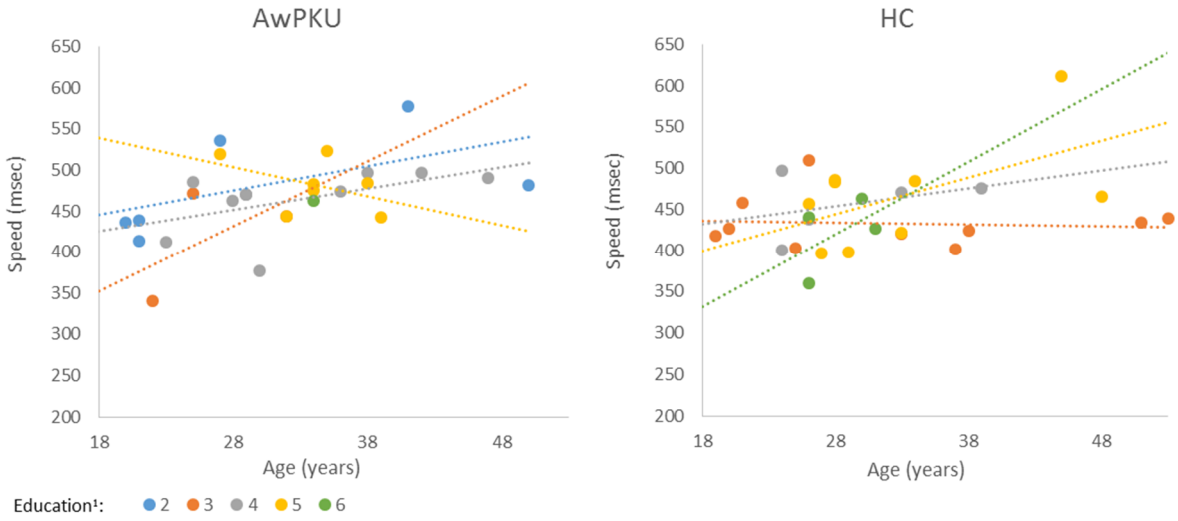
Furthermore, analysis revealed a significant interaction between group and education ($F(1,40)=5.92$, $p=.02$, $\eta_p^2=.13$; Figure 4.19), qualified by a significant group, age and education ($F(1,40)=7.91$, $p<.01$, $\eta_p^2=.17$) interaction (Figure 4.20). Post hoc comparisons showed that the moderating effects of age and education on performance on the DV

task seems to be consistent with those observed on the pattern separation task (see Figure 4.14 and Figure 4.20). No further significant main effects, covariates or interactions were observed.



[1] *Highest completed level of education ranging from 1 (primary education) to 6 (Doctoral degree)*

Figure 4.19 The relationship between education and speed of correct responses (msec) on the DV task for ET AwPKU and healthy controls (HC)



[1] *Highest completed level of education ranging from 1 (primary education) to 6 (Doctoral degree)*

Figure 4.20 The relationship between age, education and speed of correct responses (msec) on the DV task for ET AwPKU and healthy controls (HC)

4.4.2.4 Working memory

Means and standard deviations for accuracy (% correct) and speed (msec) on spatial and numeric working memory tasks for both groups are displayed in Table 4.5. SPSS Linear Mixed Models for accuracy (% correct) and speed (msec) are displayed in Appendices K and L.

4.4.2.4.1 Spatial Working Memory (SWM)

4.4.2.3.2.5 Accuracy

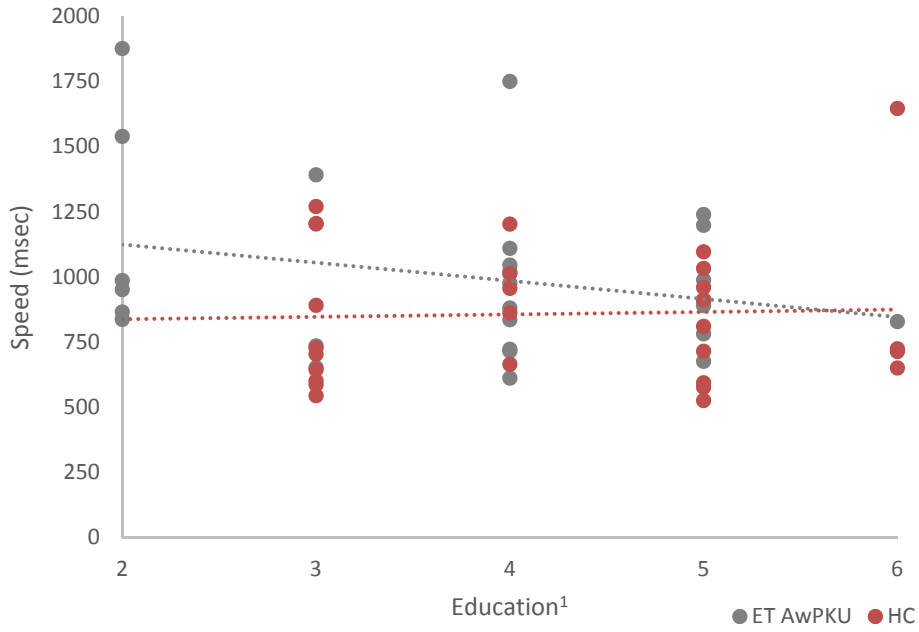
No significant main effects, covariates or interactions were observed for accuracy of performance (% correct) on the SWM task.

4.4.2.3.2.6 Speed

Analysis revealed a main effect of group on reaction times on the SWM task ($F(1,99)= 10.29$, $p=.002$, $\eta_p^2=.09$), such that ET AwPKU were slower than healthy controls (see Table 4.5). Moreover, there was a significant interaction between group and education ($F(1,99)=5.29$, $p=.02$, $\eta_p^2=.05$). ET AwPKU with lower levels of education tended to be slower than healthy controls with lower educational levels, but this difference was not observed at higher levels of education (see Figure 4.21). In addition, significant main effects of type of stimulus ($F(1,99)=5.99$, $p=.016$, $\eta_p^2=.06$) and gender ($F(1,99)=7.27$, $p=.008$, $\eta_p^2=.07$) were observed. Post-hoc analyses revealed both groups were significantly slower at identifying new versus original stimuli. Furthermore, women tended to be slower at identifying both types of stimuli (see Table 4.5).

Table 4.5 Mean (SD) accuracy (% correct) and speed (msec) of ET AwPKU and healthy controls (HC) on tasks of working memory at T2

	ET AwPKU			HC		
	Overall	**Female**	**Male**	**Overall**	**Female**	**Male**
Spatial Working Memory (SWM)						
Overall accuracy (% correct)	96.03 (6.35)	94.95 (7.40)	96.96 (5.24)	97.39 (3.41)	97.38 (3.05)	96.41 (6.46)
Overall speed (msec)	1000.16 (320.86)	1144.55 (383.34)	866.09 (173.35)	858.10 (272.85)	829.41 (231.03)	929.83 (366.13)
Original stimuli						
Accuracy (% correct)	95.91 (6.60)	95.31 (7.60)	96.43 (5.86)	97.45 (3.13)	97.50 (3.14)	97.32 (3.34)
Speed (msec)	885.00 (204.93)	985.20 (231.74)	791.97 (122.73)	842.11 (397.16)	771.46 (204.31)	1018.74 (667.22)
New stimuli						
Accuracy (% correct)	96.15 (6.21)	94.58 (7.52)	97.50 (4.70)	97.32 (3.72)	97.25 (3.02)	97.50 (5.35)
Speed (msec)	1093.80 (456.96)	1277.00 (572.29)	923.69 (244.91)	879.67 (260.49)	875.76 (270.08)	889.44 (252.25)
Numeric Working Memory (NWM)						
Overall accuracy (% correct)	95.47 (5.35)	94.40 (6.29)	96.43 (4.25)	96.91 (6.14)	96.41 (6.46)	97.08 (5.43)
Overall speed (msec)	825.55 (166.70)	874.03 (193.39)	780.54 (128.54)	686.07 (146.30)	663.43 (126.83)	742.66 (183.77)
Original stimuli						
Accuracy (% correct)	93.58 (5.99)	92.82 (7.43)	94.28 (4.42)	93.81 (9.42)	94.04 (8.28)	96.67 (7.13)
Speed (msec)	790.01 (192.83)	850.85 (241.87)	733.51 (115.00)	676.92 (145.23)	647.63 (118.46)	750.14 (186.24)
New stimuli						
Accuracy (% correct)	97.44 (3.81)	96.11 (4.46)	98.57 (2.84)	98.33 (2.94)	98.67 (2.74)	97.50 (3.45)
Speed (msec)	863.53 (177.64)	905.20 (201.25)	824.83 (149.59)	693.68 (156.28)	677.89 (143.43)	733.17 (189.39)



[1]Highest completed level of education ranging from 1 (primary education) to 6 (Doctoral degree)

Figure 4.21 The relationship between education and speed of correct responses (msec) on the SWM task for ET AwPKU and healthy controls (HC)

Furthermore, age was a significant covariate ($F(1,99)=3.96$, $p=.049$, $\eta_p^2=.04$), such that older participants were slower. Finally, analysis revealed significant gender*age ($F(1,99)=8.27$, $p=.005$, $\eta_p^2=.08$), gender*education ($F(1,99)=4.70$, $p=.033$, $\eta_p^2=.05$) and gender*age*education interactions ($F(2,99)=3.45$, $p=.036$, $\eta_p^2=.07$). The difference in speed between younger and older participants appeared to be greater for men than women (see Figure 4.22). Furthermore, the difference in reaction times was greater for older women with higher educational levels whereas this effect of a higher educational level was not observed in older male participants (see Figure 4.23). No other significant covariates or interactions were observed.

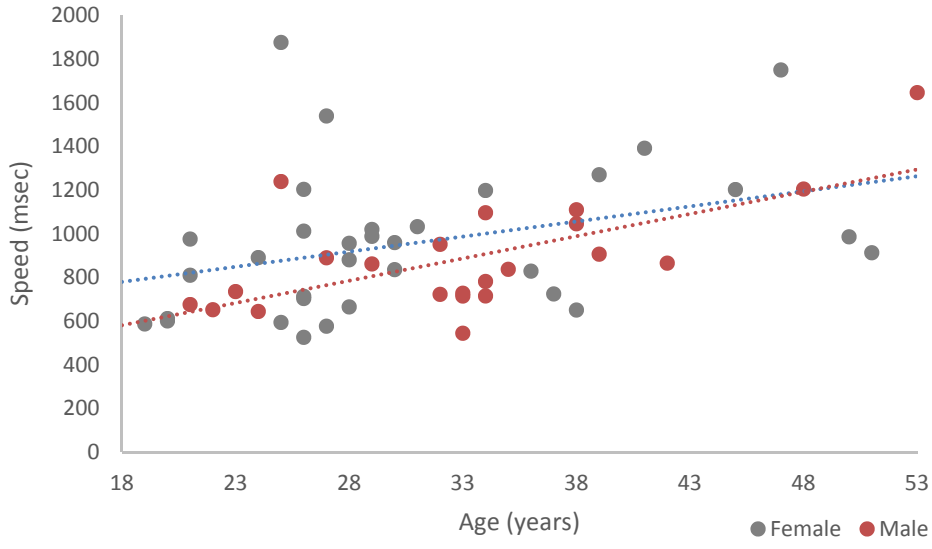
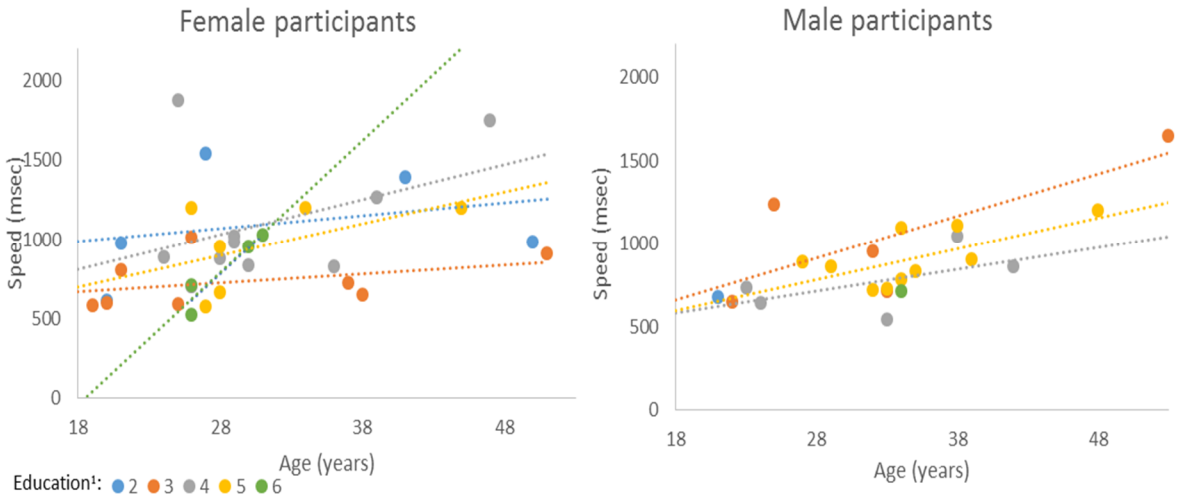


Figure 4.22 The relationship between age and speed of correct responses (msec) on the SWM task for male and female participants



[1]Highest completed level of education ranging from 1 (primary education) to 6 (Doctoral degree)

Figure 4.23 The relationship between age, education and speed of correct responses (msec) on the SWM task for male and female participants

4.4.2.4.2 Numeric Working Memory (NWM)

4.4.2.3.2.7 Accuracy

No significant main effects or covariates were found for the NWM task, but analysis did reveal a significant group*gender*type of stimulus interaction ($F(1,76)=4.42$, $p=.04$, $\eta_p^2=.06$). Accuracy (%) of identifying both types of stimuli was high (>90%) for all participants. However, with the exception of male healthy controls, whose accuracy did not differ across stimulus type, all participants were less accurate at identifying original compared to new stimuli (see Table 4.5). Moreover, female ET AwPKU were less accurate than male ET AwPKU at identifying both types of stimuli, whereas this gender difference was only apparent for the original stimuli in healthy controls (see Table 4.5). No further significant interactions were observed.

4.4.2.3.2.8 Speed

Analysis revealed no main effects of group or type of stimulus, but there was a significant main effect of gender on speed of performance on the NWM task ($F(1,100)=4.85$, $p=.03$, $\eta_p^2=.05$), such that men tended to be slower (see Table 4.5). Additionally, there was a significant group*gender interaction. Post hoc comparisons suggest that ET AwPKU were slower than healthy controls, but this difference was particularly pronounced in female ET AwPKU compared with female healthy controls (see Table 4.5). No significant covariates or further interactions were observed.

4.4.3 Metabolic control

Twenty-five ET AwPKU reported their most recent known Phe levels when completing the second test session. Self-reported Phe levels ranged from 60 to 900 µmol/L. Two ET AwPKU were either pregnant or on the pre-conception-diet. Of the reported levels, 64% were within target ranges of respondents' respective country's guidelines (Blau, van Spronsen, et al., 2010). No significant correlations were found between most recent Phe levels and any of the cognitive performance outcomes (see Appendix M).

4.5 Discussion

4.5.1 Summary of results

This study aimed to assess cognitive functioning in ET AwPKU, in particular, it aimed to explore episodic memory, which has been studied less frequently than other cognitive domains in this patient group. This was the first study reporting on performance on a pattern separation task in ET AwPKU. Results showed that, as a group, ET AwPKU were slower, but not less accurate, at identifying both original and closely similar pictures than healthy controls. However, compared to younger ET AwPKU, older ET AwPKU were less accurate but maintained similar response times. In contrast, older healthy controls were slower but maintained similar accuracy compared to younger healthy controls. There was no evidence for impaired pattern separation (the ability to discriminate between the closely similar pictures and the original pictures) in ET AwPKU compared to healthy controls. Moreover, ET AwPKU showed impairments in speed, but not accuracy, on tasks of attention and working memory. It has been suggested that the reduced processing speed observed on these tasks might represent a trade-off to maintain adequate accuracy (Romani et al. 2018).

4.5.2 Do age and education moderate speed of cognitive performance in PKU?

Even though previous research has attempted to match ET AwPKU and control groups on characteristics known to influence cognitive performance (e.g. age, IQ, SES), the subsequent statistical analyses performed often failed to account for any potential relationships between such factors and outcome measures (exception: Jahja, Huijbregts et al. (2017), who included IQ as a covariate in their analysis). The comprehensive analysis performed on the current data explored all possible relationships between the different factors and covariates, which might contribute to variance observed in the data. This indicates that age and/or level of education achieved modulate speed and

accuracy of responses in ET AwPKU and healthy controls differently. However, it has been consistently reported that ET AwPKU have similar levels of educational attainment compared to matched controls and/or the general population (Bosch et al., 2007; Jahja et al., 2016; Palermo et al., 2017). Overall, results of the current study suggest that, where the ET AwPKU as a group were able to maintain accurate performance (relative to that of healthy controls) with slower response times, older ET AwPKU were unable to compensate in the same way on the pattern separation task compared to younger ET AwPKU and older healthy controls. Moreover, slower decision making was more apparent in ET AwPKU with a higher level of education, which may reflect a higher awareness of potential cognitive issues related to PKU and a greater motivation to perform well on the tasks (Romani, MacDonald, De Felice, & Palermo, 2018). Interestingly, similar moderating effects of age and education on speed of task performance were observed on the pattern separation and DV tasks, suggesting these tasks might be engaging the same cognitive processes. Similarities between these tasks could be reflecting lapses in attention in the second part of the pattern separation task. However, educational levels were not equally represented in the ET AwPKU and healthy controls groups due to differential drop-out between the groups. This may have contributed to the observed interactions between education and group on the speed of performance revealed on all tasks except SRT. As illustrated by the plots depicting the relationship between age, education and RT on the DV, SWM and pattern separation tasks, observed interactions should be carefully interpreted as these interactions may have been skewed by the small sample size as well as the unequal representation of different educational levels. Furthermore, other factors that could have influenced (differences in) performance of ET AwPKU and healthy controls, such as IQ and nutritional status, were not measured in this research. Furthermore, the current study was a cross-sectional assessment, therefore, observed effects of age might be due to individual differences and do not necessarily reflect the trajectory of cognitive ageing in PKU. A longitudinal follow-up of participants would be required to examine this.

4.5.3 Is cognitive ageing accelerated in PKU?

Performance on tasks of attention, processing speed and working memory was similar to that found in previous research in ET AwPKU and reflects the pattern seen in age-related cognitive decline (Romani et al., 2018). In line with recent findings (Romani et al. 2018), ET AwPKU were significantly slower than healthy controls on most tasks that required decision making (CRT, DV, SWM and pattern separation, but not NWM), but not simple single response tasks (SRT). However, in contrast to findings of Romani et al. (2018), there was no greater slowing evident in reaction times on "no" compared to "yes" responses (i.e. responses to new compared to old stimuli) on any of the task. Furthermore, although there was no evidence for impaired pattern separation in this sample of ET AwPKU, the plot of accuracy on the pattern separation task for both types of stimuli in relation to age showed greater decline in accuracy of identifying closely similar pictures. This decline in accuracy across age is similar to the decline observed by Wesnes (2010) in a replication of Toner et al. (2009)'s study. However, in both the Toner et al. (2009) and Wesnes (2010) studies, this decline was observed from age 50 onwards, whereas in the current sample this decline was observed from age 20 onwards in ET AwPKU. A similar decline in accuracy with age was not observed in the healthy controls group. This warrants further investigation and future research should examine whether pattern separation may be impaired at a younger age in ET AwPKU compared to healthy controls. It should be stressed, however, that the current research is a cross-sectional study and results, therefore, observed effects of age may be due to individual differences and do not necessarily reflect the trajectory of cognitive ageing in PKU.

4.5.4 Relationship between cognitive performance and self-reported metabolic control

Although animal studies suggest that elevated levels of Phe negatively impact connectivity in hippocampal areas, no relationship was observed between performance

on the pattern separation task and self-reported recent Phe-levels. Moreover, no associations were found between self-reported recent Phe and any of the other outcome measures. Even though similar levels of metabolic control were reported here as in an audit of metabolic centres across Europe, with nearly 65% of respondents reporting Phe levels within target ranges of their countries guidelines (Ahring et al., 2011), social desirability may have led respondents to underreport their Phe levels. Moreover, it is likely that the Phe levels at the time of testing were different from the last known (reported) level.

4.5.5 Limitations and future research

In addition to the use of self-reported measures of metabolic control, a limitation of this study is its relatively small (n=27) and heterogeneous sample of ET AwPKU. Despite all being treated early, the ET AwPKU had varying levels of self-reported metabolic control and will most likely have differed in metabolic control throughout life. Access to information on-dietary management history and history of metabolic control would have allowed us to better account for these differences.

A strength of the current research is the use of a cognitive test battery comprised of cognitive tasks designed to measure core aspects of cognitive functioning. Most research in ET AwPKU has employed a variety of separate cognitive tasks, and only a limited amount of research has used test batteries such as the Amsterdam Neurological Test battery (ANT) and the Cambridge Neuropsychological Test Automated Battery (CANTAB). Like ANT, CogTrack™ provides participants with specific and clear instructions with regards to where participants should place their hands/fingers. CANTAB, on the other hand, uses touchscreen devices and provides no instructions on hand placement/mode of response, which could cause a delay in recorded response time. Additional advantages of the CogTrack™ system are that it can be used remotely (i.e. at home) and all tests included in the test battery utilised here have multiple parallel forms that can be employed across repeated assessments to avoid practice effects. Hence, CogTrack™, or a similar online cognitive test battery, could be a useful tool in the routine follow-up of individuals with PKU. The use of a remote system to

routinely assess cognitive performance in PKU would potentially limit the burden on both patients and their families (e.g. travel time, time taken off work) and clinics (e.g. time and personnel). A limitation of the CogTrack™ system, however, is that it does not currently include tests of EF other than working memory, whereas ET AwPKU have demonstrated deficits in other EF such as reasoning, planning, and cognitive flexibility (Bilder et al., 2016). Additionally, with the exception of the pattern separation task, accuracy of performance on tasks was high for both groups (on average between 92 and 99%). Therefore, the ability of CogTrack™ to detect differences in cognitive performance between ET AwPKU and healthy controls may have been limited by ceiling effects.

4.5.6 Conclusions

The results of this explorative study suggest that, in addition to well-documented issues with attention and working memory, ET AwPKU may have subtle deficits in hippocampal function reflected in episodic memory performance at a younger age than healthy controls. More research is needed to assess whether this could be related to the negative effects of Phe on synaptic transmission in the hippocampus (Doulgeraki et al., 2002; Glushakov et al., 2002; Horling et al., 2015; Martynyuk et al., 2005). Future studies would benefit from a larger sample size and access to both historical and concurrent measures of metabolic control. Moreover, future studies should better control for potential confounding factors, which might help identify any factors that could protect ET AwPKU from these deficits. In addition, more longitudinal research is needed to investigate the trajectory of cognitive function and age-related cognitive decline in ET AwPKU, particularly as this relates to hippocampal tasks such as pattern separation. Finally, results of this study support previous research showing subtle cognitive impairments in ET AwPKU. Dietary interventions, which may improve the nutritional status of ET AwPKU and consequently could improve cognitive function and QoL/wellbeing, should be investigated. Study 3 presents such an intervention.

Chapter 5 Nutritional status, quality of life and cognitive functioning in adherent and semi-adherent early treated adults with PKU (ET AwPKU; Study 3, part 1)

5.1 Introduction

Individuals with PKU who fail to adhere to their protein substitutes and have either relaxed or stopped their low protein diet are at risk of developing nutritional deficiencies (see also Chapter 1). Despite some patients referring to themselves as being 'off-diet', ET AwPKU tend to continue to avoid high protein foods such as meat and fish (Bernstein et al., 2014; MacDonald et al., 2011). As a result of following a self-restricted diet, either without or with limited consumption of protein substitutes, these 'semi-adherent' patients often 1) consume too much Phe, resulting in Phe levels above target ranges; 2) have a total protein intake below the absolute minimum recommended protein intake of 0.75g/kg/day (British Nutrition Foundation, 2018; Das et al., 2013; Hochuli et al., 2017), and 3) are likely to develop micronutrient deficiencies as a result of insufficient micronutrient intake from foods such as meat, (oily) fish, eggs, dairy products, nuts, seeds and pulses (Hochuli et al., 2017; Robinson et al., 2000; Rohde et al., 2014), some of which have been linked to functioning of the brain (see Table 5.1). In a cross-sectional study with 20 AwPKU, Hochuli et al. (2017) observed that the AwPKU who had suboptimal adherence to their protein substitutes, had an insufficient (self-reported) micronutrient intake, despite a compensatory increase in their dietary protein intake. In addition, a different cross-sectional study of adult/adolescent PKU patients reported that 50% of their patient sample were not taking any protein substitutes although they were unknowingly self-restricting their protein intake (Das et al., 2013). However, (long-term) effects of such dietary practices on nutritional status, QoL/wellbeing and cognitive functioning are not well described in literature. There is limited research on outcomes in off-diet ET AwPKU, and it is often not well-defined what is meant by "off-diet" i.e. the dietary practices of these patients are often unclear. To this end, this chapter aims to compare nutritional status, QoL/wellbeing and cognitive functioning of adherent and semi-adherent ET AwPKU.

Table 5.1 Overview of the role of selected nutrients in brain function and their main food sources – adapted from Montoya Parra, Singh, Cetinyurek-Yavuz, Kuhn, & MacDonald, 2018

Nutrient	Role in brain function	Main food sources
Calcium	Important intra-cellular brain messenger required for synaptic plasticity and secretion of neurotransmitters.	Dairy products.
Cholesterol	Essential component of neuronal membranes, involved in signalling, synaptic plasticity, learning and memory. Also converted to bioactive oxysterols and vitamin D.	Eggs and fat containing foods.
DHA, EPA and phospholipids (PL)	DHA is abundant in the brain. DHA and EPA are components of different PL in synaptic cell membranes. Involved in membrane fluidity and function.	DHA and EPA: oily fish. PL: soya, rapeseed, sunflower, eggs, milk.
Iron	Important in oxygen transport for optimal cognitive function.	Meat, fish, cereals, legumes, nuts, egg yolks, some vegetables, potatoes and fortified foods.
Selenium	Critical role as antioxidant in the brain.	Meat, fish, legumes, grains (variable content in soil).
Vitamin A	Critical role as antioxidant in the brain.	Offal, dairy products, eggs, carrots and dark green leafy vegetables.
Vitamins B6, B_{12} and folate (vitamin B9)	Vitamin B6: neurotransmitter synthesis. Vitamin B12 and folate: important for oxygen transport for optimal cognitive function. Vitamin B_{12} is also involved in myelin synthesis.	Vitamin B6: grains, legumes, nuts, seeds, potatoes, meat and fish. Vitamin B12: meat (especially offal), fish, dairy products, eggs. Folate: dark green leafy vegetables, legumes, fruits and fortified cereals.
Vitamin D	Neuro-steroid, modulates neurotransmission. Helps maintain calcium balance and signalling. Contributes to synaptic plasticity.	Limited dietary sources; mainly oily fish, egg, fortified foods.
Vitamin E	Critical role as antioxidant in the brain.	Nuts, seeds, oily fish, egg yolk and whole grain cereals.
Zinc	Critical role as antioxidant and neurosecretory product in the synaptic vesicles of specific neurons.	Meat, legumes, eggs, fish, grains.

5.1.1 Nutritional status in adults with PKU

In the literature on nutritional status in PKU, data on adults is often not reported separately from data on younger age groups and study samples tend to consist of a mixture of both on-diet AwPKU and AwPKU who are either off-diet or following a relaxed diet. Table 5.2 provides an overview of literature reporting various measures of nutritional status in AwPKU (e.g. AA, vitamins, minerals). The table includes any papers in which results for adults were reported separately, or from which it was possible to extract results for adults from the presented data.

Micronutrient deficiencies that have been observed in ET AwPKU include zinc (Couce et al. (2016), but not Crujeiras, Aldámiz-Echevarría, Dalmau, Vitoria, Andrade, Roca, Leis, Fernandez-Marmiesse, et al. (2015) or Rohr, Munier, and Levy (2001)), selenium (Crujeiras, Aldámiz-Echevarría, Dalmau, Vitoria, Andrade, Roca, Leis, Fernandez-Marmiesse, et al. (2015) and Demirdas et al. (2017), but not Gokmen-Ozel et al. (2009) or Hochuli et al. (2017)), vitamin D (Crujeiras et al., 2015a, 2015b; Nagasaka et al., 2011), homocysteine (Couce et al., 2016; Stølen, Lilje, Jørgensen, Bliksrud, & Almaas, 2013), and copper (Couce et al., 2016).

Table 5.2 Summary of studies assessing nutrient status in AwPKU

Author (year)	Country	Study sample	Study design	Assessments	Results	Comments/Conclusions
Aung et al. (1997)	USA	19 year old male with PKU on a self-restricted diet since age 14	Case report	Routine laboratory examination	- patient reported progressive tiredness, forgetfulness and inability to concentrate - serum vitamin B12: 125pg/ml (normal 200–900pg/ml), serum folate: 3.4ng/ml (normal 1.5–20.6ng/ml) - 1 month after initiation of oral vitamin B12 therapy the patient's general wellbeing improved and laboratory results returned to normal	- self-restricted PKU diets lack vitamin B12 because they exclude meat and dairy products - In the event of neurological dysfunction, both vitamin B12 deficiency and the effect of an elevated phenylalanine level should be considered in the differential diagnosis
Couce et al. (2016)	Spain	141 patients with hyperphenylalaninaemia (HPA) 100 with PKU 43 AwPKU + 6 adults with HPA (≥18 years) Age / gender for adult patients not reported separately Results not reported separately for HPA and PKU	Cross-sectional	Annual median blood Phe levels, Phe tolerance, anthropometric measures, blood pressure, triglycerides (TGC), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoproteins A and B (ApoA and ApoB), vitamin B12, total homocysteine (tHcy), methionine (Met), C-Reactive Protein (CRP)	<u>n adults with HPA/PKU w/ deficiencies:</u> - TC, zinc: 5 (10.2%)[1] - TGC: 1 (2%) - HDL-C, tHcy: 8 (16.3%) [1] - Copper: 14 (28.6%) <u>n adults with HPA/PKU w/ levels above target range:</u> - TC, ApoB, tHcy, vitamin B12: 2 (4.1%)[1] - TGC: 16 (32.7%) - LDL-C, copper: 3 (6.1%)[1] - CRP: 5 (10.2%)	Overall, results suggest an association between lipid profile parameters and good adherence to the diet in PKU patients.

Author (year)	Country	Study sample	Study design	Assessments	Results	Comments/Conclusions
Cruijeiras et al. (2015a)	Spain	156 patients with HPA (43 AwPKU) n=34 mild HPA, n=86 classical PKU, n=36 mild PKU; n=12 late diagnosed (LD) 83 female, 73 male Aged 7 months–42 years Results not reported separately for ET AwPKU, but extracted from Cruijeiras et al. (2015b)	Cross-sectional	Annual median blood Phe, Phe tolerance, anthropometric measurements, biochemical parameters (total protein, pre-albumin, electrolytes, selenium, zinc, vitamin B12, folic acid, ferritin, vitamin D)	- 22 AwPKU had inadequate adherence to diet Group results: - total calcium, phosphorus, vitamin B12, ferritin and zinc levels were within the normal range in almost all patients - pre-albumin was decreased in 34.6% of patients *(3 AwPKU)* - selenium was diminished in 25% of patients *(11 AwPKU)* - vitamin D was altered in 14% of total patients *(9 AwPKU)* - folic acid was above reference level in 39% of patients *(20 AwPKU)* - total protein status and pre-albumin were significantly positively correlated with age and Phe levels - calcium and phosphorus were significantly negatively correlated with age - phosphorus and vitamin B12 were significantly negatively correlated with Phe levels	This study shows a high percentage of pre-albumin *(only 3 AwPKU)* and selenium *(11 AwPKU)* deficiencies as well as an increased level of folic acid in treated PKU patients *(20 AwPKU).*
Das et al. (2013)	Germany	51 ET PKU patients 32 female, 19 male Mean age 26.6 (±6.6) years (range 16-44)	Cross-sectional	Dietary protocol, quality of life questionnaire, blood, urine, body impedance plethysmography and cerebral magnetic resonance imaging (MRI).	- 42% followed a low-protein diet with amino acid mixtures (AAM) - 8% vegan diet with AAM - 14% vegan diet without AAM - 36% self-reported normal diet without AAM; but dietary protocols and blood urea levels indicated protein intake was restricted in this group	- 50% of adolescent/adult PKU did not take AAM but still (unknowingly) self-restricted protein intake - this group had no overt nutritional deficits - however, long-term brain function may be compromised - need for specialized metabolic care in PKU during adulthood

Author (year)	Country	Study sample	Study design	Assessments	Results	Comments/Conclusions
Demirdas et al. (2017)	the Netherlands	60 ET PKU patients (5 off-diet) 15 ET AwPKU 10 female, 5 male Median age 29 years (IQR 20.8-35.8) Majority of results not reported separately for ET AwPKU	Cross-sectional	Dietary intake (questionnaire), blood concentrations of micronutrients and essential fatty acids (EFA), bone mineral density (BMD) and fracture history	All ET PKU: - selenium dietary intake and serum concentrations were low in 14% and 46% of ET PKU, respectively - low serum vitamin D (D2 + D3) in 14% of patients, while 20% had low vitamin D intake - despite adequate intake, zinc was deficient in 14% of ET PKU - elevated folic acid serum concentration as well as intake observed - despite safe total protein and fat intake, arginine and erythrocyte eicosapentaenoic acid were below reference values in 19% and 6% of patients - elevated intake of magnesium, vitamin B6 and B12 observed ET AwPKU: - plasma arginine and asparagine levels were below reference ranges in 11 and 10 out of 15 ET AwPKU, respectively	- zinc deficiency might be due to low/no intake of animal protein, which may reduce absorption of zinc - low selenium serum levels in patients achieving adequate intake may be due to less efficient absorption of the inorganic form used in protein substitutes for PKU - Dutch patients with PKU on long-term dietary treatment have a near normal nutrient status - supplementation of micro-nutrients of which deficiency may be deleterious (e.g., vitamin D and selenium) should be considered
Gokmen-Ozel et al. (2009)	UK	34 PKU patients 13 AwPKU 17 females, 17 males Median age 14.9 years (range 7.2-53.8)	Intervention Baseline data summarised here	Plasma Phe, anthropometric and nutritional biochemistry and haematological markers	<u>For AwPKU (>18 years):</u> - vitamin B12, calcium, albumin and selenium were within reference ranges	

Author (year)	Country	Study sample	Study design	Assessments	Results	Comments/Conclusions
Hanley et al. (1996)	Canada	18 year old female with PKU 0n-diet (irregular intake of AAM)	Case report	Routine (laboratory) examination	- patient presented with spastic paraparesis, tremor, disorientation, slurred speech, distractibility, deteriorating mental function and megaloblastic anaemia - vitamin B12: 65.8 pmol/L (normal 150-670) - treatment with oral vitamin B12 quickly corrected her anaemia and there was a gradual improvement in speech, gait, tremor, disorientation and mood but mild spastic diplegia remained	Authors recommend that complete blood count, serum vitamin B12, red blood cell (RBC) folate, methylmalonic acid (MMA) and homocysteine (Hcy) be routinely measured in adolescents and young adults with PKU.
		37 adolescents and AwPKU 28 on-diet, 9 off-diet 20 females, 11 males Mean age 21.6 years (range 11-35)	Cross-sectional		- 6 patients (2 off-diet) had suboptimal vitamin B12 levels (<150pmol/L) and another 6 (3 off-diet) borderline low levels (15-200) - no neurological signs or other symptoms of vitamin B12 deficiency	
Hvas et al. (2006)	Denmark	31 AwPKU 15 female, 16 male (n=7 unrestricted diet without AAM, n=24 relaxed diet with LNAA supplements) Median age 28 years (range 18-43)	Cross-sectional	Laboratory tests, clinical information and detailed information on food consumption (food frequency questionnaire and 7-day food diary).	- 75% of the AwPKU had early biochemical sign of vitamin B12 deficiency - 9 (29%) AwPKU had a plasma Hcy >12 µmol/L despite a normal folate status - 11 (39%) AwPKU consumed less than the recommended daily vitamin B12 intake - 20 (71%) consumed less than the recommended vitamin B6 intake - 11 (39%) AwPKU took a vitamin pill daily and these patients had significantly lower plasma Hcy	- AwPKU on a less restricted diet are at increased risk of developing vitamin B12 deficiency - PKU patients should continue dietary guidance throughout life - considering the risks, costs and potential benefits, daily vitamin supplementation seems justified in AwPKU on a relaxed diet

Author (year)	Country	Study sample	Study design	Assessments	Results	Comments/Conclusions
Hochuli et al. (2017)	Switzerland	20 AwPKU Classified into 2 groups: [A] Regular intake of AAM [B] AAM intake below calculated requirements	Cross-sectional	<u>Nutrient intake:</u> structured food record Laboratory assessment of nutritional status	Group B: - relatively higher consumption of natural protein, but their total protein intake was below the recommended amounts in 60% of AwPKU (vs. 7% in group A) - higher fat intake - intake of selenium, folate and vitamin B12 were below the recommended intake - serum selenium, folate and vitamin B12 remained within the normal range (both groups) - plasma tyrosine correlated with AAM intake, and hydroxyproline correlated with natural protein consumption	Relaxed AAM intake resulted in insufficient nutrient supply, despite a compensatory increase in consumption of natural protein.
Modan-Moses (2005)	Israel	31 ET AwPKU (all classical) 18 female, 13 male Mean age 25(±5.3) years (range 19-41) Split into adherent (n=17) and non-adherent (n=14) based on patients' own report.	Cross-sectional	Bone mineral density (BMD). <u>Food intake</u> (3-day food diaries/24 hour recall): energy, protein, fat, Phe, calcium. Blood minerals, Phe, vitamin D, calcium and alkaline phosphatase (PO_4).	- non-adherent ET AwPKU off-diet 7.4 (±4.9) years - only 8/17 adherent AwPKU (32.2%) had Phe levels within target ranges (< 726 µmol/L) - no significant differences serum vitamin D, calcium, PO_4 or BMD between groups, but BMD decreased in ET AwPKU (based on z-scores) - significantly lower protein and calorie intake non-adherent AwPKU - significantly higher calcium intake in non-adherent ET AwPKU	Peak bone mass is decreased in (adherent and non-adherent) ET AwPKU, but does not seem to be linked to (micro)nutrient status.

Author (year)	Country	Study sample	Study design	Assessments	Results	Comments/Conclusions
Moseley et al. (2002)	USA	25 adults and 2 adolescents with PKU; (25 on-diet, n=11 LD) 18 female, 9 male Mean age 31 years (range 10-50) 120 non-PKU adult controls Mean age 37.7 (±12.2) years (range 22-78)	Cross-sectional	Plasma Phe, cholesterol, lipoproteins, triglycerides and fatty acid (FA) profiles (seven FA in plasma and RBC).	- 7 patients had significantly elevated cholesterol/HDL-C ratios; triglyceridaemia was documented in 5 of these - slight but significant reductions in long-chain polyunsaturated fatty acids (LCPUFA) in PKU - In 6 AwPKU plasma docosahexaenoic acid (DHA) or arachidonic acid (ARA) concentrations were <50% of controls	Since DHA and ARA have important physiological roles, including brain and retinal function, it is recommended that blood lipid concentrations be monitored in all patients with PKU (including AwPKU).
Nagasaka (2011)	Japan	34 AwPKU 21 female, 13 male Aged 20-35 years 36 age-matched controls 22 female, 14 male	Cross-sectional	Vitamin D, parathyroid hormone (PTH), bone turnover markers, and daily nutrient intake (3-day dietary history).	- AwPKU had lower daily energy and protein intake - No group differences in fat, vitamin D and calcium intake - Vitamin D and PTH in female PKU significantly higher than female controls - Vitamin D in male PKU significantly lower than male controls - Urinary calcium levels and bone resorption (but not formation) markers were significantly higher in AwPKU than controls - None of the bone parameters correlated significantly with serum Phe or nutrient intake	AwPKU exhibited lower vitamin D status and more rapid bone resorption despite normal calcium and vitamin D intakes. Additional investigations must be undertaken to determine the factors of bone metabolism status in PKU.
Nagasaka (2013)	Japan	33 AwPKU Aged 21-38 years 20 age-matched controls	Cross-sectional	Blood oxysterols, blood Phe, lipids and vitamin D.	- Total LDL-C and vitamin D significantly decreased in AwPKU. - brain peripheral and hepatic cholesterol elimination were significantly decreased in the AwPKU (25-40%) - oxidative stress was increased significantly in the AwPKU group	Blood oxysterol changes predominate over blood cholesterol changes and influence vitamin D status in AwPKU. Unfavourable effects of altered oxysterol metabolism on the nervous system and bone health need to be further investigated.

Author (year)	Country	Study sample	Study design	Assessments	Results	Comments/Conclusions
Rohr et al. (2001)	USA	9 PKU patients 6 AwPKU 6 female, 3 male 10-32 years	Intervention Baseline data summarised here	Plasma Phe, serum ferritin, folate, serum vitamin B12 and serum zinc.	At baseline: - Phe levels >1000µmol/L for all AwPKU - 3 AwPKU had ferritin levels above reference range, 2 within range (1 missing) - 3 AwPKU had folate levels above reference range, 3 within range - all 6 AwPKU had vitamin B12 levels within the reference range - 4 AwPKU had zinc concentrations above the reference range (2 missing)	
Stølen et al. (2014)	Norway	56 PKU patients 22 AwPKU (n=7 LD)	Cross-sectional	<u>Dietary interview</u> Intake of folic acid and vitamin B12 from AAM (for patients adherent with their AAM). Intakes of folic acid and vitamin B12 were compared with plasma levels of folate, vitamin B12, and Hcy.	- Median plasma folate was well above the upper reference level in AwPKU - 73% of AwPKU had plasma folate above the upper reference level - Median plasma vitamin B12 was in range in AwPKU - 23% of AwPKU had plasma B12 above the upper reference level - median plasma Hcy was at the lower reference range in adults - 18% of AwPKU had plasma Hcy below the reference range	Many PKU patients have a very high level of plasma folate related to the very high content of folic acid of their AAM. There is no evidence of risk associated with high intakes of folate from natural sources, but a high intake of folic acid from AAM may mask haematological symptoms caused by vitamin B12 deficiency.
Vugteveen et al. (2011)	the Netherlands	75 ECT PKU patients	Cross-sectional	Serum vitamin B12, plasma Hcy, MMA, and blood Phe.	- 8 ECT PKU (6 adults) had vitamin B12 concentrations below normal. Of these 8 patients, 2 AwPKU had elevated MMA and/or Hcy concentrations; - 10 other patients (3 adults) with normal vitamin B12 concentrations had elevated MMA and/or Hcy levels	A vitamin B12 concentration within the reference range does not automatically imply sufficient vitamin B12 availability.

Key: AAM: amino acid mixture; ApoA: apolipoprotein A; ApoB: apolipoprotein B; ARA: arachidonic acid; BMD: bone mineral density; CRP: C-reactive protein; DHA: docosahexaenoic acid; (E)AA: (essential) amino acids; (ET) AwPKU: (early treated) adults with phenylketonuria; (E)FA: (essential) fatty acids; HDL-C: high-density lipoprotein cholesterol; HPA: hyperphenylalaninaemia; LD: late diagnosed; LCPUFA: long-chain polyunsaturated fatty acids; LDL-C: low-density lipoprotein cholesterol; Met: methionine; MMA: methylmalonic acid; PO_4: alkaline phosphatase; RBC: red blood cell(s); TC: total cholesterol; (t)Hcy: total homocysteine; TGC: triglycerides

Notes: [1] not necessarily the same patients

In addition, Demirdas et al. (2017) reportedly observed deficiencies in arginine (Arg) and asparagine (Asn) in approximately two thirds of their sample of ET AwPKU. Moseley et al. (2002) observed low docosahexaenoic acid (DHA) and arachidonic acid levels in approximately 25% of the AwPKU in their study sample. Furthermore, increased levels of folate (vitamin B9) and its synthetic form folic acid (Crujeiras et al., 2015a, 2015b; Stølen et al., 2013), triglycerides (Couce et al., 2016), high-density lipoprotein cholesterol (HDL-C) and total cholesterol (Couce et al., 2016; Moseley, Koch, & Moser, 2002) have been reported in ET AwPKU.

The majority of studies that assessed vitamin B12 status reported that their sample of AwPKU had levels within the reference range (Couce et al., 2016; Crujeiras et al., 2015a; Gokmen-Ozel et al., 2009; Rohr et al., 2001; Stølen et al., 2013), despite reports of inadequate (self-reported) vitamin B12 intake (Hochuli et al., 2017). However, several papers have highlighted the risk of developing vitamin B12 deficiencies on a relaxed PKU diet (Aung, Klied, McGinn, & McGinn, 1997; Hanley, Feigenbaum, Clarke, Schoonheyt, & Austin, 1996; A. M. Hvas, Nexo, & Nielsen, 2006; Robinson et al., 2000). Moreover, it has been shown that vitamin B12 levels within the reference range do not automatically imply sufficient vitamin B12 status (Vugteveen et al., 2011). Vitamin B12 acts as a co-factor in enzymatic reactions converting homocysteine and methylmalonic acid (MMA) into methionine (Met) and succinyl co-enzyme A (succinyl-CoA), respectively. Therefore, elevated homocysteine and/or MMA could indicate a functional vitamin B12 deficiency even when vitamin B12 levels are within range (Wiersinga, de Rooij, Huijmans, Fischer, & Hoekstra, 2005).

Elevated levels of homocysteine were observed in approximately 29% of a sample of Danish AwPKU who were either following an unrestricted diet without protein substitutes or a relaxed diet with LNAA supplements (Hvas et al., 2006). Moreover, in the same sample, it was found that AwPKU who took a vitamin supplement daily had significantly lower plasma homocysteine levels than those who did not (Hvas et al., 2006). In addition to an insufficient intake of vitamin B12, Hochuli et al. (2017) reported that self-reported intake of selenium and folate were below the RDI, but serum selenium and folate levels both remained within the normal range (Hochuli et al., 2017). Insufficient intake of micronutrients (with the exception of vitamin B12) was also observed in a sample of 10 children and 1 adult with PKU on a relaxed diet (Rohde et al.,

2014). However, in contrast to research reported by Hochuli et al. (2017), intake of protein, including EAA was found to be sufficient (Rohde et al., 2014).

5.1.2 Relationship between nutritional status and quality of life (QoL)

Limited research on the QoL of AwPKU suggests that HRQoL of AwPKU does not differ widely from that of healthy controls or data from a reference population (see Chapter 1). However, little is known about the effects of differing adherence to dietary treatment on QoL in PKU. Elevated Phe levels observed in ET AwPKU with poor dietary adherence, could adversely affect well-being by limiting Tyr uptake to the brain and impairing dopamine synthesis. Clacy et al. (2014) and Sharman et al. (2012) have reported significant associations between depressive symptoms, anxiety and stress and lifetime Phe and Tyr levels as well as the Phe:Tyr ratio. Increasing depressive symptoms were also associated with poorer EF (Sharman, Sullivan, Young, & McGill, 2012). In addition to the potential effects of elevated Phe, decreased Tyr and an altered Phe:Tyr ratio on mental health, deficiencies in vitamin B12 and omega-3 fatty acids have been linked to depression (Lin & Su, 2007; Tiemeier et al., 2002) and deficiencies in zinc and vitamin D have been associated with poor mood (Barnard & Colón-Emeric, 2010; Sawada & Yokoi, 2010; Wilkins, Sheline, Roe, Birge, & Morris, 2006). Hence, deficiencies in these micronutrients could potentially contribute to issues with depression and mood observed in AwPKU.

5.1.2.1 Assessing quality of life (QoL) in PKU

The majority of studies that assessed QoL in AwPKU (see Table 1.3, Chapter 1) employed generic HRQoL questionnaires (Bosch et al., 2007; Cazzorla et al., 2014; Cotugno et al., 2011; Das et al., 2013; Demirdas et al., 2013; E. Simon et al., 2008). It has been suggested that these generic HRQoL questionnaires may be not be sensitive enough to detect subtle issues with HRQoL that are specific to PKU (Briançon, Gergonne, Guillemin, Empereur, & Klein, 2002; Patrick & Deyo, 1989; Regnault et al., 2015). Therefore, Regnault and colleagues (2015) recently developed PKU specific QoL questionnaires (PKU-QoLQ). They postulated that their questionnaires would be able to detect subtle

PKU-specific deficits in HRQoL as well as potential improvements in HRQoL resulting from therapeutic interventions. Moreover, they argued that a PKU-specific HRQoL questionnaire would make more sense to individuals with PKU, which may lead to more honest and accurate responses to the questionnaire (Regnault et al., 2015). Results from a large international study using the PKU-QoLQ support the ability of the questionnaires to identify PKU-specific HRQoL issues and measure differences in HRQoL in relation to both the severity and management (e.g. treatment received) of the disorder (Bosch et al., 2015). These questionnaires may also be sensitive enough to be able to detect differences in QoL that are a result of differing levels of adherence.

5.1.3 Relationship between nutritional status and cognitive functioning

Going from a restricted diet during childhood to following a less restricted or relaxed diet in adolescence or adulthood, with limited or no supplementation of protein substitutes, may expose patients to sub-optimal nutritional intake and deficiencies which may impact normal brain function. As shown in Chapter 3 (systematic review), deficits in cognitive functioning in ET AwPKU have most frequently been reported in relation to sustained attention, working memory and motor skills. Deficits in performance on tasks of attentional capacity, verbal fluency, complex language skills, complex EF and inhibitory control have been reported, albeit less consistently. However, only a limited amount of research has reported on cognitive performance of off-diet ET AwPKU or ET AwPKU following a relaxed diet and these samples were either small or consisted of a mixed sample of on-diet and off-diet ET AwPKU. Furthermore, previous research often failed to clearly characterise (the dietary practices of) the off-diet ET AwPKU included. Therefore, the effect of following a self-restricted diet with poor adherence on cognitive functioning remains unclear.

5.1.3.1 Relationship between metabolic control and cognitive functioning

Deficits in cognitive functioning believed to be related to patients' Phe levels at several stages throughout life (e.g. concurrent Phe levels, lifetime Phe levels, variation in Phe levels) and several studies in ET AwPKU have reported associations between high Phe levels and deficits in cognitive functioning across domains (see Chapter 3). Moreover, an intervention assessing the effects of Phe in ECT AwPKU showed deficits in performance on a sustained attention task after a 4-week Phe load (ten Hoedt, de Sonneville, et al., 2011). However, the authors reported no significant effects of the Phe load on any of the other measures of cognitive performance employed (processing speed, visuospatial processing, working memory, inhibition and set shifting, and (visuo)motor skills).

In addition, limited research on the association between the Phe:Tyr ratio and EF in PKU suggests that high lifetime ratios rather than average Phe levels are more closely associated with observed deficits in EF (Luciana et al., 2001; Sharman et al., 2009).

However, due to methodological issues related to the measurement of Phe and Tyr, as well as discrepancies in reporting of these measures in the literature, these observed correlations may not be very reliable (see also Chapter 3). Furthermore, these issues make it difficult to compare study outcomes and, therefore, it is still unclear how metabolic control throughout life influences cognition in ET AwPKU.

5.1.3.2 Relationship between micronutrients and cognitive functioning

As is clear from Table 5.1, in addition to elevated Phe levels, decreased Tyr levels, and an altered Phe:Tyr ratio potentially negatively affecting cognitive performance of ET AwPKU following a nutritionally incomplete diet, the majority of the (micro)nutrients which are likely to be lacking in such a diet have been linked to cognitive functioning (Montoya Parra et al., 2018). Vitamin B12 deficiency, for example, could potentially cause neurological impairment (MacDonald et al., 2010). In fact, Brenton and Pietz (2000) pointed out that neurological deterioration observed in PKU patients who ceased treatment or had poor adherence to treatment is similar to neurological symptoms of vitamin B12 deficiency. Vitamin B12 is involved in myelin synthesis and vitamin B12,

folate and iron are all involved in oxygen transport needed for optimal cognitive functioning (Walter, 2011). Zinc and selenium are both important antioxidants (Frederickson, Suh, Silva, Frederickson, & Thompson, 2000). In addition, zinc is involved in neurotransmission (Frederickson et al., 2000) and has been previously been related to attention, learning and memory (Sandstead, Frederickson, & Penland, 2000). Vitamin D has also been shown to be involved in neurotransmission and helps maintain calcium balance and signalling (Harms, Burne, Eyles, & McGrath, 2011). Finally, cholesterol, DHA and eicosapentaenoic acid (EPA) are all essential components of (neuronal) cell-membranes, are involved in signalling and synaptic plasticity (Calder, 2012; Dyall, 2015; Martin, Pfrieger, & Dotti, 2014), and have been linked to learning, memory and motor functions (Agostoni et al., 2003; Schreurs, 2010). Hence, deficiencies in these micronutrients may contribute to cognitive deficits observed in AwPKU.

5.2 Study aims

The aim of the study presented in this chapter was to explore the effects of following a self-restricted low-protein diet in combination with poor or no adherence to protein substitutes compared to good dietary adherence on nutritional status, QoL and cognitive performance by comparing these outcomes in a group of semi-adherent ET AwPKU and a group of adherent ET AwPKU.

5.3 Method

5.3.1 Design

Study 3, part 1 was a cross-sectional comparison of adherent and semi-adherent ET AwPKU. The semi-adherent ET AwPKU were subsequently enrolled onto a 12-week intervention with a new protein substitute (see Chapter 6).

5.3.2 Participants

Ten adherent and ten semi-adherent ET AwPKU were recruited from the Mark Holland Metabolic Unit at Salford Royal NHS Foundation Trust (SRFT). Eligible ET AwPKU were identified by specialised metabolic dietitians in the Metabolic Unit. Identified patients were invited to participate in the trial via recruitment letters or verbally, by their specialised metabolic dietitians and clinicians during visits to the clinic.

Adherence to dietary management is regularly assessed by the metabolic dietitians during patient visits to the clinic using 24-hour dietary recall. Patients were considered to be adherent if this assessment showed that their total protein intake (from natural protein and Phe-free AA protein substitutes) was ≥0.75 g/kg bodyweight and their Phe levels were within UK target ranges (<700 µmol/L). If the patient's total protein intake was <0.75 g/kg bodyweight and their Phe levels were >1000 µmol/L, they were considered to be semi-adherent to their dietary management.

5.3.2.1 Participant inclusion criteria

AwPKU who were diagnosed through new-born screening were considered for participation in the trial. Dietary management of both adherent and semi-adherent AwPKU had to be initiated within the first 3 months of life and followed until at least 16 years of age. Furthermore, adherent patients had to be adherent to their dietary management (protein-restricted diet and Phe-free AA protein substitutes) for at least 6 months prior to participation in the study (see Table 5.3). Semi-adherent patients had to be on a self-restricted low-protein diet and be non-adherent to the Phe-free AA protein substitutes for at least 6 months at the time of inclusion. Finally, all patients had

to be able and willing to provide informed consent, happy to undergo blood tests and otherwise comply with the study protocol.

Table 5.3 Inclusion criteria for adherent and semi-adherent ET AwPKU

Adherent ET AwPKU	Semi-adherent ET AwPKU
Phe levels at screening <700 µmol/L	Phe levels at screening >1000 µmol/L
Male or female	Male or female
Age ≥16 years	Age ≥16 years
Diagnosed at birth (new-born screening)	Diagnosed at birth (new-born screening)
Early[1] and continuously managed (by means of a protein-restricted diet and Phe-free AA protein substitutes) until (at least) 16 years of age	Early[1] and continuously managed (by means of a protein-restricted diet and Phe-free AA protein substitutes) until (at least) 16 years of age
Adherent to dietary management (protein-restricted diet and Phe-free AA protein substitutes) for at least 6 months prior to participation: total protein intake (natural protein and protein from Phe-free AA protein substitutes) ≥0.75 g/kg body weight	Semi-adherent to dietary management (non-adherent to Phe-free protein substitutes and following a self-restricted diet: restriction of high-protein foods) for at least 6 months prior to participation: total protein intake <0.75 g/kg bodyweight.
Able/willing to provide informed consent	Able/willing to provide informed consent

Note: [1] dietary management initiated within first 3 months of life

5.3.2.2 Participant exclusion criteria

Women who were pregnant or planning to become pregnant were excluded from participation in the study, as these patients would have been following a very strict (pre-conception/ pregnancy) diet, most likely supplemented with several of the micronutrients that were outcome variables in this research. It was planned that any women who became pregnant during the study would be excluded immediately, although this did not occur. Similarly, participants fitted with a pacemaker were to be excluded from bio-impedance measures, but this was not applicable to any of the included participants. For full exclusion criteria, see Table 5.4.

Table 5.4 Exclusion criteria for adherent and semi-adherent ET AwPKU

Adherent ET AwPKU	Semi-adherent ET AwPKU
Phe levels at screening >700 µmol/L	Phe levels at screening <1000 µmol/L
Aged < 16 years	Aged < 16 years
Late diagnosed PKU	Late diagnosed PKU
Pregnant or planning to get pregnant	Pregnant or planning to get pregnant
Discontinued diet before 16 years of age	Discontinued diet before 16 years of age
Unable/unwilling to provide informed consent	Unable/unwilling to provide informed consent

5.3.3 Anthropometric measures

Participants' weight and body composition (% body fat) were measured using a portable bio-impedance scale (Tanita SC-240, Tanita Europe B.V., Amsterdam, the Netherlands). To obtain participants' BMI, height was also measured using a stadiometer.

5.3.4 Nutritional status

5.3.4.1 Venous blood measures

A venous blood sample (~19mL whole blood) was obtained on the test day via venepuncture. Samples were analysed for a full AA profile, protein status (albumin, pre-albumin and transferrin), C-reactive protein (CRP) and a range of micronutrients and related measures (e.g. enzymes, hormones).

Samples were separated upon collection by means of centrifugation. Separated serum and plasma were pipetted into individual microtubes. Serum and plasma samples were stored in a -80 °C freezer at the Human Appetite Research Unit (HARU), School of Psychology, University of Leeds until analysis.

Full AA profiles were analysed using a Biochrom 30 plus amino acid analyser (Biochrom Ltd., Cambridge Science Park, England) at the Biochemical Genetics laboratory at St James's University Hospital, Leeds Teaching Hospitals NHS Trust (LTHT). Tryptophan (Trp) levels were not analysed and reported as the assay employed was not sensitive

enough to detect Trp. Pre-albumin levels were analysed at Sheffield Teaching Hospitals NHS Trust. All other assays were performed at specialized laboratories at LTHT. To aid interpretation, reference ranges for all measures of nutritional status are reported within the tables reporting results. All reference ranges were provided by the appropriate laboratories.

5.3.4.2 Dried blood spots (DBS)

Finger prick blood sampling was used to obtain DBS for the analysis of blood Phe and Tyr levels at the time of screening. Analyses were performed at the Biochemical Genetics laboratory at St James's University Hospital, LTHT.

5.3.5 Intelligence Quotient (IQ)

Full scale IQ (FSIQ) was estimated using the Wechsler Abbreviated Scale of Intelligence (WASI). The WASI consists of four subtests – Vocabulary, Block Design, Similarities and Matrix Reasoning – which tap into various facets of intelligence, such as verbal knowledge, visual information processing, spatial and nonverbal reasoning and crystallized and fluid intelligence (Horn & Cattell, 1966).

5.3.5.1 Vocabulary subtest

The WASI Vocabulary subtest is a 42-item task similar to the Vocabulary subtests of the WISC-III and the WAIS-III, except that the WASI subtest includes picture items. Items 1-4 of the Vocabulary subtest require the naming of pictures, which are displayed one at a time. Items 5-42 are orally and visually presented words that the examinee orally defines. Vocabulary is a measure of the individual's expressive vocabulary, verbal knowledge and fund of information. Additionally, it is a good measure of crystallized intelligence (the ability to use skills, knowledge, and experience) and general intelligence. It also taps other cognitive abilities, such as memory, learning ability and concept and language development (Sattler, 1988).

5.3.5.2 Block Design subtest

The WASI Block Design subtest consists of a set of 13 modelled or printed two-dimensional geometric patterns that the examinee replicates within a specified time limit using two-colour cubes. The subtest assesses abilities related to spatial visualization, visual-motor coordination and abstract conceptualization. It is a measure of perceptual organization and general intelligence.

5.3.5.3 Similarities subtest

The WASI Similarities subtest contains 4 picture items (Items 1-4) and 22 verbal items. For each of Items 1-4, the examinee is shown a picture of three common objects on the top row and four response options on the bottom row. The examinee responds by pointing to the one response option that is similar to the three target objects. For each verbal item, a pair of words is presented orally, and the examinee explains the similarity between the common objects or concepts that the two words represent. This subtest provides a measure of verbal concept formation, abstract verbal reasoning ability and general intellectual ability.

5.3.5.4 Matrix Reasoning subtest

The WASI Matrix Reasoning subtest is a series of 35 incomplete patterns on a grid that the examinee completes by pointing to or stating the number of the correct response from five possible choices. Matrix Reasoning is a measure of nonverbal fluid reasoning and general intellectual ability.

The WASI was administered in accordance with the instructions provided in the WASI Manual (PsychCorp 1999). Administration of all four subtests is a means of quickly estimating an individual's verbal, non-verbal and general cognitive functioning in approximately 30 minutes.

5.3.6 Cognitive performance testing

The cognitive performance of adherent and semi-adherent ET AwPKU was assessed using selected tasks from the Amsterdam Neurological Tasks (ANT) battery. The ANT battery has been used to assess cognitive functioning in various clinical conditions,

including PKU (Huijbregts et al., 2003; Huijbregts, de Sonneville, Licht, Sergeant, et al., 2002; Huijbregts, de Sonneville, van Spronsen, et al., 2002; Jahja, Huijbregts, et al., 2017). Various ANT paradigms have been shown to be sensitive to differences in cognitive functioning between ECT AwPKU with differing levels of metabolic control (Jahja, Huijbregts, et al., 2017; Jahja, van Spronsen, et al., 2017) as well as changes in cognitive performance following dietary interventions in both children (Huijbregts, de Sonneville, Licht, van Spronsen, & Sergeant, 2002) and adults (Schmidt et al., 1994; ten Hoedt, de Sonneville, et al., 2011) with PKU. Table 5.5 provides an overview of the selected tasks, cognitive domains assessed and outcome measures.

Tasks were administered by one investigator using a laptop. The input device for the participants' responses was a standard mouse with symmetrical buttons. Participants were instructed verbally and were shown task-specific target stimuli on the screen. Subsequently, prior to task administration, participants practiced until the tasks were understood.

Participants were instructed to use their left and right index fingers to press the left and right mouse buttons, respectively. They were told to keep their index fingers lightly resting on the appropriate mouse buttons and respond as quickly and accurately as possible. For tasks consisting of separate parts for the left and the right hand (e.g. BS), the part which required participants to use their non-dominant hand was always administered first. In tasks where the mouse buttons represented 'yes' and 'no' answers (e.g. MS2D), the left mouse button was the 'yes'-button for left-handed participants, and the right mouse button represented 'yes' for right handed participants. In 'two-button tasks' where the keys had no 'yes'/'no' function (e.g. FL), instructions were the same for all participants.

All tasks were self-paced. The post response interval, that is, the period between the response and next stimulus onset, was 250 msec in the SAD task and 1200 msec in the MS2D task (standard ANT settings). The valid response window, that is, reaction times (RT) that were considered valid, was set at 200–6000 msec post stimulus onset.

Table 5.5 Overview of the ANT test battery used to assess the cognitive functioning of adherent and semi-adherent ET AwPKU

Cognitive Task	Task ID	Cognitive domain	Outcome measures
Baseline Speed	BS	Alertness of attention (reaction times)	Mean and standard deviation (SD) reaction times of the left and right hand, mean (SD) overall reaction times, number of omissions
Flanker	FL	Selective/directed attention Inhibition (EF)	Mean (SD) reaction times for correct responses per response type (neutral vs. compatible; compatible vs. incompatible), number of errors per response type
Memory Search 2D Objects	MS2D	Divided attention Working memory (EF)	Mean (SD) reaction times for correct responses per response type, number of errors (misses and false alarms*)
Sustained Attention Dots	SAD	Sustained attention	Mean (SD) reaction times for correct responses, number of errors (misses and false alarms; false alarms to fewer (low) or more (high) dots)
Shifting Attentional Set - Visual	SSV	Inhibition of pre-potent responses (EF) Attentional flexibility (set shifting, EF)	Mean (SD) reaction times for correct responses per response type (compatible vs. incompatible), number of errors
Tracking	TR	Fine motor function*	Mean deviation (from ideal trajectory) left, right and overall and percentage of movements within target area
Pursuit	PU	Fine motor function*	Mean deviation (from target), percentage of movements within 3, 6 and 12 mm from target

** Tasks differ in a low (TR) vs. high (PU) demand on executive function (EF); the difference in performance on both tasks differentiates the EF of the responder*

5.3.6.1 Baseline Speed (BS)

This 2-part (separate parts for the left and the right hand) task provided a baseline level of response speed. At the start of this task, a cross was visible in the centre of the screen. Participants were instructed to watch the cross closely, as it would turn into a square at random intervals. Participants were asked to press the relevant mouse button (i.e. left for part 1, right for part 2) as fast as possible whenever the square appeared on the screen. Upon the participants' response, the square would immediately turn back into a cross (see Figure 5.1). Participants were told to only press the mouse button when they saw the square, not when they expected the square to appear on the screen. They completed the task twice, once for their non-dominant hand and once with their dominant hand. Each part consisted of 32 trials. Reaction times (RT; msec), number of omissions and number of premature responses were recorded.

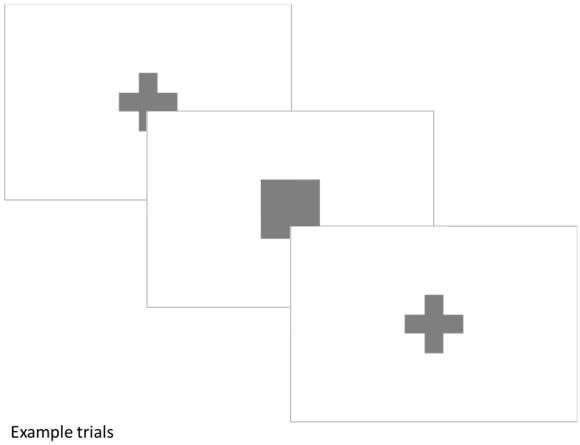


Figure 5.1 Trials on the BS task

5.3.6.2 Flanker (FL)

This task provided a measure of selective / directed attention and inhibition and consisted of two parts. The stimulus used was a 3x3 matrix, consisting of a central square surrounded by 8 squares ('flankers'). Participants were required to focus on the central stimulus, whilst ignoring the flankers. The colour of the central square determined the required response. In part 1 (40 trials), the central square was surrounded by compatible (same colour; 20 trials) or neutral flankers (20 trials), in part 2 (80 trials) it was surrounded by compatible (40 trials) or incompatible flankers (colour linked to the opposite hand; 40 trials). Example stimuli and trials are shown in Figure 5.2.

The differences in errors (n) and RT (msec) between compatible and incompatible flanker stimuli (part 2) provided a measure of inhibitory control / interference suppression. Number of errors and RT (msec) of correct responses for neutral, compatible and incompatible trials were recorded.

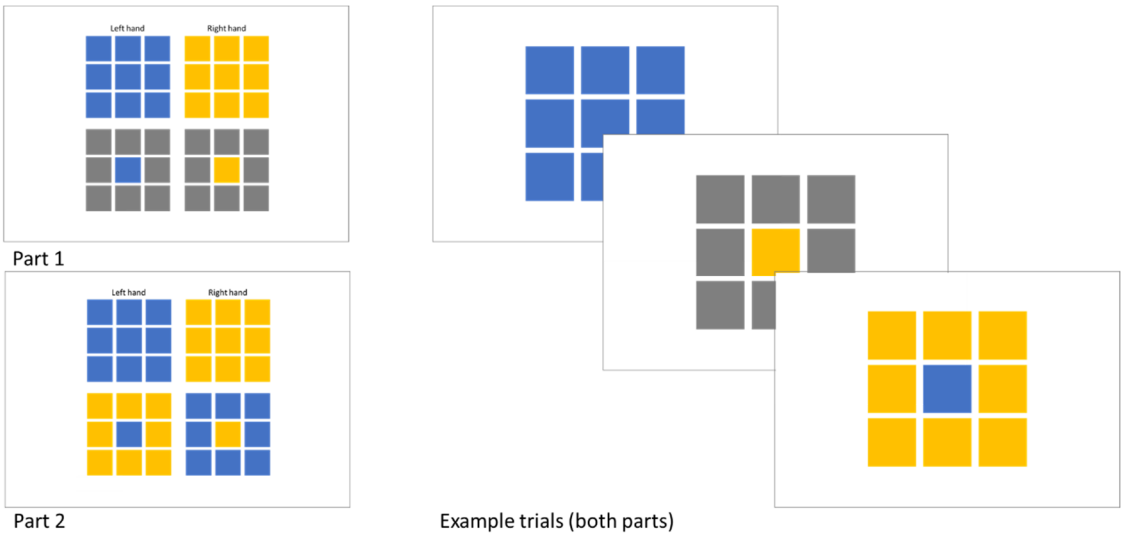


Figure 5.2 Example target stimuli (left) and example trials (right) for both parts of the FL task

5.3.6.3 Memory Search 2D Objects (MS2D)

The MS2D task provided a measure of divided attention and working memory and consisted of two parts. The stimuli used were 4 different shapes: a square, circle, triangle and cross. In each trial, the four shapes would appear in different shape-colour (blue, red, yellow or green) combinations. In part 1 (48 trials, with 24 containing the target stimulus) participants had to identify one memorized target stimulus (e.g. a blue triangle) with two specific features (shape and colour) out of four stimuli displayed on the screen. Participants were instructed to return a 'yes' response as quickly as possible when the target stimulus was present and a 'no' response when it was not. A 'yes' response was given by pressing the mouse button under the index finger of the dominant hand, the index finger of the non-dominant hand returned 'no' responses. In part 2 (48 trials, with 24 containing a target stimulus), three different target stimuli (e.g. a yellow cross, a red circle, and a green triangle) had to be remembered, of which one

had to be present in the display for a 'yes' response. If none of the target stimuli were present, participants were required to return a 'no' response. Figure 5.3 shows examples of target stimuli and trials for both parts of the MS2D task. Number of errors and RT (msec) of correct responses were recorded.

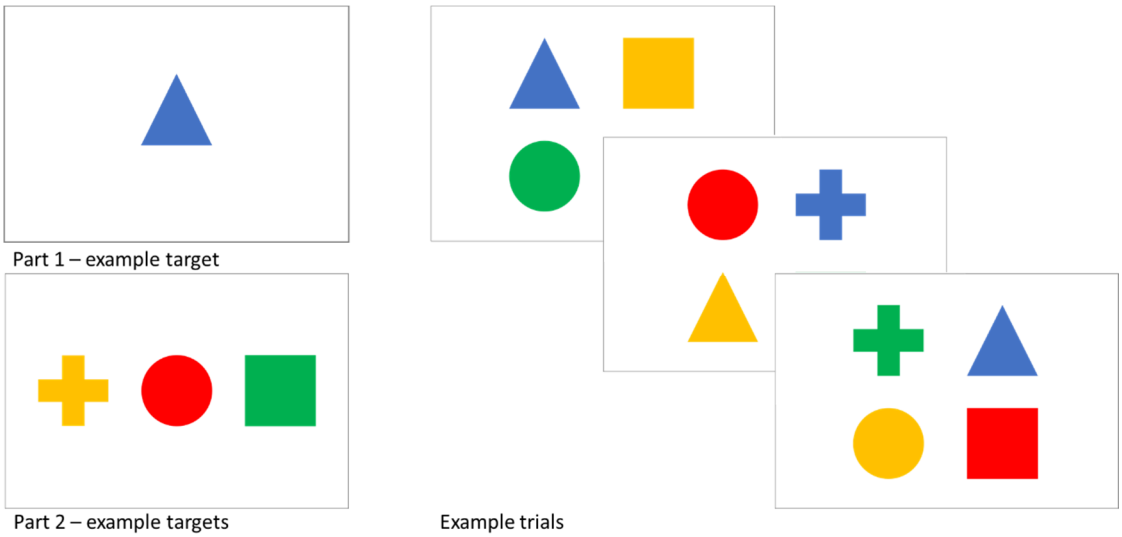


Figure 5.3 Example targets (left) and example trials (right) for the MS2D task

5.3.6.4 Sustained Attention Dots (SAD)

The SAD task measured inhibitory control and sustained attention. It included 600 trials, in which dots were presented in random configurations on the computer screen: 200 trials with three dots, 200 with four dots and 200 with five dots (see Figure 5.4 for example trials). Participants were instructed to return a 'yes' response (dominant hand) when four dots appeared on the screen, and a 'no' response (non-dominant hand) in the case of three or five dots. As a 'no' response is required twice as often in this task, it becomes the automatic or pre-potent response as the task progresses. Therefore, inhibitory control of a pre-potent response was required on 'yes' trials (four dot trials). Auditory feedback (a beep) was given when an error was made. Inhibitory control was measured by the overall number of errors and Mean Series Time (MST; the mean RT of 50 series of 12 trials, each with three-, four-, and five-dot trials). A comparison of error rate and MST during the first and last 120 trials was made in order to examine performance over the course of the task, hence measuring sustained attention.

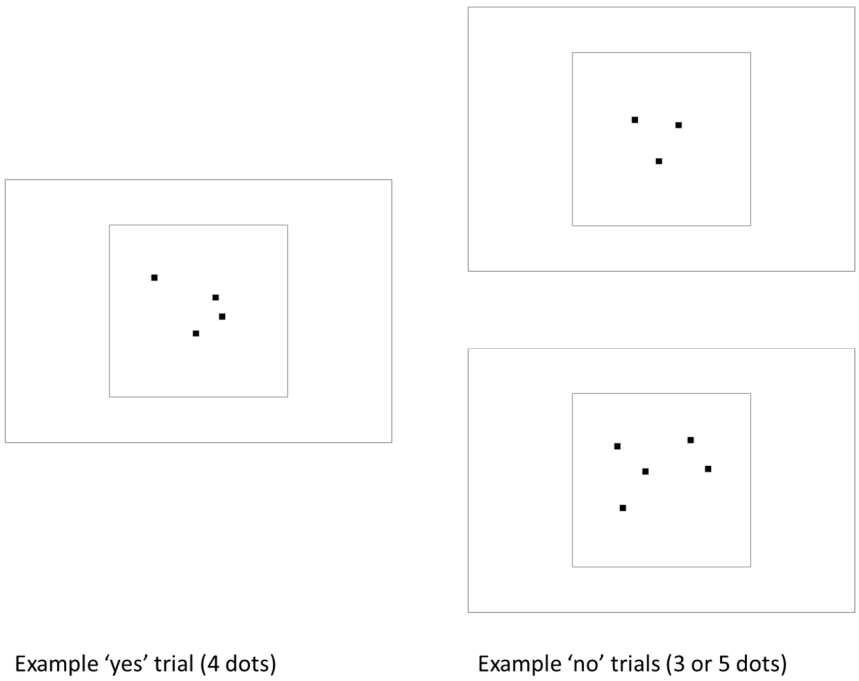


Figure 5.4 Example trials of the SAD task

5.3.6.5 Shifting Attentional Set – Visual (SSV)

The SSV task was used to measure inhibition of pre-potent responses and attentional flexibility (set shifting). It consisted of 3 parts. The stimulus was a bar, consisting of 10 blocks. One of these blocks was coloured and randomly jumped to the left or right. The colour of the block determined the required response (see Figure 5.5). In part 1, (40 trials) participants were required to give a compatible response, that is, they were required to press the left mouse button when the block (green in the example in Figure 5.5) jumped left and the right mouse button when the block jumped right. In part 2 (40 trials), the block had a different colour (e.g. red) and participants were required to give an incompatible response: they were required to press the left mouse button whenever the block jumped right and the right mouse button when the block jumped left. Part 3 consisted of a mix of 40 compatible (e.g. green block) and 40 incompatible (e.g. red block) trials, requiring participants to switch between response types. Number of errors and RT (msec) of correct responses for compatible and incompatible trials were recorded.

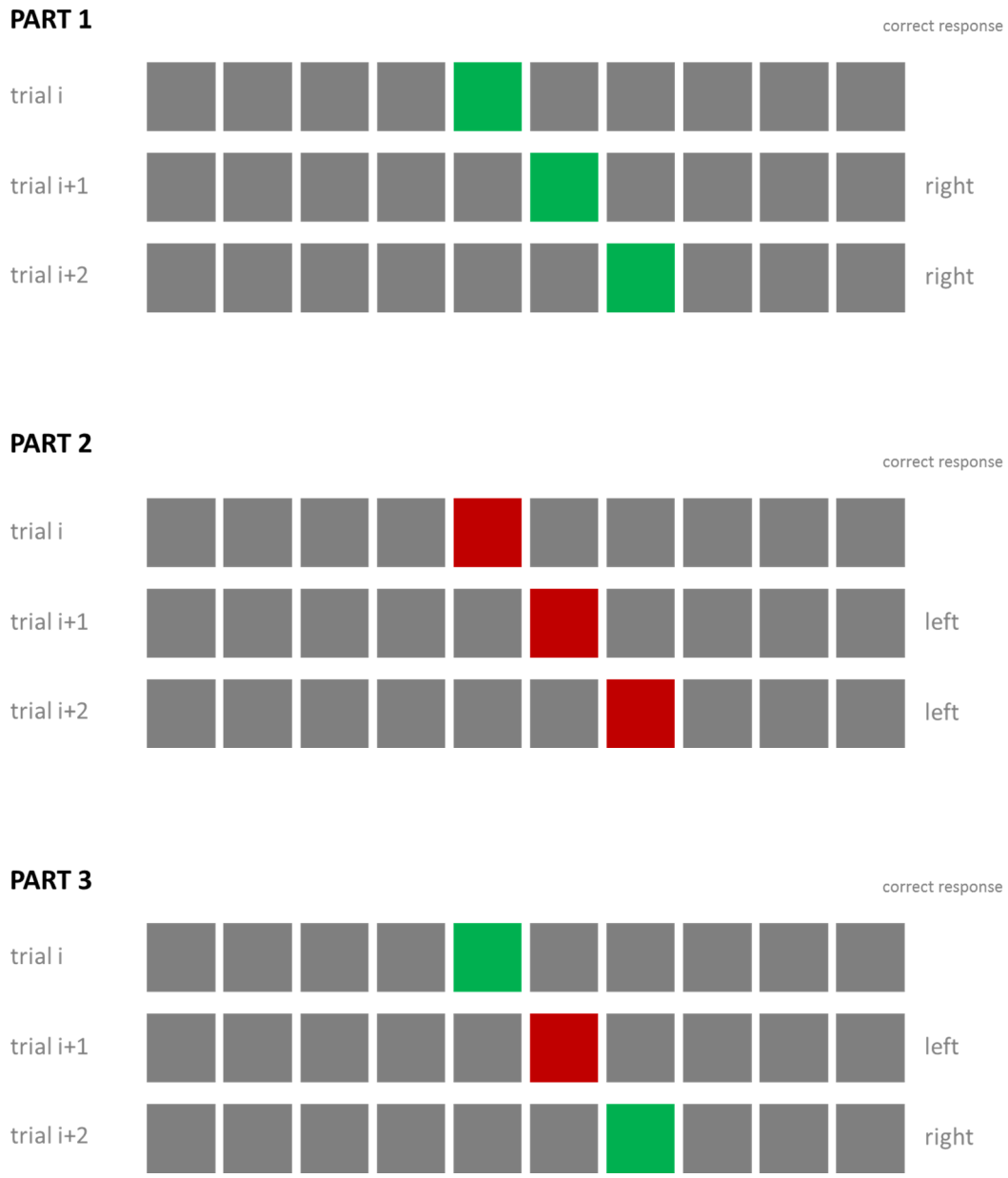


Figure 5.5 Example trials for all parts of the SSV task

5.3.6.6 Tracking (TR)

The tracking task required participants to trace the mouse cursor in between an outer (radius 8.5 cm) and an inner circle (radius 7.5 cm) presented on the computer screen (see Figure 5.6). Participants were required to trace the circle in a clockwise direction with their right hand (part 2) and in a counter-clockwise direction with their left hand (part 1). The part which required participants to use their non-dominant hand was always administered first. The ANT program divided the trajectory into 60 radially equal segments and computed the mean distance between the cursor trajectory and the

midline per segment, resulting in 60 deviation scores. Time taken to complete the task, the mean deviation (mm; accuracy of movement) and the standard deviation (mm; stability of movement) of the trajectory that was followed were recorded.

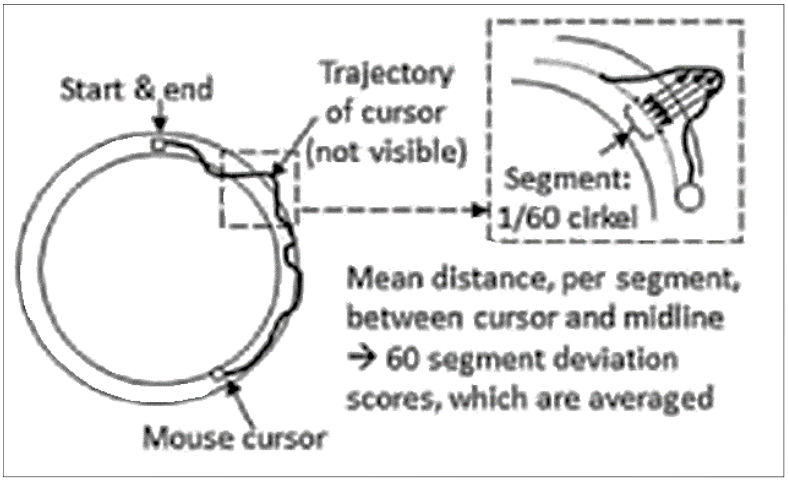


Figure 5.6 TR task (ANT)

5.3.6.7 Pursuit (PU)

The pursuit task required participants to follow a target (an asterisk) that randomly moved across the computer screen with the mouse cursor as closely as possible for 60 seconds (see Figure 5.7). Participants had to complete the task twice, once for each hand. The ANT program computed the mean distance between the mouse cursor and the moving target per second, resulting in 60 deviation scores. The mean deviation of the moving target (mm; accuracy of movement) and the standard deviation (mm; stability of movement) of the trajectory that was followed were recorded.

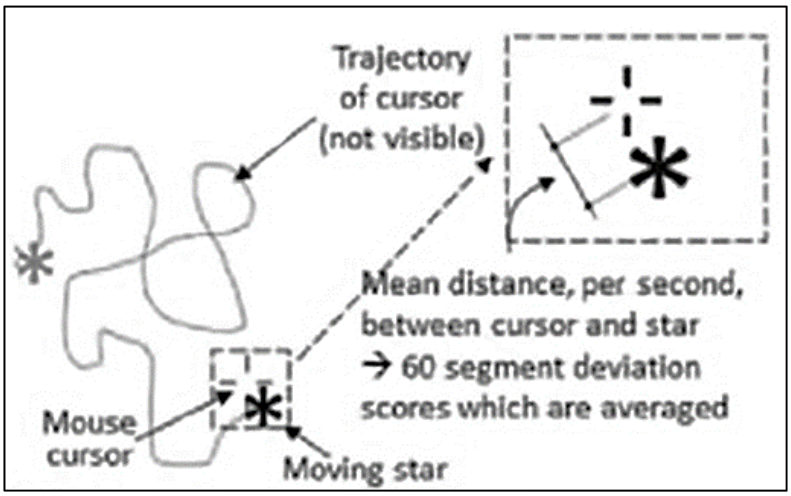


Figure 5.7 PU task (ANT)

5.3.7 Questionnaires

5.3.7.1 PKU specific Quality of Life Questionnaire (PKU-QoLQ)

Participants' QoL was assessed using a PKU specific QoL questionnaire (PKU-QoLQ) (Regnault et al., 2015). The PKU-QoLQ is comprised of four modules: 'PKU symptoms', 'PKU in general', 'administration of Phe-free protein supplements' and 'dietary protein-restrictions'.

The **'PKU symptoms'** module consists of single-item symptom scores (i.e. self-rated health, headaches, stomach aches, tiredness, lack of concentration, slow thinking, trembling hands, irritability, aggressiveness, moodiness, sadness and anxiety).

The other modules are subdivided into several domains:

- The **'PKU in general'** module includes the following PKU impact scores: practical, social, emotional and overall impact of PKU, anxiety due to blood tests and anxiety due to high blood Phe levels.
- The module **'administration of Phe-free protein supplements'** includes scores of adherence to Phe-free protein substitutes, guilt due to poor adherence to Phe-free protein substitutes and impact of Phe-free protein substitutes on daily life (practical impact) and family.
- The domains related to the **'dietary protein-restrictions'** module include scores on food temptations, adherence to dietary protein restrictions, overall difficulty following dietary restrictions, guilt if the diet is not followed, social impact of the diet, practical impact of the diet, food enjoyment and taste of specialty low-protein food products.

Each item of the questionnaire yields a score ranging from 0 to 4. Domain scores range from 0 to 100. Domain scores for the PKU-QOLQ were calculated by summing the item scores and applying linear transformation (see Figure 5.8). A domain score was only calculated if at least 70% of the items on that domain had been completed.

$$\text{domain score} = \frac{\text{Sum of item scores within the domain}}{\text{Number of non missing item scores within the domain}} * 25$$

Figure 5.8 Formula for the calculation of PKU-QoLQ domain scores

For symptom scores, a higher domain score is associated with more frequent symptoms; for adherence scores, a higher domain score is associated with a poorer adherence; and for other aspects, a higher score is associated with a greater impact.

5.3.8 Procedure

5.3.8.1 Screening

After identification of eligible adherent and semi-adherent ET AwPKU by specialised metabolic dietitians (see 5.3.2), patients who agreed to take part in the study were invited for a screening visit at either the Clinical Research Facility (CRF) at SRFT or the HARU, School of Psychology, University of Leeds. At screening, participants were given written and verbal information about the purpose of the study, all procedures involved, and what was required of them during participation (see Appendices O and P). Informed consent from each participant was obtained in writing prior to commencement of the study (Appendices Q and R). Following this, participants were required to complete a screening questionnaire (Appendix S), which asked for demographic information (including age, gender, highest completed level of education, occupation) and dietary habits. Furthermore, a DBS sample was obtained to measure Phe and Tyr levels at the time of screening and FSIQ was assessed using the WASI (see 5.3.5). Once eligibility was confirmed, patients were invited to attend the CRF or HARU, whichever was most convenient, for their test day.

5.3.8.2 Test day

The test day procedure was the same regardless of participant group (see Figure 5.9). Patients were asked not to eat or drink anything apart from water during the 2 hours leading up to the test session. During the session, participants completed the ANT cognitive test battery (see 5.3.6). Given the length of the test battery and the

concentration required to complete the tests, participants had two breaks from the laptop screen (after completing the BS, FL and MS2D tasks, and after completing the SAD task). During the first break, their height, weight, BMI and percentage body fat were measured. During the second break, participants completed the PKU-QoLQ (see 5.3.7.1). At the end of the test session, a blood sample was obtained via venepuncture. The blood sample was obtained last to ensure patients were sufficiently fasted and to limit any interference of perceived stress related to blood sampling with any of the other measures.

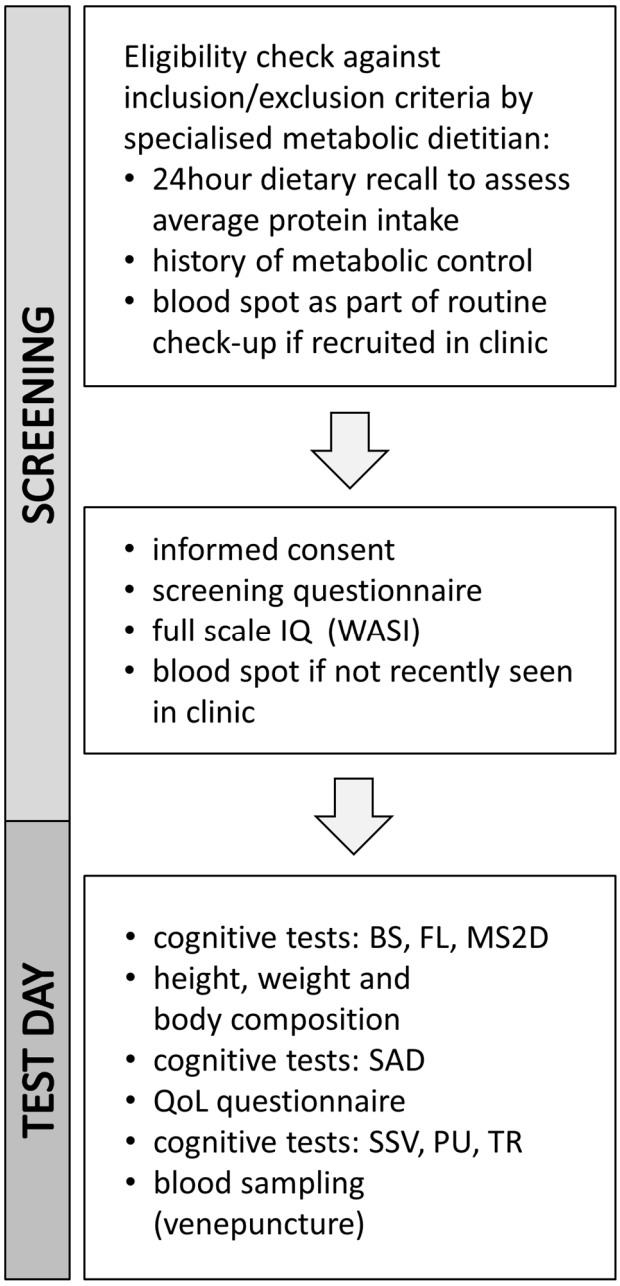


Figure 5.9 Study procedure flow diagram for Study 3 part 1

5.3.9 Ethical considerations

This study was approved by the Yorkshire & The Humber - Sheffield Research Ethics Committee (REC; 16/YH/0273, 15/08/2016), Leeds Teaching Hospitals NHS Trust R&D (20/07/2016) and Salford Royal NHS Foundation Trust R&D (17/10/2016). This research was conducted in accordance with the ethical principles expressed in the Declaration of Helsinki (World Medical Association, 2013) and the principles of good clinical practice (GCP; National Institute for Health Research, 2016).

The informed consent of each participant was obtained in writing prior to commencement of the study (see Appendices Q and R). At screening, participants were given written and verbal information about the purpose of the study, and all procedures involved, and what was required of them during participation (see Appendices O and P). Participants' travel costs were reimbursed.

5.3.10 Statistical analysis

Cognitive test data were extracted from ANT and entered intro Excel. Furthermore, subjective data and results from blood assays were entered into Excel. All data were checked for accuracy prior to analysis with SPSS Version 22. Results for both groups were reported using means, standard deviations (SD) and 95% confidence intervals (95% CI). Baseline characteristics, obtained at screening, were compared using independent t-tests or Chi-squared tests as appropriate. Differences in nutritional status and measures of QoL between adherent and semi-adherent ET AwPKU were explored using independent t-tests. Differences in speed (msec) and accuracy (n errors) on selected ANT tasks were explored using independent t-tests. RT of correct responses (msec) and number of errors (n) on the MS2D task were subject to a 2 (group: adherent vs. semi-adherent ET AWPKU) x 2 (type of stimulus: low (1 target) vs. high (3 targets) working memory load) ANOVA. Similarly, differences in speed of correct responses (msec) and number of errors (n) on the second part of the FL task and the third part of the SSV task were assessed using 2 (group: adherent ET AwPKU vs. semi-adherent ET AwPKU) x 2 (type of stimulus: compatible trials vs. incompatible trials) ANOVAs. To assess

differences in sustained attention, MST (RT) and number of errors (n) during the first and last 10 series (120 trials) on the SAD task were subject to a 2 (group: adherent vs. semi-adherent ET AWPKU) x 2 (time: first 120 trials vs. last 120 trials) ANOVA. Motor skills under lower (TR) and higher (PU) controlled processing demands were explored by subjecting the mean deviation of the moving target (mm; accuracy of movement) and the standard deviation (mm; stability of movement) of the trajectory that was followed on both tasks to a 2 (group: adherent vs. semi-adherent ET AWPKU) x 2 (task: TR vs. PU) ANOVA. Where applicable, post-hoc comparisons were made using the Tukey-Kramer method.

5.4 Results

5.4.1 Participant characteristics

A total of 10 adherent and 10 semi-adherent ET AwPKU met all eligibility criteria, provided their informed consent, and completed the screening and test session.

Table 5.6 gives an overview of participant characteristics of both groups at screening. Dietary protein intake, protein intake from protein substitutes and total protein intake for each individual participant can be found in Appendix T. The adherent and semi-adherent ET AwPKU did not differ significantly in terms of gender, age, IQ, highest level of education achieved, or occupational status. Tyr levels obtained at screening did not differ significantly between groups. As expected, the semi-adherent ET AwPKU had significantly higher Phe levels, a significantly higher Phe:Tyr ratio, and a significantly lower total protein intake.

Table 5.6 Participant characteristics of adherent and semi-adherent ET AwPKU at screening

		Adherent ET AwPKU	Semi-adherent ET AwPKU	Adherent vs. semi-adherent ET AwPKU
n (female)		10 (6)	10 (6)	$X^2(1)<.001$, $p=1.00$
age (years)	*mean (SD)*	36.63 (9.63)	33.04 (10.53)	$t(18)=.80$, $p=.44$
	range	18.57-50.72	19.51-53.84	
Phe[1] (µmol/L)	*mean (SD)*	522.70 (110.10)	1180.50 (158.83)	**$t(18)=-10.76$, $p<.001$**
	range	283-692	1027-1489	
Tyr (µmol/L)	*mean (SD)*	43.44 (14.82)	33.43 (23.68)	$t(18)=1.04$, $p=.32$
	range	26-66	19-86	
Phe:Tyr	*mean (SD)*	12.89 (8.08)	38.66 (21.99)	**$t(18)=-2.95$, $p=.02$**
	range	4.03-26.62	8.96-61.29	
Protein intake[2] (g/kg/day)	*mean (SD)*	1.11 (0.34)	0.66 (0.06)	**$t(18)=4.14$, $p=.002$**
	range	0.80-1.79	0.59-0.74	
IQ	*mean (SD)*	116.86 (13.13)	116.95 (14.53)	$t(18)=-.01$, $p=.99$
	range	89-134	92-136	
Educational[3] level (n)	*Primary*	0	1	$X^2(1)=1.54$, $p=.82$
	Secondary	3	3	
	College	4	2	
	Bachelor	2	3	
	Master	1	1	
	Doctorate	0	0	
Occupational status	*Employed*	7	5	$X^2(1)=2.33$, $p=.51$
	Unemployed	0	2	
	Student	1	1	
	Housewife/ homemaker	2	2	

[1] Phe levels had to be <700 (adherent) or >1000 (semi-adherent) µmol/L to be eligible for participation;

[2] Total protein intake had to be ≥0.75 (adherent) or <0.75 (semi-adherent) g/kg/day to be eligible for participation;

[3] Highest completed level of education

5.4.2 Nutritional status

Table 5.7 and Table 5.8 provide an overview of mean (SD, 95% CI) concentrations of (micro)nutrients and AA assessed in adherent and semi-adherent ET AwPKU. The reference range for each measure is provided. All measures are explored in more detail in this section.

5.4.2.1 Protein status: albumin, pre-albumin and transferrin

There were no significant differences in mean serum albumin or transferrin concentrations between both groups of ET AwPKU, but semi-adherent ET AwPKU had significantly lower serum pre-albumin levels compared to the group of adherent ET AwPKU (see Table 5.7). All adherent ET AwPKU had albumin and pre-albumin concentrations within range (35-50 g/L) and one adherent ET AwPKU had a transferrin level (1.13 g/L) below the reference range (2.00-3.20 g/L). In the semi-adherent group, one ET AwPKU had deficient serum albumin (33 g/L) and transferrin (1.81 g/L) levels and two of the participants had levels above the upper reference level (51 and 52 g/L). Despite having significantly lower levels, none of the semi-adherent ET AwPKU had a pre-albumin deficiency.

5.4.2.2 Micronutrients and related measures

No overall group deficiencies were observed for any of the measures, but adherent male ET AwPKU (n=4) had elevated iron levels (see Table 5.7). As a group, semi-adherent ET AwPKU had higher mean homocysteine and vitamin B12 levels.

5.4.2.2.1 C-reactive protein (CRP)

C-reactive protein (CRP) levels typically rise in response to inflammation (Bennett, 2006). Because plasma vitamin E levels are only valid when CRP is below its reference range (<mg/L) (Barski, 2015), vitamin E concentrations of participants with elevated CRP levels were excluded from analysis. Selenium concentrations may also be affected by elevated CRP (Barski, 2018). Therefore, CRP levels are reported in the case of altered selenium levels. Three semi-adherent and one adherent ET AwPKU had CRP levels above the reference range (<5 mg/L). .

Table 5.7 Mean (SD; 95% CI) measures of nutritional status of adherent and semi-adherent ET AwPKU (with reference ranges)

	Reference range	Adherent ET AwPKU			Semi-adherent ET AwPKU			
		mean	SD	95% CI	mean	SD	95% CI	*t, p* and *d*
Albumin† (g/L)	35-50	43.00	1.49	41.93-44.07	44.10	5.63	40.08-48.12	*t*(10.26)=.60, *p*=.56, *d*=.27
Pre-albumin† (g/L)	0.20-0.50	0.32	0.06	0.28-0.36	0.26	0.04	0.23-0.29	***t*(17)=2.65, *p*=.02, *d*=1.18**
Transferrin† (g/L)	2.00-3.20	2.20	0.42	1.89-2.50	2.52	0.37	2.26-2.79	*t*(18)=1.85, *p*=.08, *d*=.81
Alkaline Phosphatase† (U/L)	30-130	83.20	52.19	45.86-120.54	78.30	13.55	68.61-87.99	*t*(18)=-.29, *p*=.78, *d*=.13
Calcium† (mmol/L)	2.20-2.60	2.24	0.12	2.16-2.33	2.32	0.17	2.20-2.44	*t*(18)=1.21, *p*=.24, *d*=.54
Copper‡ (µmol/L)	11-22	13.39	3.20	11.10-15.68	17.00	5.78	12.86-21.14	*t*(18)=1.73, *p*=.20, *d*=.77
Homocysteine‡ (µmol/L)	<18	7.25	1.05	6.38-8.12	9.43	3.34	5.81-13.04	***t*(17)=2.56, *p*=.02, *d*=.88**
Iron† (µmol/L)	14-31 (♂)	**34.03**	11.58	15.60-52.45	21.68	11.27	3.74-39.61	*t*(6)=-1.53, *p*=.18, *d*=1.08
	11-29 (♀)	16.66	7.58	7.25-26.07	15.23	4.85	10.14-20.33	*t*(9)=-.38, *p*=.71, *d*=.22
Phosphate† (mmol/L)	0.80-1.50	1.05	0.13	0.95-1.15	1.06	0.14	0.96-1.16	*t*(18)=.96, *p*=.35, *d*=.07
Parathyroid hormone‡ (PTH) (pmol/L)	1.50-7.60	3.66	1.40	2.66-4.66	3.96	1.33	2.93-4.98	*t*(18)=.14, *p*=.89, *d*=.22
Selenium‡ (µmol/L)	0.80-2.00	0.93	0.16	0.82-1.04	0.92	0.13	0.83-1.01	*t*(18)=-.09, *p*=.93, *d*=.07
Vitamin A† (µmol/L)	1.05-3.39	1.87	0.54	1.49-2.26	1.85	0.54	1.43-2.27	*t*(17)=-.10, *p*=.92, *d*=.04
Vitamin B12† (ng/L)	211-911	521.78	158.38	400.03-643.52	403.56	134.89	299.87-507.24	*t*(16)=-1.71, *p*=.11, *d*=.80
Vitamin D† (nmol/L)	<75	**73.35**	21.90	57.69-89.01	**57.47**	37.97	28.28-86.65	t(17)=-1.13, *p*=.27, *d*=.51
Vitamin E† (µmol/L)	12-42	22.62	6.83	17.38-27.87	22.73	6.84	15.56-29.91	*t*(17)=.03, *p*=.98, *d*=.02
Zinc‡ (µmol/L)	9.80-17.90	10.39	1.17	9.55-11.23	10.33	3.36	7.92-12.74	*t*(18)=-.05, *p*=.96, *d*=.02

Notes: † serum; ‡ plasma

5.4.2.2.2 Zinc

As can be seen in Table 5.7, groups did not differ significantly in their plasma zinc levels. Zinc levels indicative of deficiency were observed in one adherent and five semi-adherent ET AwPKU.

5.4.2.2.3 Selenium

Plasma selenium levels did not significantly differ between adherent and semi-adherent ET AwPKU (see Table 5.7). Three adherent and two semi-adherent ET AwPKU had plasma selenium levels indicative of deficiency (reference range 0.80-2.00 µmol/L). One of the semi-adherent patients with deficient selenium status had elevated CRP levels (14.5 mg/L; reference range: <5 mg/L), which may have affected their selenium level.

5.4.2.2.4 Vitamin A

There were no significant differences in vitamin A concentrations between groups (see Table 5.7). One semi-adherent ET AwPKU was deficient in vitamin A. All other participants had vitamin A levels within the reference range.

5.4.2.2.5 Vitamin B12 and homocysteine

Vitamin B12 and homocysteine levels of one of the semi-adherent ET AwPKU were excluded from the data as they had recently received a vitamin B12 booster injection. Their levels were within target ranges. All adherent and semi-adherent ET AwPKU had serum vitamin B12 concentrations within the reference range(211-911 ng/L), but mean levels tended to be slightly but non-significantly lower in semi-adherent ET AwPKU (see Table 5.7).

Mean total homocysteine levels of semi-adherent ET AwPKU were significantly higher compared to those of adherent ET AwPKU (see Table 5.7).

5.4.2.2.6 Vitamin D

Data from one semi-adherent ET AwPKU was excluded as they reported taking vitamin D supplements. Vitamin D levels did not differ significantly between groups with, both adherent and semi-adherent ET AwPKU showing deficient mean concentrations (see Table 5.7). Five adherent and seven semi-adherent patients had levels below the reference range, indicating vitamin D depletion.

5.4.2.2.7 Vitamin E

Vitamin E results from participants with elevated CRP levels were excluded from analysis. There were no significant differences in vitamin E concentrations between groups (see Table 5.7). Furthermore, one semi-adherent ET AwPKU was deficient in vitamin E. All other participants had levels within the reference range.

5.4.2.2.8 Calcium, parathyroid hormone (PTH) and phosphate

Serum calcium, PTH and phosphate levels did not differ significantly between adherent and semi-adherent ET AwPKU (see Table 5.7). None of the ET AwPKU had PTH or phosphate levels outside of the reference range. However, deficient plasma calcium levels were observed in three adherent and two semi-adherent ET AwPKU.

5.4.2.2.9 Copper

Plasma copper levels did not differ significantly between groups. However, three adherent and one semi-adherent ET AwPKU had levels below the reference range andone semi-adherent patient's copper level was elevated (i.e. above the reference range).

5.4.2.2.10 Iron

Although male adherent ET AwPKU had an average serum iron level above the reference range (see Table 5.7), serum iron levels did not differ significantly between adherent and semi-adherent ET AwPKU. Three adherent male ET AwPKU had serum iron levels above reference ranges and one adherent female ET AwPKU had a deficient serum iron concentration. Conversely, three (two female, one male) of the semi-adherent ET AwPKU had deficient serum iron levels and one male semi-adherent ET AwPKU had a serum iron concentration above the upper reference level.

5.4.2.2.11 Alkaline phosphatase

Alkaline phosphatase concentrations did not differ between adherent and semi-adherent ET AwPKU (see Table 5.7). One of the adherent ET AwPKU had increased levels, all other levels were within reference range.

5.4.2.3 Full amino acid profile

Table 5.8 provides a summary of mean (SD, 95% CI) AA concentrations of adherent and semi-adherent ET AwPKU. Reference ranges are provided and group deficiencies are highlighted. Both adherent and semi-adherent ET AwPKU showed overall group deficiencies in essential AA (EAA) histidine (His) and lysine (Lys). Moreover, as a group, semi-adherent ET AwPKU were deficient in Tyr. Furthermore, both groups were deficient in the non-essential AA, aspartic acid (Asp), glutamate (Glu), and ornithine (Orn). Adherent ET AwPKU showed a deficiency in asparagine (Asn) and proline (Pro) deficiency was evident in the semi-adherent ET AwPKU.

Results from the full AA (FAA) profile assays of the adherent and semi-adherent ET AwPKU are described in more detail below.

Table 5.8 Mean (SD, 95% CI) amino acid concentrations (µmol/L) of adherent and semi-adherent ET AwPKU (with reference ranges)

	Reference range (µmol/L)	Adherent ET AwPKU			Semi-adherent ET AwPKU			
		mean	SD	95% CI	mean	SD	95% CI	*t*, *p* and *d*
Alanine (Ala)	*248-778*	269.40	54.08	230.71-308.09	263.33	76.06	204.87-321.80	*t*(18)=-.34, *p*=.74, *d*=.09
Arginine (Arg)	*30-198*	54.60	18.23	41.56-67.64	43.33	15.45	31.46-55.21	*t*(18)=-1.57, *p*=.13, *d*=.67
Asparagine (Asn)	*35-128*	**31.40**	7.59	25.97-36.83	38.67	11.14	30.11-47.23	*t*(18)=1.95, *p*=.07, *d*=.76
Aspartic acid (Asp)	*45-125*	**10.30**	1.83	8.99-11.61	**9.30**	7.97	3.60-15.00	*t*(17)=-.47, *p*=.65, *d*=.17
Citrulline (Cit)	*13-52*	30.30	8.39	24.30-36.30	31.56	9.32	24.40-38.72	*t*(18)=.39, *p*=.70, *d*=.14
Cysteine (Cys)	*17-124*	40.90	12.07	32.27-49.53	40.67	8.70	33.98-47.36	*t*(18)=-.29, *p*=.77, *d*=.02
Glutamate (Glu)	*46-428*	**30.00**	14.02	19.97-40.03	**39.22**	17.20	26.00-52.45	*t*(18)=1.49, *p*=.15, *d*=.59
Glutamine (Gln)	*270-1159*	445.30	53.74	406.86-483.74	433.00	99.21	356.74-509.26	*t*(18)=-.34, *p*=.74, *d*=.15
Glycine (Gly)	*185-552*	254.70	64.90	208.27-301.13	244.89	99.25	168.60-321.18	*t*(18)=-.34, *p*=.74, *d*=.12
Histidine (His)†	*81-193*	**67.50**	10.41	60.06-74.94	**65.22**	14.70	53.92-76.52	*t*(18)=-.38, *p*=.71, *d*=.18
Isoleucine (Iso)†	*35-127*	45.70	18.25	32.65-58.75	49.22	15.62	37.22-61.23	*t*(18)=.29, *p*=.77, *d*=.21
Leucine (Leu)†	*80-229*	96.10	25.24	78.05-114.15	102.00	21.21	85.69-118.31	*t*(18)=.55, *p*=.59, *d*=.25
Lysine (Lys)†	*165-378*	**134.10**	17.57	121.53-146.67	**135.67**	30.78	112.01-159.33	*t*(18)=1.57, *p*=.83, *d*=.06
Methionine (Met)†	*9-52*	14.40	4.06	11.50-17.30	13.22	4.18	10.01-16.43	*t*(18)=.38, *p*=.71, *d*=.29
Ornithine (Orn)	*117-279*	**42.90**	12.44	34.00-51.80	**41.33**	17.30	28.04-54.63	*t*(18)=-.23, *p*=.82, *d*=.10
Phenylalanine (Phe)†	*120-700*	**713.70**	203.96	567.80-859.60	**1114.00**	198.12	972.27-1255.73	***t*(18)=4.45, *p*<.001, *d*=1.99**
Proline (Pro)	*123-451*	125.50	41.65	95.71-155.29	**112.33**	36.70	84.12-140.54	*t*(17)=-.73, *p*=.48, *d*=.34
Serine (Ser)	*68-256*	93.30	20.37	78.73-107.87	89.22	23.55	71.12-107.33	*t*(18)=-.32, *p*=.75, *d*=.19
Taurine (Tau)	*80-344*	91.30	21.58	75.86-106.74	87.44	70.47	33.28-141.61	*t*(10.82)=-.35, *p*=.74, *d*=.07
Threonine (Thr)†	*60-231*	112.10	32.82	88.62-135.58	88.11	26.31	67.89-108.33	*t*(18)=-1.88, *p*=.08, *d*=.81
Tyrosine (Tyr)‡	*57-110*	66.10	29.22	45.19-87.01	**47.00**	20.56	31.20-62.80	*t*(18)=-1.81, *p*=.09, *d*=.76
Valine (Val)†	*117-359*	216.70	59.55	174.10-259.30	212.56	45.54	177.55-247.56	*t*(18)=-.36, *p*=.72, *d*=.08

[1] recommended reference range for AwPKU in the UK prior to implementation of new European guidelines (van Wegberg et al., 2017); †Essential Amino Acid; ‡Conditionally Essential Amino Acid (in PKU)

5.4.2.3.1 Phenylalanine, tyrosine and phenylalanine/tyrosine ratio

At the time of testing, some of the ET AwPKU had Phe levels above (adherent: n=5) or below (semi-adherent: n=3) inclusion criteria (see Figure 5.10). Four adherent ET AwPKU had Phe levels within the recommended target ranges included in the new European guidelines (120-600 µmol/L). As expected, Phe levels differed significantly between both groups (p<.001; see Table 5.8).

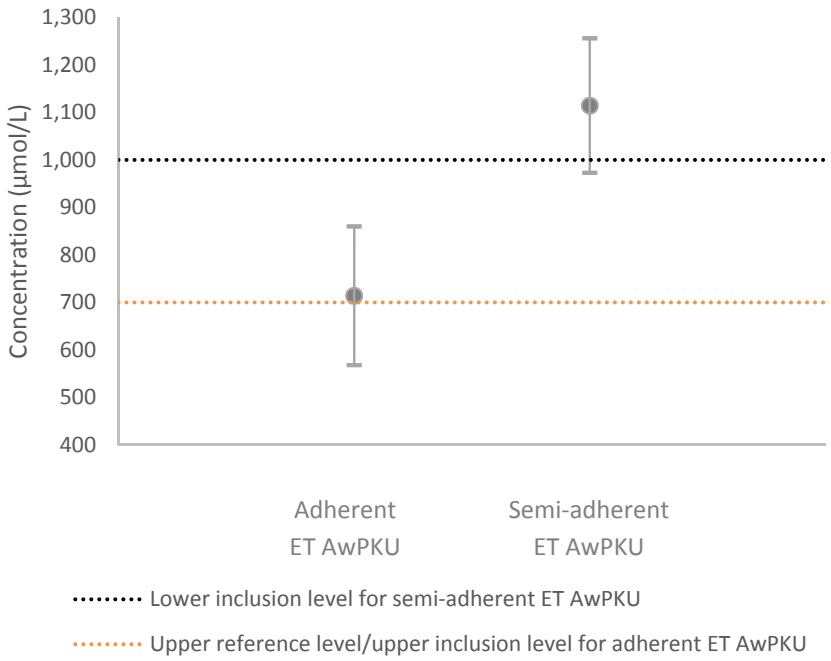


Figure 5.10 Mean phenylalanine concentrations (µmol/L) of adherent and semi-adherent ET AwPKU (error bars represent 95% confidence intervals)

Tyr levels were below reference range in three adherent and eight semi-adherent patients. Mean Tyr level was lower in semi-adherent ET AwPKU than adherent ET AwPKU (see Figure 5.11) but this difference was not significant (see Table 5.8).

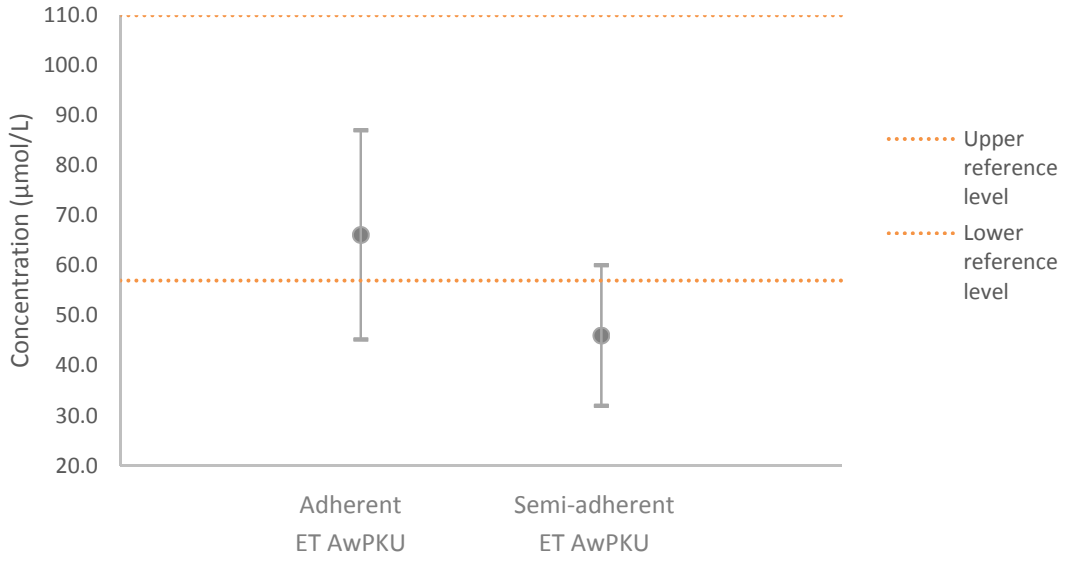


Figure 5.11 Mean tyrosine concentrations (µmol/L) of adherent and semi-adherent ET AwPKU (error bars represent 95% confidence intervals)

Finally, analysis revealed that the mean Phe:Tyr ratio was significantly higher in semi-adherent ET AwPKU (27.53 (±10.89)) than adherent ET AwPKU (13.20 (±9.56); t(18)=-3.128, p=.006).

5.4.2.3.2 (Other) essential amino acids

Threonine (Thr) levels tended to be lower in semi-adherent ET AwPKU than adherent ET AwPKU (see Table 5.8). One adherent ET AwPKU had a Thr level just below the reference range (60-231 µmol/L; their level was 58 µmol/L). Thr levels of all other participants were within the reference range.

Mean levels of Met, valine (Val), isoleucine (Iso), leucine (Leu), His and Lys did not differ significantly between both groups of ET AwPKU (see Table 5.8). One adherent and one semi-adherent ET AwPKU had deficient Met, Iso and Leu concentrations. Two further adherent ET AwPKU showed deficiencies in Leu and an additional two adherent patients were deficient in Iso. Of the latter two, one also had a Thr level below the reference range. One further semi-adherent patient showed deficiencies in Iso and Leu. With the exception of one adherent and one semi-adherent ET AwPKU, concentrations of His were below the reference range in all participants. Finally, two semi-adherent ET AwPKU

had Lys levels within the reference range, but all other ET AwPKU had deficient Lys concentrations.

5.4.2.3.3 Non-essential amino acids

Levels of non-essential AA did not differ significantly between groups (see Table 5.8). Moreover, all ET AwPKU had Arg, cysteine (Cys), glutamine (Gln), and Citrulline (Cit) levels within reference ranges. Furthermore, all ET AwPKU were deficient in Orn and Asp. Several deficiencies in non-essential AA were observed amongst adherent and semi-adherent ET AwPKU: two adherent and four semi-adherent ET AwPKU had alanine (Ala) levels below the lower reference level; three adherent and six semi-adherent ET AwPKU were deficient in Pro (n=1 unknown); three semi-adherent ET AwPKU had glycine (Gly) levels below the normal range; eight adherent and six semi-adherent patients had deficient Glu concentrations; one adherent and two semi-adherent patients had serine (Ser) levels below the lower reference level; three adherent and eight semi-adherent ET AwPKU were deficient in taurine (Tau) and, finally, six adherent and three semi-adherent patient had deficient Asn levels.

5.4.3 Quality of Life (QoL)

5.4.3.1 Self-rated health and symptoms

Mean (SD, 95% CI) self-reported ratings of health and symptoms related to PKU for adherent and semi-adherent ET AwPKU are summarised in Table 5.9. Differences between groups for each single-item score are reported below.

Table 5.9 Mean (SD, 95% CI) self-reported health and symptom PKU-QoLQ scores[1] (range 0-100) for adherent and semi-adherent ET AwPKU

	Adherent ET AwPKU			Semi-adherent ET AwPKU			
	mean	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	***t, p* and *d***
Self-reported health	22.50	14.19	12.35-32.65	62.50	21.25	47.30-77.70	***t*(18)=4.95, *p*<.001, *d*=2.21**
Aggressiveness	10.00	17.48	-2.50-22.50	15.00	21.08	-0.08-30.08	*t*(18)=.58, *p*=.57, *d*=.26
Anxiety	35.00	39.44	6.79-63.21	65.00	31.62	42.38-87.62	*t*(18)=1.88, *p*=.08, *d*=.84
Headaches	15.00	17.48	2.50-27.50	37.50	33.85	13.29-61.71	*t*(18)=1.87, *p*=.08, *d*=.84
Irritability	32.50	31.29	10.12-54.88	50.00	28.87	29.35-70.65	*t*(18)=1.30, *p*=.21, *d*=.58
Lack of concentration	22.50	29.93	1.09-43.91	55.00	25.82	36.53-73.47	***t*(18)=2.60, *p*=.02, *d*=1.16**
Moodiness	27.50	24.86	9.72-45.28	57.50	23.72	40.53-74.47	***t*(18)=2.76, *p*=.01, *d*=1.23**
Sadness	22.50	21.89	6.84-38.16	47.50	14.19	37.35-57.65	***t*(18)=3.03, *p*=.007, *d*=1.36**
Slow thinking	20.00	25.82	1.53-38.47	45.00	28.38	24.70-65.30	*t*(18)=2.06, *p*=.05, *d*=.92
Stomach aches	2.50	7.91	-3.16-8.16	17.50	28.99	-3.24-38.24	*t*(10.33)=1.58, *p*=.15, *d*=.71
Tiredness	40.00	29.34	19.01-60.99	80.00	19.72	65.89-94.11	***t*(18)=3.58, *p*=.002, *d*=1.60**
Trembling hands	15.00	31.62	-7.62-37.62	40.00	37.64	13.07-66.93	*t*(18)=1.61, *p*=.13, *d*=.72

[1] *a lower score is associated with a lower severity of PKU symptoms*

5.4.3.1.1 Self-reported health

Mean (95% CI) self-reported ratings of health for both groups are displayed in Figure 5.12. Adherent ET AwPKU perceived their health to be significantly better than semi-adherent ET AwPKU.

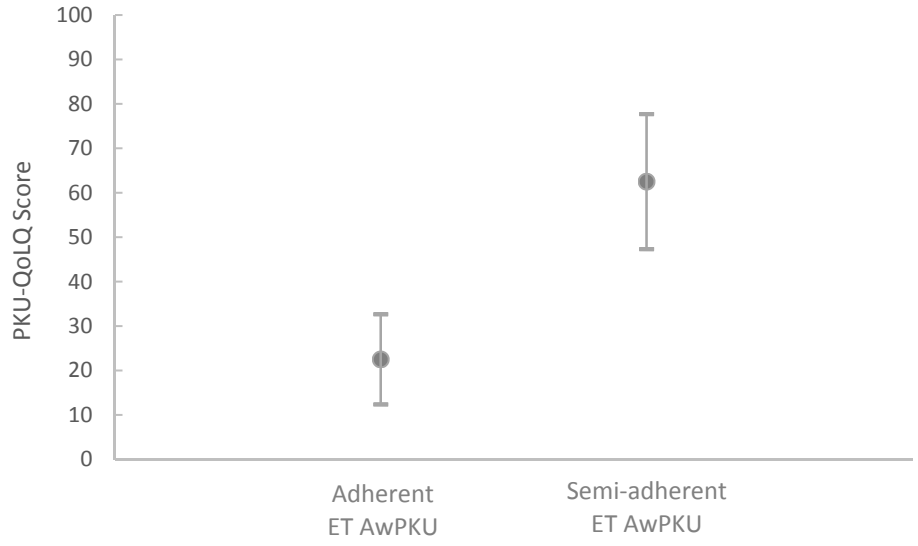


Figure 5.12 Mean self-reported PKU-QoLQ scores of self-rated health (range 0-100) for adherent and semi-adherent ET AwPKU at baseline (error bars represent 95% confidence intervals)

5.4.3.1.2 PKU-related symptoms

The semi-adherent ET AwPKU group reported significantly higher scores for lack of concentration, moodiness, sadness and tiredness (see Table 5.9). Furthermore, semi-adherent ET AwPKU tended to report higher levels of anxiety, headaches and slow thinking, but differences between groups were not significant.

There were no significant differences in self-reported ratings of aggressiveness, irritability, and 'trembling hands' between groups of ET AwPKU. Finally, as can be seen in Figure 5.13, the self-reported occurrence of stomach aches in the semi-adherent ET AwPKU group showed considerable variance amongst participants, whereas adherent ET AwPKU's self-reported ratings for stomach aches were consistently low. As a group, however, semi-adherent ET AwPKU did not differ significantly from adherent ET AwPKU (see Table 5.9).

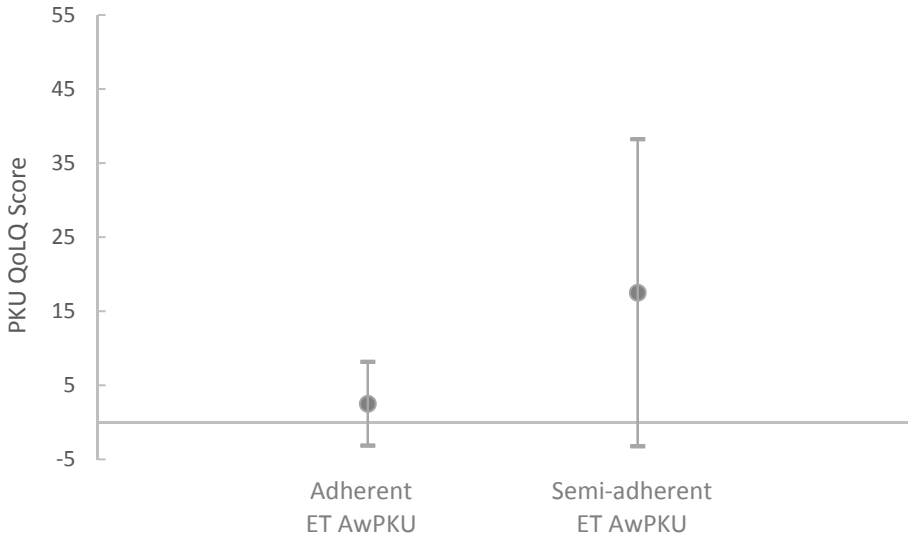


Figure 5.13 Mean self-reported PKU-QoLQ scores of 'stomach aches' (range 0-100) of adherent and semi-adherent ET AwPKU at baseline (error bars represent 95% confidence intervals)

5.4.3.1.3 Perceived impact of PKU

Table 5.10 gives an overview of mean (SD, 95% CI) self-reported PKU-QoLQ impact scores for adherent and semi-adherent ET AwPKU.

Table 5.10 Mean (SD, 95% CI) self-reported PKU-QoLQ impact scores[1] for adherent and semi-adherent ET AwPKU

	Adherent ET AwPKU			Semi-adherent ET AwPKU			
Impact	**mean**	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	***t, p* and *d***
Emotional	33.00	6.11	19.18-46.82	57.50	7.65	40.20-74.80	***t*(18)=2.50, *p*=.02, *d*=3.54**
Social	12.08	4.29	2.38-21.79	27.29	8.07	9.03-45.55	***t*(18)=2.48, *p*=.03, *d*=2.35**

[1] *a lower score is associated with lower perceived impact of PKU*

Compared to adherent ET AwPKU, semi-adherent ET AwPKU reported a significantly greater perceived impact of PKU on their lives, in terms of both emotional (self-esteem, unfairness, worries about the future/future children) and social (impact on relationships with family/partner, difficulties making friends, feeling embarrassed or left out) impact (see Table 5.10).

5.4.4 Cognitive performance

Table 5.12 shows mean (SD, 95% CI) speed (msec) on the BS task as well as speed of correct responses (msec) and number of errors (n) for all types of trials on both parts of the FL task.

5.4.4.1 Baseline Speed (BS)

Adherent and semi-adherent ET AwPKU did not differ in speed (msec) on the BS task (see Table 5.11).

5.4.4.2 Flanker (FL)

Compared to adherent ET AwPKU, the semi-adherent ET AwPKU made significantly more errors on the compatible, but not incompatible, trials, of the second part of the FL task (see Table 5.11). The analysis did not reveal a significant main effect of group on speed (msec) or number of errors (n) on part 2 of the task (see Table 1, Appendix U). There was a significant main effect of type of stimulus on speed of correct responses ($F(1,18)=23.10$, $p<.001$, $\eta_p^2=.56$) and number of errors ($F(1,18)=10.32$, $p=.005$, $\eta_p^2=.36$), such that both groups of ET AwPKU were slower and less accurate on the incompatible versus the compatible trials. There were no significant group*type of stimulus interactions on either of the measures (see Table 1, Appendix U).

5.4.4.3 Memory Search 2D Objects (MS2D)

Mean (SD, 95% CI) RT (msec) and number of hits, correct responses, false alarms and misses of adherent and semi-adherent ET AwPKU on both parts of the MS2D task are shown in Table 5.12. Analysis revealed no main effect of group (see Table 2, Appendix U). As expected, there was a main effect of type of stimulus on speed ($F(1,18)=242.86$, $p<.001$, $\eta_p^2=.93$) and number of errors ($F(1,18)=24.47$, $p<.001$, $\eta_p^2=.58$), such that both groups of ET AwPKU were slower and made more errors under a high (3 targets) compared to low (1 target) working memory load. No significant group*type of stimulus interactions for speed (msec) or accuracy (n errors) were observed (see Table 2, Appendix U).

Table 5.11 Mean (SD, 95% CI) speed of correct responses (msec) and number of errors (n) on the BS and FL tasks

	Adherent ET AwPKU			Semi-adherent ET AwPKU			
	mean	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	***t, p* and *d***
Baseline Speed (BS)							
Speed (msec)	262.40	38.12	235.13-289.67	265.50	43.59	234.32-296.68	*t*(18)=.17, *p*=.87, *d*=.08
Flanker (FL)							
Part 1 – Compatible							
Speed (msec)	459.60	91.06	394.46-524.74	445.30	86.08	383.72-506.88	*t*(18)=-.36, *p*=.72, *d*=.16
Errors (n)	0.30	0.48	-0.05-0.65	0.60	0.97	-0.09-1.29	*t*(18)=.88, *p*=.39, *d*=.39
Part 1 - Neutral							
Speed (msec)	471.30	74.21	418.21-524.39	465.90	90.02	401.50-530.30	*t*(18)=-.15, *p*=.88, *d*=.07
Errors (n)	0.50	0.71	-0.01-1.01	1.40	1.26	0.50-2.30	*t*(18)=1.96, *p*=.07, *d*=.88
Part 2 - Compatible							
Speed (msec)	517.70	108.99	439.73-595.67	485.80	97.51	416.05-555.55	*t*(18)=-.69, *p*=.50, *d*=.31
Errors (n)*	0.40	0.70	-0.10-0.90	1.90	1.85	0.57-3.23	***t*(18)=2.40, *p*=.03, *d*=1.07**
Part 2 - Incompatible							
Speed (msec)	535.80	107.95	458.58-613.02	542.70	141.91	441.19-644.21	*t*(18)=.12, *p*=.90, *d*=.05
Errors (n)	1.20	1.32	0.26-2.14	3.30	3.83	0.56-6.04	*t*(18)=1.64, *p*=.12, *d*=.73

Table 5.12 Mean (SD, 95% CI) outcome measures on both parts of the MS2D task

	Adherent ET AwPKU			Semi-adherent ET AwPKU			
	mean	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	***t, p* and *d***
Part 1							
Hits (n)	23.30	0.95	22.62-23.98	23.30	1.06	22.54-24.06	$t(18)<.01$, $p=1.00$, $d<.001$
Speed (msec)	612.80	124.70	523.59-702.01	554.30	116.41	471.03-637.57	$t(18)=-1.08$, $p=.29$, $d=.48$
Correct (n)	23.20	0.92	22.54-23.86	23.30	0.95	22.62-23.98	$t(18)=.24$, $p=.81$, $d=.11$
Speed (msec)	695.90	154.77	585.18-806.62	671.30	225.96	509.66-832.94	$t(18)=-.28$, $p=.78$, $d=.13$
Misses (n)	1.40	0.89	0.29-2.51	1.75	0.96	0.23-3.27	$t(18)<.01$, $p=1.00$, $d=.38$
Speed (msec)	743.00	253.22	428.58-1057.42	521.00	162.52	262.39-779.61	$t(7)=-1.51$, $p=.18$, $d=1.04$
False alarms (n)	1.33	0.82	0.48-2.19	1.75	0.50	0.95-2.55	$t(18)=.24$, $p=.81$, $d=.62$
Speed (msec)	629.50	238.00	379.74-879.26	472.50	112.98	292.73-652.27	$t(8)=-1.21$, $p=.26$, $d=.84$
Part 2							
Hits (n)	17.80	3.33	15.42-20.18	18.00	4.08	15.08-20.92	$t(18)=.12$, $p=.91$, $d=.05$
Speed (msec)	2002.80	447.08	1682.98-2322.62	2219.20	619.00	1776.40-2662.00	$t(18)=.90$, $p=.38$, $d=.40$
Correct (n)	19.40	4.30	16.32-22.48	21.50	4.90	17.99-25.01	$t(18)=1.02$, $p=.32$, $d=.46$
Speed (msec)	2795.30	669.21	2316.57-3274.03	2942.60	690.19	2448.87-3436.33	$t(18)=.49$, $p=.63$, $d=.22$
Misses (n)	6.20	3.33	3.82-8.58	6.67	3.71	3.82-9.52	$t(18)=.12$, $p=.91$, $d=.13$
Speed (msec)	2647.70	1068.15	1883.59-3411.81	2740.67	844.65	2091.41-3389.92	$t(17)=.21$, $p=.84$, $d=.10$
False alarms (n)	5.75	4.03	2.38-9.12	5.00	6.20	-2.70-12.70	$t(18)=-1.02$, $p=.32$, $d=.14$
Speed (msec)	2734.88	590.75	2240.99-3228.76	2981.00	1424.61	1212.12-4749.88	$t(11)=-.44$, $p=.67$, $d=.23$

5.4.4.4 Sustained Attention Dots (SAD)

MST (the mean RT of a set number of series of 12 trials; msec) and number of errors (n) for all 50 series (600 trials), the first 10 series (120 trials), and the last 10 series (120 trials) on the SAD task are shown in Table 5.13.

5.4.4.4.1 Inhibition

There were no significant differences in MST or number of errors on the SAD task between adherent and semi-adherent ET AwPKU (see Table 5.13).

5.4.4.4.2 Sustained attention

There was no main effect of group on MST nor number of errors on the SAD task (see Table 3, Appendix U). Analysis revealed a significant effect of time on MST ($F(1,18)=8.23$, $p=.01$, $\eta_p^2=.31$), but not number of errors, such that participants were significantly slower towards the end of the task (last 10 series vs. first 10 series). There was no significant interaction between group and time on either outcome measure (see Table 3, Appendix U).

Table 5.13 Mean (SD, 95% CI) Mean Series Time (MST; msec) and number of errors (n) on the SAD task

	Adherent ET AwPKU			Semi-adherent ET AwPKU			
	mean	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	***t*, *p* and *d***
Inhibition							
MST[1] (msec) – all 50 series	10.88	3.70	8.23-13.53	9.98	2.35	8.30-11.66	*t*(18)=.65, *p*=.52, *d*=.29
Errors (n) – all 50 series	23.30	19.30	9.49-37.11	34.80	21.26	19.59-50.01	*t*(18)=-1.27, *p*=.22, *d*=.57
Sustained attention							
MST (msec) – first 10 series	10.94	3.50	8.43-13.45	9.48	1.93	8.10-10.86	*t*(18)=1.15, *p*=.26, *d*=.52
Errors (n) – first 10 series	4.10	3.90	1.31-6.89	5.90	4.95	2.36-9.44	*t*(18)=-.90, *p*=.38, *d*=.40
MST (msec) – last 10 series	11.67	4.53	8.43-14.91	11.52	3.42	9.07-13.96	*t*(18)=.08, *p*=.93, *d*=.04
Errors (n) – last 10 series	5.70	5.83	1.53-9.87	7.40	5.36	3.57-11.23	*t*(18)=-.68, *p*=.51, *d*=.30

[1] MST: average RT over a number series (each series contains 12 trials)

5.4.4.1 Set Shifting Visual (SSV)

Table 5.15 provides an overview of speed of correct responses (msec) and number of errors (n) made by adherent and semi-adherent ET AwPKU on both types of trial (compatible and incompatible) on the SSV task. There was no effect of group for speed or number of errors on part 2 of the task (see Table 4, Appendix U). There was a significant main effect of type of stimulus for both speed of correct responses ($F(1,17)=10.77$, $p=.004$, $\eta_p^2=.39$) and number of errors ($F(1,17)=4.56$, $p=.048$, $\eta_p^2=.21$), such that as expected, both groups of ET AwPKU were slower and less accurate on the incompatible versus the compatible trials. No significant group*type of stimulus interactions were observed on either outcome (see Table 4, Appendix U).

5.4.4.2 Motor skills: tracking (TR) and pursuit (PU)

Mean (SD, 95% CI) accuracy (mm; mean deviation of the trajectory that was followed) and the stability (mm; standard deviation of the trajectory that was followed) on the TR and PU tasks are shown in Table 5.16.

Analysis revealed no main effects of group (see Table 5, Appendix U), but there was a main effect of task (TR vs. PU) on both the accuracy ($F(1,18)=89.57$, $p<.001$, $\eta_p^2=.83$) and stability ($F(1,18)=13.84$, $p=.002$, $\eta_p^2=.44$) of movement, such that participants had better accuracy and stability on the TR compared to the PU task. Furthermore, no significant group*task interactions for the accuracy or stability of movement were observed (see Table 5, Appendix U).

Table 5.14 Mean (SD, 95% CI) speed of correct responses (msec) and number of errors (n) on the SSV task

	Adherent ET AwPKU			Semi-adherent ET AwPKU			
	mean	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	***t, p* and *d***
Part 1 - Compatible							
Speed (msec)	432.10	100.09	360.50-503.70	436.60	105.32	361.26-511.94	$t(18)=-.10$, $p=.92$, $d=.04$
Errors (n)	0.70	1.25	-0.20-1.60	0.70	1.06	-0.06-1.46	$t(18)<.01$, $p=1.00$, $d<.001$
Part 2 - Incompatible							
Speed (msec)	782.30	158.44	668.96-895.64	817.90	425.63	513.42-1122.38	$t(11.45)=-.25$, $p=.81$, $d=.11$
Errors (n)	3.40	3.63	0.81-5.99	1.70	1.64	0.53-2.87	$t(18)=-1.35$, $p=.19$, $d=.60$
Part 3 - Compatible							
Speed (msec)	992.00	279.32	777.29-1206.71	1028.10	304.31	810.41-1245.79	$t(18)=.27$, $p=.79$, $d=.12$
Errors (n)	3.22	3.11	0.83-5.62	1.50	1.43	0.47-2.53	$t(18)=-1.58$, $p=.13$, $d=.71$
Part 3 - Incompatible							
Speed (msec)	1180.89	385.76	884.37-1477.41	1123.70	435.37	812.26-1435.14	$t(17)=-.30$, $p=.77$, $d=.14$
Errors (n)	3.89	3.79	0.98-6.80	2.70	2.83	0.68-4.72	$t(17)=-.78$, $p=.45$, $d<.001$

Table 5.15 Mean (SD, 95% CI) accuracy (mm) and stability (mm) on the TR and PU tasks

	Adherent ET AwPKU			Semi-adherent ET AwPKU			
	mean	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	***t, p* and *d***
Tracking (TR)							
Accuracy[1] (mm)	1.17	0.79	0.61-1.74	0.87	0.97	0.17-1.56	*t*(18)=.78, *p*=.45, *d*=.34
Stability[2] (mm)	1.86	0.79	1.30-2.42	1.56	0.92	0.91-2.22	*t*(18)=-.78, *p*=.45, *d*=.35
Pursuit (PU)							
Accuracy[1] (mm)	4.46	1.29	3.54-5.39	3.86	0.64	3.40-4.31	*t*(18)=-1.33, *p*=.20, *d*=.59
Stability[2] (mm)	3.06	1.86	1.73-4.39	2.55	0.81	1.97-3.14	*t*(18)=-.79, *p*=.44, *d*=.36

[1] Accuracy (mm): mean deviation of the trajectory that was followed; [2] Stability (mm): standard deviation of the trajectory that was followed

5.5 Discussion

5.5.1 Summary of results

The study presented in this chapter aimed to assess the effects of poor adherence to protein substitutes in combination with self-restricted protein intake in ET AwPKU considered "semi-adherent" on several aspects of their health. To this end, nutritional status, QoL and cognitive functioning in semi-adherent ET AwPKU was compared with that of adherent ET AwPKU.

Apart from expected differences in Phe levels and Phe:Tyr ratios, there were no significant differences in Tyr levels, age, gender or IQ between the two groups of ET AwPKU. Furthermore, adherent and semi-adherent ET AwPKU did not differ with respect to occupational status or achieved level of education. Similarly, Das et al. (2013) found no differences in educational level between 4 groups of ET AwPKU who were either following a strict PKU diet, a vegan diet with or without protein substitutes, or eating 'normally'. In addition they reported that those on a 'normal' diet had significantly higher Phe levels than those on the PKU diet and found no significant differences in Tyr levels between groups. They did not report age and gender of these groups separately and did not assess Phe:Tyr ratio, IQ or occupational status.

5.5.1.1 Nutritional status

Despite insufficient total protein intake (<0.75g/kg/day), with the exception of pre-albumin status, the group of semi-adherent ET AwPKU did not significantly differ from the adherent ET AwPKU with respect to other measures of protein status and key vitamins and minerals. Both adherent and semi-adherent ET AwPKU were deficient in vitamin D. Moreover, although still within the target range, the semi-adherent ET AwPKU had significantly higher levels of homocysteine than adherent ET AwPKU. Semi-adherent ET AwPKU had significantly higher levels of Phe and Phe:Tyr ratios than the adherent ET AwPKU, reflecting poorer metabolic control. Das et al. (2013) also reported significantly higher levels of Phe in AwPKU who were not following a low protein diet with protein substitutes compared to those who were. In addition, they reported significantly higher levels of Val in these AwPKU, which did not differ here.

Interestingly, despite several reports of an increased risk of developing vitamin B12 deficiency for AwPKU on a less restricted diet (Aung et al., 1997; Hanley et al., 1996; Hvas et al., 2006), none of the ET AwPKU showed any signs of vitamin B12 deficiency. However, one of the semi-adherent ET AwPKU received a vitamin B12 booster before taking part in the study because they had developed a deficiency in vitamin B12. Hvas et al. (2006) reported that 75% of their sample of AwPKU showed early signs of vitamin B12 deficiency. The AwPKU studied were either following an unrestricted diet without any protein substitutes or a relaxed diet with LNAA supplements, which were not fortified with vitamin B12. It is possible that the limited amount of protein substitutes consumed by the semi-adherent ET AwPKU in the study reported here provided enough vitamin B12 for their levels to stay within normal reference ranges. The semi-adherent patients who were not taking any protein substitutes reported they were eating small amounts of meat. Folate levels were not assessed in the current study but elevated levels of folate have been suggested to mask functional vitamin B12 deficiency (de Benoist, 2008). High levels of folate have been reported in AwPKU in the past (Crujeiras et al., 2015b; Demirdas et al., 2017; Stølen et al., 2013). However, it is thought that increased folate levels are related to the high folic acid content of their protein substitutes (Stølen et al., 2013). Given the low intake of protein substitutes in the semi-adherent ET AwPKU, it is unlikely that their folate levels were elevated. Only one of the semi-adherent ET AwPKU had a slightly elevated homocysteine level, possibly indicative of a vitamin B12 deficiency. All other participants had homocysteine levels within reference ranges.

5.5.1.2 Quality of Life (QoL)

Overall, the adherent ET AwPKU had better self-reported health and lower severity of PKU-related symptoms compared to the semi-adherent ET AwPKU. The latter reported significantly more severe mood-related symptoms (moodiness and sadness) as well as increased tiredness and lack of concentration. A recent explorative study reported reduced quality of sleep, increased latency of falling asleep, and more sleepiness during the day in a group of PKU patients compared to their first degree relatives (Bruinenberg, Gordijn, MacDonald, van Spronsen, & Van der Zee, 2017). The same researchers

observed an increased fragmentation (i.e. the switch between active and inactive behaviour) and a shift in diurnality (i.e. a shift of a part of resting behaviour into the active phase) in PKU mice. Taken together, their results suggest that sleep is disturbed in PKU. Important modulators of sleep are dopamine, norepinephrine and serotonin (Eban-Rothschild, Rothschild, Giardino, Jones, & de Lecea, 2016; Holst, Valomon, & Landolt, 2016) and altered sleep has been linked to disturbances in mood (Meerlo, Havekes, & Steiger, 2015; Short & Louca, 2015) as well as cognitive functioning (Banks, 2007; Couyoumdjian et al., 2010). It is possible that differences in self-reported tiredness, lack of concentration, moodiness and sadness are the result of elevated Phe levels and are modulated by sleep disturbances. Furthermore, research has shown that patients with PKU who have insufficient protein intake tend to have insufficient total energy intake (Das et al., 2013; Modan-Moses et al., 2007) and a higher intake of fat (Das et al., 2013; Hochuli et al., 2017) compared to those who are adherent to the PKU diet. Therefore, the increased tiredness in the semi-adherent ET AwPKU could be related to insufficient intake of protein, insufficient energy intake and/or increased fat intake and likely to be related to the self-reported lack of concentration and issues with mood in this group.

In addition to differences in PKU-related symptoms, the self-reported emotional and social impact of PKU was significantly higher in the ET AwPKU with poor compared to good adherence.

These findings are in contrast with findings from Das et al. (2013), who reported no significant differences in QoL or mood between AwPKU with differing dietary practices. However, Das et al. (2013) used a generic tools to assess QoL and mood and, as suggested in Section 5.1.2.1, generic questionnaires may not be sufficiently sensitive to detect PKU-related issues with QoL and wellbeing (Regnault et al., 2015). Results from a different study using generic HRQoL measures, however, suggest that HRQoL in ET AwPKU is negatively associated with metabolic control (Huijbregts et al., 2018). In addition, Cazzorla et al. (2014) reported that the HRQoL was better in PKU patients who had been adherent to their treatment for a longer duration relative to the time of assessment. Neither of these studies looked at the dietary practices of their participants. Finally, during a short-term, double-blind intervention examining the effects of Phe levels on mood and cognitive functioning in ET AwPKU, ten Hoedt et al. (2011) observed

significantly lower mood states during a 4-week Phe loading period (as reported by the patients, and relatives or friends). ET AwPKU were more depressed and more fatigued during this period compared to 4 weeks during which they took a placebo.

5.5.1.3 Cognitive performance

With the exception of differences in accuracy of performance on the task measuring inhibition, no significant differences in cognitive performance on tasks of processing speed, inhibition, flexibility, working memory, sustained attention and motor skills were observed between adherent and semi-adherent ET AwPKU. Semi-adherent ET AwPKU made significantly more errors on compatible trials of the second part of the FL task, but there was no evidence for impaired inhibition in the semi-adherent ET AwPKU compared to the adherent ET AwPKU. Previous studies have inconsistently reported differences in processing speed, sustained attention, inhibition, working memory, and motor skills, but not complex EF between ET AwPKU with good and poor metabolic control (see Section 3.2.1.2.1). Furthermore, limited research has shown associations between concurrent Phe levels and performance on tasks of sustained attention, working memory, and motor skills with higher levels of Phe being related to worse task performance (see Section 3.2.1.2.2). It is possible that the current sample was too small and therefore lacked the power to detect any differences in cognitive functioning between adherent and semi-adherent ET AwPKU. The level of adherence in the adherent group is questionable since only five of the group had Phe levels <700 µmol/L at the time of testing, although this might be due to inadequate calorie intake prior to the test day. Failure to detect a significant differences between groups does not necessarily mean that these patients do not suffer from any cognitive deficits previously reported in ET AwPKU, and they may have been impaired compared to a healthy control group not included in this study. Future research should therefore, include an age and gender matched healthy (non-PKU) control group to investigate cognitive outcomes in semi-adherent ET AwPKU this further.

An examination of performance across all the ANT tasks included in this study indicated that there was considerable variation in speed and accuracy of performance across cognitive tasks even within each group. High within group variation reduces the

likelihood of detecting a significant difference, particularly when sample size is small (Tabachnick & Fidell, 2013). Anecdotally, there were also clear differences in the ability of participants to understand verbal task instructions which seemed unrelated to their Phe levels such that some patients within both groups seemed to catch on to the requirements of the tasks and test battery (e.g. which buttons to press to start the next task) quickly, whereas others did not. A recent systematic review of neurological, psychological and behavioural symptoms in untreated and late-treated (after 7 years of age) PKU highlighted the existence of "unusual" PKU patients, i.e. PKU patients with unexpected favourable intellectual outcomes (van Vliet et al., 2018). In relation to this, van Vliet et al. (2018) discussed three hypotheses first proposed two decades ago, which suggest that these patients may have some sort of protective mechanism, making them less vulnerable to high levels of Phe (Möller, Weglage, Wiedermann, & Ullrich, 1998; Weglage et al., 1998). The proposed mechanisms which might underlie inter-individual differences in brain vulnerability to high plasma Phe concentrations between PKU patients include 1) a difference in the transport of Phe and other LNAA across the BBB, 2) a difference in the transport of Phe and other LNAA across different cell-membranes in the brain, and 3) a difference in the vulnerability to one or more neurotoxic mechanisms of elevated brain Phe levels (see Figure 1.2) (van Vliet et al., 2018). Even though some (17%) of these "unusual" PKU patients described by van Vliet et al. (2018) still had one or more problems in neuropsychological or social functioning, differences in individual vulnerability to high levels of Phe might explain within group variation observed in the current study. Some of the semi-adherent patients, who had Phe levels that were twice as high as the upper target range in the UK guidelines, did not have any noticeable deficits in their cognitive functioning i.e. they were not outliers on any of the tasks nor were they less able to understand what was required of them during the cognitive test battery. In contrast, some of the adherent ET AwPKU, with levels around or below the upper target treatment range had difficulties understanding verbal instructions. This raises the question as to whether previously reported deficits in cognitive functioning (see Chapters 3 and 4) apply to all ET AwPKU, or whether some ET AwPKU, who potentially are less vulnerable to effects of elevated Phe, are unaffected and may even be over treated? Whereas others, not included in the literature, may have worse outcomes.

5.5.2 Limitations and future research

The study reported in this chapter had some issues with recruitment, such that only 10 ET AwPKU were finally recruited to each group. Despite the fact that consultants at the Mark Holland Metabolic Unit in Salford reported that approximately 50% of the 300+ AwPKU under their care follow a self-restricted diet with no or insufficient supplementation with protein substitutes, few patients that were seen in clinic during recruitment which ran for a period of 18 months (from December 2016 to May 2018) were both eligible for participation and willing to participate. Only a few AwPKU responded to the recruitment letters that were sent out to them. Unfortunately, the clinic did not follow up the recruitment letters with phone calls as agreed and the investigator had no access to personal information or contact information, until patients expressed an interest in participating and provided informed consent. Moreover, the no-show or DNA rate of AwPKU at the Mark Holland Metabolic Unit was high during recruitment. One possible reason for this could be the perceptions and attitudes of these patients with regards to PKU (e.g. not accepting the disorder, not seeing the need for treatment). In addition, the unit was understaffed at the time of recruitment and had recently undergone some staff-changes in the metabolic team which may have impacted on patient satisfaction and reduced the continuity of care. These circumstances may have been a barrier to their willingness to participate in research. In support of this proposition it has been demonstrated that perceived lack of professional support is associated with poor dietary adherence in PKU (Bilginsoy et al., 2005) and attending routine follow-up visits is an important factor in promoting adherence to the dietary management of PKU (Cazzorla et al., 2018). Those attending clinic visits are likely to be more engaged with their diet and have a better adherence to the dietary management. The majority of those ET AwPKU with poor dietary adherence, who still regularly attend their routine follow-ups, are aware they should change their dietary practices and often enquire about new developments in the management of PKU (personal communication metabolic team at SRFT). Hence, the semi-adherent ET AwPKU included in the current study are likely to be a self-selected sample and could differ from semi-adherent ET AwPKU who are less engaged with their PKU and dietary management who one might predict would be likely to have a poorer nutritional status and QoL than observed here. Adherence to diet is likely to fluctuate on a day to day basis in ET AwPKU

as routines vary and circumstances change. Identifying semi-adherent ET AwPKU is therefore not a precise process and there may have been variations in both the adherent and semi-adherent ET AwPKU diet at the time of recruitment, the honesty with which they reported their dietary behaviour and the ability of the dietitians to accurately determine their level of adherence. All of these factors could explain differences observed in metabolic control between screening and the test session. For instance, even though all adherent ET AwPKU had Phe levels <700 µmol/L at screening, 50% had levels >700 µmol/L at the time of their test session; similarly, all semi-adherent ET AwPKU had Phe levels >1000 µmol/L at screening but 3 had levels between 700 and 1000 µmol/L at the time of testing.

To address these difficulties with recruitment, future research might benefit from multicentre studies, thereby increasing the potential participant pool. Furthermore, instead of using most recent Phe level as the main criterion for inclusion , multiple Phe levels in the months leading up to inclusion should be considered to ensure consistency in metabolic control as an inclusion criterion .

Another limitation of the research reported here is that, other than total protein intake, the dietary intake of the patients was not assessed. More information about the dietary practices of individual patients would allow for more comprehensive interpretation of 1) the observed 'lack' of differences in nutritional status between adherent ET AwPKU and patients following seemingly nutritionally incomplete diets as well as 2) deficiencies observed at an individual level. Hochuli et al. (2017) failed to find many nutritional deficiencies in AwPKU despite the patients' self-reports suggesting that nutrient intake was deficient It is possible that, in addition to insufficient total protein intake, intake of several important nutrients was deficient in the semi-adherent ET AwPKU which may have long-term consequences for several aspects of their health. Moreover, it is possible that the limited intake of protein substitutes by the semi-adherent ET AwPKU provided sufficient nutrients. ET AwPKU who are self-restricting their protein intake but are no longer taking any protein substitutes are likely to be at an increased risk of developing nutritional deficiencies and future research should assess the consequences of such dietary practice on nutritional status and wellbeing of these patients.

The study presented in this chapter allows some reflection on the appropriateness of measures to assess QoL in PKU. The PKU QoLQ was sensitive to PKU-specific issues with

HRQoL in the current study. It therefore is an appropriate tool for comparing outcomes of different dietary practices in PKU. However, it does not allow for comparison between HRQoL of individuals with and without PKU nor comparisons with the majority of existing literature. For instance, using a generic measure of QoL, Das et al. (2013) observed no differences in HRQoL between groups of AwPKU with differing dietary practices and normative data from the general population. Hence, even though it is apparent that HRQoL is negatively associated with poor adherence, it is not clear whether the adherent and/or semi-adherent PKU patients in this study have a lower QoL than individuals without PKU. Future research might benefit from employing a generic HRQoL questionnaire alongside the PKU QoLQ.

5.5.3 Conclusions

Apart from expected differences in Phe levels and the Phe:Tyr ratio, no major differences in nutritional status were observed between adherent and semi-adherent ET AwPKU. However, even without clear nutritional deficiencies resulting from a self-restricted low-protein diet with insufficient intake from protein substitutes, such dietary practice might have long-term consequences for the health and wellbeing of these patients. Furthermore, ET AwPKU who do not consume any protein substitutes but still follow a self-restricted diet are likely to be at a greater risk of developing nutritional deficiencies than the semi-adherent ET AwPKU included in the research reported here. In addition, in spite of significantly higher Phe levels, the semi-adherent ET AwPKU showed no significant differences in cognitive performance on a range of cognitive tests, compared to adherent ET AwPKU. This could be due to lack of power in the current research, individual differences in vulnerability to elevated Phe, or even absence of differences in nutrients that are related to brain function.

Despite the fact that there were no clear differences in terms of nutritional status and cognitive performance, results revealed clear differences in the self-reported QoL of ET AwPKU with good and poor adherence. Poorer QoL observed in the semi-adherent ET AwPKU could be related to increased Phe levels as well as insufficient protein intake. Increasing the protein intake, and thereby most likely increasing the intake of other

nutrients and energy might improve the QoL and wellbeing of this group of patients. There is therefore a need to promote use of dietary protein intake in semi-adherent ET AwPKU and the factors identified in Chapter 2 suggest that taste and convenience of protein substitutes are key drivers of (non-)adherence. These semi-adherent ET AwPKU attended clinics and took part in the study indicating interest and motivation related to their PKU. There is a potential for the use of more palatable protein substitutes to nutritionally complete the diet of these patients. Chapter 6 which follows, presents a trial of such a newly developed product.

Chapter 6 Acceptability of a CGMP-based protein substitute in semi-adherent ET AwPKU and effects on nutritional status, quality of life and cognitive functioning

6.1 Introduction

Chapter 5 compared differences in nutritional status, QoL/wellbeing and cognitive functioning between groups of adherent and semi-adherent ET AwPKU. The semi-adherent ET AwPKU had a significantly poorer self-reported QoL compared to the adherent ET AwPKU. Furthermore, results revealed no clinically relevant differences in nutritional status or cognitive performance between groups. However, because all semi-adherent ET AwPKU in this sample had a self-restricted dietary protein intake and insufficient intake of protein substitutes, their total protein intake was below the minimal recommended intake (see Appendix T). When intake of total protein is inadequate, there will not be sufficient AA for protein synthesis. As a result, the body will break down muscle tissues (catabolism), which will cause Phe to be released from body cells, increasing blood Phe concentrations (van Spronsen et al., 2017). To ensure adequate protein intake, as well as adequate intake of energy and other essential nutrients, it is critical to encourage semi-adherent ET AwPKU to consume a nutritionally complete diet (Giżewska et al., 2016; van Spronsen et al., 2017). The restricted low-protein diet in conjunction with protein substitutes provides a nutritionally complete diet and resumption of the PKU diet will likely have beneficial effects on cognitive functioning and QoL/wellbeing. The study reported in Chapter 6 examines the acceptability of a newly developed CGMP-based protein substitute (CGMP-AA Protein Substitute) in the group of semi-adherent ET AwPKU who participated in the baseline study reported in Chapter 5, with the aim to supplement their diets. It aims to assess adherence to CGMP-AA Protein Substitute over the course of 12(±1) weeks as well as effects on nutritional status, QoL/wellbeing and cognitive functioning.

6.1.1 Resumption of the PKU diet: effects on cognitive functioning

Several short-term interventions assessing the effects of blood Phe concentrations on cognitive functioning in off-diet AwPKU reported improvements in performance on tasks measuring processing speed, sustained attention and flexibility, but not motor skills, after 3-4 weeks on the low-protein diet with protein substitutes (see Table 6.1) (Lou, Lykkelund, Gerdes, Udesen, & Bruhn, 1987; Pietz et al., 2008; Schmidt et al., 1994).

6.1.2 Resumption of the PKU diet: effects on quality of life and well-being

Only two studies have assessed the effects of the resumption of the PKU diet on quality of life and well-being of AwPKU (see Table 6.2). Out of 53 AwPKU returning to a restricted diet after following a relaxed diet for at least 3 months, only 55% were able to remain on-diet for 3 months and only 19% completed the 9 month intervention (Bik-Multanowski et al., 2008). The authors reported that, prior to reintroduction of the diet, their sample of AwPKU had interpersonal differences in QoL: some retained good QoL on a relaxed diet, whereas others suffered from severe emotional distress. Resumption of the diet improved subjective well-being in the majority of AwPKU who showed moderate-severe distress at baseline, especially with regards to depressive mood and anxiety (Bik-Multanowski et al., 2008). In spite of this, only 47% of AwPKU maintained adequate metabolic control approximately one year after returning to diet.

Table 6.1 Summary of studies assessing effects of resumption of the dietary management of PKU on cognitive functioning in AwPKU (adapted from Bilder et al. (2016))

Author (year)	Country	Study sample	Design	Intervention and duration	Blood Phe (µmol/L) Mean (range)	Cognitive measures	Results
Lou et al. (1987)	Denmark	9 AwPKU (5 ET, 4 LD) 3 female, 6 male Mean age 18.4 (±3.3) years (range 15-24) Off-diet	Intervention	3 weeks on PKU diet	Off-diet: 1477 (980-2050) On-diet: 758 (456-1052)	Processing speed: continuous RTs (~ simple RT test using a red stimulus light) Motor skills: finger tapping test	6 of 7 AwPKU with abnormally long continuous visual RTs on a regular diet showed improved RTs on PKU diet.
Pietz et al. (1993)	Germany	5 ET AwPKU 2 female, 3 male Mean age 20.6 (±1.5) years (range 19-22) Off-diet	Intervention	4 weeks on PKU diet	Off-diet: 1600 (1290-2130) On-diet: 753 (515-1023)	<u>Sonneville Visual Attention Tasks (SVAT):</u> Sustained attention: Dot Pattern Exercise (DPE) Flexibility: Colour Pattern Exercise (CPEX)	On the PKU diet, AwPKU showed improved scores on DPE and CPEX tasks.
Schmidt et al. (1994)	Germany	19 ET AwPKU 11 female, 8 male Mean age 20.5 years (range 17-24) Off-diet	Intervention	4 weeks on PKU diet	Off-diet: 1332 (569-1949) On-diet: 636 (121-1017)	<u>SVAT:</u> Sustained attention: DPE	On the PKU diet, AwPKU showed significantly improved scores for attention (DPE task).

Key: AwPKU: adults with PKU; CPEX: Colour Pattern Exercise; DPE: Dot Pattern Exercise; ET: early treated; LD: late diagnosed; RT: reaction time; SVAT: Sonneville Visual Attention Tasks

Table 6.2 Summary of studies investigating the effects of the resumption of the dietary management of PKU on QoL and well-being

Author (year)	Country	Study sample	Design	Follow-up relative to resumption of diet	Quality of Life measures	Results	Conclusions
Bik-Multanowski et al. (2008)	Poland	53 ET AwPKU (all classical PKU) on relaxed diet (>3 months) 25 female, 28 male Mean age 24 years (range 18-32) aged 18–32 years; mean age 24 years)	Intervention: resumption of diet Target Phe: <720 µmol/L	9 months	<u>Quality of Life (QoL):</u> Psychological General Well-Being Index (anxiety, depression, sense of positive well-being, self-control, and general health and vitality) bi-weekly blood Phe	<u>Baseline:</u> 55% positive wellbeing 28% moderate distress 17% severe distress After 3 months: - 29 AwPKU maintained diet (~55%) <u>After 9 months:</u> - 10 AwPKU completed study (~19%) - average blood Phe decreased by 420 µmol/L) - improvement of subjective wellbeing observed in majority of AwPKU with moderate/severe distress at baseline (especially anxiety and depression)	- interpersonal differences between AwPKU on relaxed diet: some retain good QoL, others suffer from severe emotional distress - Returning to diet is likely to increase QoL in the majority of patients

Author (year)	Country	Study sample	Design	Follow-up relative to resumption of diet	Quality of Life measures	Results	Conclusions
Gassio et al. (2003)	Spain	15 AwPKU (all classical PKU) 10 female, 5 male 8 LD (3 in adulthood) Mean age 27.5 years (range 16.4-37.5)	Cross-sectional After resumption (n=12) or introduction (n=3) of PKU diet	≥ 1 year	<u>QoL:</u> 24-item questionnaire based on Health Questionnaire SF-36, the Quality of Life Questionnaire QOLIE-10 and the Conner's Behaviour Scales for Teachers and Parents <u>Index of dietary control (IDC):</u> mean of median Phe measured at 6 month intervals after diet introduction/resumption good IDC: <480 µmol/mL regular IDC: 480-600 µmol/mL poor IDC: >600 µmol/mL	IDC was poor (median Phe: 954 µmol/L) in 8/15 patients, regular (Phe: 514 µmol/L) in 4/15 and good (Phe: 354 µmol/L) in 3/15 patients. - self-reported state of health: very good (53%), good (47%) - 40% felt that their present health on-diet was better than it had been off-diet - 53% calmer, quieter and less easily upset when on-diet 40% more alert and were more able to maintain attention while on-diet - 33% happier & 27% more vital - 20% less impulsive, aggressive, and argumentative when on-diet -60% improved QoL compared with the situation off-diet	More than half of the patients believed that their QoL improved with a Phe-restricted diet; they reported feeling calmer, quieter, and less easily upset. Only 47% attained regular to good dietary control.

Key: AwPKU: Adults with Phenylketonuria; IDC: Index of Dietary Control (Phe level); LD: Late Diagnosed; QoL: Quality of Life

6.1.3 Resumption of the PKU diet: challenges

As illustrated in the studies summarised above, over 50% of AwPKU who return to diet fail to maintain adequate adherence over a prolonged period of time. There are many possible explanations for this. For example, returning to the diet requires changes in the individual's behaviour that require planning and organisation: planning and preparing low-protein meals, remembering to take protein substitutes to work or on holiday etc. Several studies have shown that, compared to healthy controls, ET AwPKU have deficits in performance on cognitive tasks that require planning and organisation (Brumm et al., 2004; Nardecchia et al., 2015; Palermo et al., 2017). Moreover, as discussed previously (Chapter 2) even though AwPKU, who have relaxed their diet, often still avoid high-protein foods such as meat and fish, they will have introduced and developed a liking for foods that are not compatible with a strict low-protein diet. As a result, the temptation to stray from the strict diet will likely be greater than when these AwPKU were previously on-diet. Avoiding foods that are higher in protein requires self-control, an aspect of inhibitory control. Jahja, Huijbregts et al. (2017) reported that ET AwPKU with high lifetime Phe levels showed deficits in both speed and accuracy of performance on a task assessing inhibitory control As proposed previously in this thesis, ET AwPKU that participate in research are likely to be a self-selected sample of ET AwPKU who are more engaged with their dietary management which could positively bias findings. Deficits in planning, organisation and inhibitory control might well be more severe in those who are less adherent to their dietary management. Furthermore, by developing a liking for a wider variety of more palatable foods, AwPKU tend to especially struggle with the taste and after taste of AA-based protein substitutes, which poses another challenge to adherence to the strict diet. Protein substitutes based on CGMP might be more acceptable to these individuals (see Chapter 2).

The behaviour change involved in returning to diet is multifaceted: as semi-adherent ET AwPKU typically relax their diet (i.e. self-restrict, but increase protein intake) and either take no or too little protein substitutes, returning to diet involves both restricting their dietary protein intake as well as adhering to their prescribed amount of protein substitutes.

Trying to implement multiple behaviour changes at once can be challenging (Sweet & Fortier, 2010). However, there are likely to be individual differences (Burgermaster, Contento, Koch, & Mamykina, 2018) with some individuals who might prefer 'going cold turkey', whereas for others a step-by-step approach where one behaviour is targeted at a time may be more effective.

Finkelson et al. (2001) interviewed 21 AwPKU who discontinued their diet during childhood and returned to diet as adults. Approximately 50% reported they were still on-diet at the time of assessment. No information was provided about the duration between resumption of diet and interviews (Finkelson et al., 2001). The on-diet AwPKU had a strong support-network, whereas those off-diet had issues mainly with barriers the diet imposed on their social lives (82%), forgetting to take their protein substitutes (45%) and costs of/access to dietary product (45%). Finally, it is likely that the motivation to return and remain on-diet varies amongst ET AwPKU too. As pointed out by Bik-Multanowski et al. (2008), some of the ET AwPKU on a relaxed diet had good self-reported QoL. It is likely that these ET AwPKU whose QoL is good do not see the need and/or importance of following dietary recommendations.

6.1.4 The use of Casein Glycomacropeptide (CGMP) in the dietary management of PKU

CGMP has a unique profile of EAA when compared to typical dietary proteins, containing 2-3 times the amount of Iso and Thr (Etzel, 2004). However, it contains limited amounts of other EAA; His, Leu, Met, Trp and Tyr (Ney et al., 2009). Therefore, in order to ensure that the AA profile of CGMP-based food and drinks meets the World Health Organisation (WHO) protein and AA requirements in human nutrition (World Health Organisation, 2007), such products should be supplemented with these EAA (LaClair, Ney, MacLeod, & Etzel, 2009). In addition, Ney et al. (2009) observed low plasma Arg concentrations in their study with CGMP and recommended that Arg should be added to CGMP-based dietary products as well.

CGMP is a bland, neutral tasting product the functional properties of which mean it can be easily incorporated into a number of foods both sweet and savoury. It can be used in either a liquid or powdered form, has good heat stability and is soluble in acid. The versatility of the product means it has the potential to be incorporated into food and drinks without adverse effects on taste. In the past decade, several animal studies and short-term human studies and case descriptions, reporting the evaluation of safety, acceptability and efficacy of CGMP in the nutritional management of PKU, have been published. Outcomes of these studies and the proposed health benefits of CGMP are summarized in sections 6.1.4.1 and 6.1.4.2.

6.1.4.1 Proposed benefits of CGMP in PKU: animal studies

Table 6.3 provides an overview of animal studies on the use of CGMP in the treatment of PKU. Early research with CGMP in a PKU mouse model showed mice fed with a CGMP-based diet supplemented with the 6 EAAs mentioned above had similar growth to those fed an AA-based diet (Ney, Hull, van Calcar, Liu, & Etzel, 2008).

Moreover, plasma and brain Phe levels were lower in the CGMP-fed mice than the AA-fed mice (Ney et al., 2008), suggesting CGMP could be a safe alternative protein source for the dietary management of PKU. Further studies on CGMP in PKU mouse models have reported an improved bone mineral density (BMD) and renal function in *Pah*-mice on a CGMP diet compared to those on an AA or casein based diet (Ney, 2013; Solverson, Murali, Litscher, Blank, & Ney, 2012). In addition, *Pah*-mice on a CGMP-diet showed reduced inflammation compared to AA-fed *Pah*-mice (Solverson, Murali, Brinkman, et al., 2012), as well as reduced *Desulfovibrio* bacteria and increased short-chain fatty acids (SCFA) (Sawin et al., 2015), suggesting CGMP might have prebiotic[1] properties

[1] a prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Thammarutwasik et al., 2009)

Table 6.3 Summary of animal studies on the effects of CGMP on several aspects of health in PKU

Author (year)	Study sample	Test groups	Composition	Duration	End points	Results	Conclusions
Ney (2008)	30 WT & PKU mice (C57BL/6-Pahenu2)	Casein	20% casein & 0.3% cysteine	42 days	Growth, feed intake, relative organ mass (kidney, heart, liver), plasma AA, brain AA	- Similar gain in body weight and feed utilisation. - Higher kidney mass in AA-fed mice feed the than CGMP-fed mice. - CGMP-fed mice had a significant 11% decrease in plasma Phe-level compared to the AA diet. - CGMP-fed mice diet had a 20% decrease in overall brain Phe compared AA-fed mice.	The CGMP diet showed similar growth and lower plasma and brain phe level than the AA diet.
		AA	17.5% free AA				
		CGMP	20% CGMP & 6 EAAs				
Sawin (2014)	284 WT & PKU mice (C57BL/6-Pahenu2)	Casein	20% casein & 0 .3% L-cysteine	~18 weeks	Growth, plasma AA, brain, neuro-transmitters, behavioural tests	- CGMP and AA diets produced similar growth. - CGMP diet resulted in higher relative brain mass in female mice than the AA diet. - Male mice fed the CGMP diet had greater vertical activity than male mice fed the AA diet	A low-phe diet (free AA and CGMP based) was able to improve some behavioural impairments and, in some behavioural tests, normalize it to that of the WT casein control mice. The study supports the view that the low-phe diet be maintained for life to improve cognitive functioning in PKU patients.
		AA	17.5% free AA				
		CGMP	20% CGMP & 5 EAA				

Author (year)	Study sample	Test groups	Composition	Duration	End points	Results	Conclusions
Sawin (2015)	WT & PKU mice (C57BL/6J-Pahenu2)	Casein	20% casein & 0.3% L-cysteine	[1] 3-5 weeks [2] + [3] 17-19 weeks	[1]. Gut microbiota, SCFA and cytokine (plasma) [2]. Growth, spleen mass, and mass of jejunum and colon [3]. Splenocyte and blood cell T-cell populations	PKU mice significantly higher spleen and jejunum mass than WT	In PKU mice, jejunum adapts to larger mass to facilitate nutrition absorption, this response was attenuated in CGMP-fed mice. Greater faecal mass consistent with (putative) prebiotic effects of CGMP. Furthermore, CGMP diet led to modulation of GI microbiota (reduced *Desulfovibrio*), increased caecal SCFA and lower indexes of inflammation compared to AA/Casein diets. Functional foods made with CGMP may be beneficial in the management of PKU, obesity, inflammatory bowel disease (IBD)
		AA	17.5% free AA				
		CGMP	20% CGMP & 1.5x NRC requirement 5 EAA			<u>CGMP-fed mice:</u> - significantly lower spleen, jejunum and colon mass than mice on casein/AA diets - significantly higher faecal mass than mice on casein/AA diets - significantly reduced caecal Proteobacteria phylum. - significant increase in Bacteriodetes (PKU mice) - significant increase in Firmicutus (WT mice) - significantly different gut microbiome in caecum and faeces: reduction in *Desulfovibrio* - increase in caecal SCFT (acetate, propionate and butyrate) concentrations - significant reduction of inflammatory cytokine levels in the spleen (IFN-ɣ) and plasma (IFN-ɣ, TNF-α, IL-1β and IL-2)	
		AA	17.5% free AA				
		CGMP	20% CGMP (Lacprodan® CGMP-20) & 1.5x NRC requirement 5 EAA				

Author (year)	Study sample	Test groups	Composition	Duration	End points	Results	Conclusions
Solverson (2012a)	217 WT & PKU mice (C57BL/6J-Pahenu2/enu2)	Casein	20% casein & 0.3% cysteine	20 weeks	Plasma Phe, growth, bone mineral content, bone strength	The AA diet reduced femoral cross-sectional area and consequent maximal load compared with the CGMP diet. The AA diet yielded a more brittle and weaker bone compared to the CGMP diet.	Skeletal fragility, as reflected in brittle and weak femora, is an inherent feature of PKU. This PKU bone phenotype is attenuated by a CGMP diet compared with an AA diet.
		AA	17.5% free AA				
		CGMP	20% CGMP & 5 EAA				
Solverson (2012b)	180 WT & PKU mice (C57BL/6-Pahenu2)	Casein	20% casein & 0.3% cysteine	20 weeks	Growth, body composition (lean mass, fat mass), energy balance, plasma AA, energy expenditure	- PKU mice fed the CGMP diet showed a significantly lower % body fat compared to PKU mice fed the AA diet. - The AA-fed mice had a higher spleen mass than those on the CGMP diet, suggesting systemic inflammation - The CGMP diet reduced renal workload compared to the AA diet - Inflammatory cytokines were elevated in male PKU mice fed the AA diet compared to the CGMP diet. - The CGMP diet increased fat oxidation (reduced the respiratory exchange ratio (CO_2 produced/O_2 consumed))	PKU mice exhibited increased energy expenditure and showed evidence of systemic inflammation when fed an AA diet, the inflammation was reduced with the CGMP diet. The study suggests that a CGMP diet provides a more physiological source of low-Phe protein compared with an AA diet because it reverses the metabolic stress reflected in increased renal workload and immune stimulation that is observed in PKU mice fed an AA diet.
		AA	17.5% free AA				
		CGMP	20% CGMP & 5 EAA				

Key: AA: amino acids; CGMP: casein glycomacropeptide; EAA: essential amino acids; IFN-ɣ: interferon-gamma; IL-1β: interleukin 1β; IL-2: interleukin 2; SCFA: short-chain fatty acids; TNF-α: tumor necrosis factor α; WT: wild-type

6.1.4.2 Proposed benefits of CGMP in PKU: human studies

Table 6.4 provides a summary of research on the use of CGMP in the dietary management of PKU in both children and adults. van Calcar et al. (2009) studied 11 AwPKU in a cross-over trial comparing a CGMP-based protein substitute with an AA-based protein substitute and concluded that CGMP was a safe alternative protein source for use in the dietary management of AwPKU. The authors reported that CGMP improved both protein retention and Phe utilization compared with free AA (van Calcar et al., 2009). Similarly, Ahring et al. (2018) compared the short-term effects of different drink mixes containing AA, CGMP or a mixture of both on biomarkers of satiety and Phe levels over the day and concluded that a mixture of CGMP and AA provides a suitable protein substitute for AwPKU. In addition to improved (after)taste and, as a result, acceptability of CGMP-based protein substitutes compared to AA-based protein substitutes (see Chapter 2), proposed benefits of the use of CGMP-based protein substitutes in the management of PKU include positive effects on satiety, metabolic control, cognitive functioning and bone and dental health. Beneficial properties of CGMP are discussed in the next sections.

6.1.4.2.1 Satiety and body weight management

Ney et al. (2016) reported improved GI symptoms and less hunger in participants on the CGMP-arm of a 3 week cross-over trial with CGMP and AA-based protein substitutes. CGMP may be more satiating due to slower absorption (Pinto et al., 2017) and has been shown to enhance satiety by suppressing ghrelin (MacLeod, Clayton, van Calcar, & Ney, 2010; van Calcar et al., 2009). Possible satiating effects of CGMP might improve appetite control, which could be used in the prevention of overweight and obesity in PKU. This is of importance because obesity in female PKU patients has been of growing concern over the last few years (Burrage et al., 2012; Gokmen-Ozel et al., 2014).

Table 6.4 Summary of studies investigating the use of CGMP in the dietary management of PKU

Author (year)	Study sample	Study product(s)	Composition	Design	Duration	End Points	Results	Conclusions
Ahring (2018)	8 PKU patients 7 females, 1 male Mean age 33.25 (±11.21) years (range 15-48)	Drink mixes (DM) 1-4	DM1: Lacprodan® CGMP-20 DM2: AA DM3: Lacprodan® CGMP-20 + FSAA DM4: AA	Intervention	4 x 240 minutes	Plasma AA, ghrelin, BUN, glucose, GLP-1, PYY, CCK Taste & Satiety (VAS) BMI and % body fat	- Intake of DM was 25% of daily recommendation (1g protein/kg bodyweight/ day) - majority of serum AA concentrations peaked after 15min for DM2+4 and 30min for DM1+3 (slower absorption of DM containing CGMP) - no significant changes in plasma Phe despite residual amount of Phe in CGMP - no significant differences in biomarkers of satiety - no significant differences subjective ratings of taste or fullness (VAS)	- CGMP and AA had the same short-term effect on biomarkers, including Phe. - The mixture of AA and CGMP provides a suitable protein substitute to use in the dietary management of PKU.

Author (year)	Study sample	Study product(s)	Composition	Design	Duration	End Points	Results	Conclusions
Browne (2018) SIMD conference poster	12 PKU patients 9 female, 3 male Mean age 28 years (range 5-50); n=8 AwPKU All taking AA-based medical foods (MF)	CGMP-AA MF	PhenylAde® GMP drink mix: 15.3mg Phe/10g protein equivalent (PE)	Intervention	31 days (3 days baseline; 28 days CGMP-AA MF)	Blood AA profile in particular: Phe, Tyr, BCAA (Ile, Leu & Val) Adherence (self-reported)	- Self-reported adherence: 96% (SD 1.6) - Blood Phe and BCAA remained stable over study period - Blood Tyr significantly increased - No significant change Phe:Tyr	- No significant changes in blood AA, with the exception of a significant increase (improvement) in Tyr - BCAA remained within recommended ranges
Daly (2017)	22 PKU patients 9 female, 13 male median age 11 years (range 6-16)	Phe-free L-AA MF (n=9)	Usual patient Phe-free L-AA formula	Prospective pilot study	6 months	Phe, Tyr, Phe:Tyr	<u>CGMP-AA group</u> (compared to pre-study): - significant increase in Phe - significant decrease in Tyr - significant increase in Phe:Tyr <u>L-AA group</u>: - non-significant decrease in Phe - no changes in Tyr or Phe:Tyr	- Decline in blood Phe control on CGMP-AA MF observed, but Phe remained within target range. - Observed increase in blood Phe possibly the result of Phe in CGMP-AA MF. - CGMP-AA MF were more acceptable than L-AA MF.
		CGMP-AA MF (n=12; 1 withdrawal) Partially or wholly replacing Phe-free L-AA	CGMP supplemented with AA to meet WHO/FAO/UNU requirements. Addition of CHO, fat and micronutrients. 30mg Phe/20g PE					

Author (year)	Study sample	Study product(s)	Composition	Design	Duration	End Points	Results	Conclusions
MacLeod (2010)	11 PKU patients	AA-based breakfast	Usual patient AA formula	Cross-over	8 days (4 day AA-diet, 4 day CGMP-diet)	Plasma AA, insulin, ghrelin Satiety (VAS)	<u>CGMP- vs. AA-diet:</u> - Postprandial ghrelin concentration was significantly lower with CGMP - Lower postprandial ghrelin concentrations were associated with greater feelings of fullness after breakfast	Results show sustained ghrelin suppression, and suggest greater satiety with ingestion of a meal containing CGMP compared with AAs.
		CGMP-based breakfast	CGMP & His, Leu, Met, Trp					
LaClair (2009)	4 female AwPKU Aged 19 to 29 years	AA-MF	Usual patient AA formula + diet	Cross-over	8 days (4 days AA-MF, 4 days CGMP-MF)	Plasma AA (2.5 hours after eating breakfast)	Similar Phe levels	Supplementation of CGMP with limiting AA was required. CGMP-MF met or exceeded the DRI for all indispensable AA. Production of increased purity CGMP (low enough Phe content) at the commercial scale holds promise to improve QoL related to the dietary management of PKU.
		CGMP-MF	Patient AA formula replaced by formula containing CGMP & His, Leu, Met, Trp The 2 diets were isocaloric and con- tained the same amount of protein and Phe					

Author (year)	Study sample	Study product(s)	Composition	Design	Duration	End Points	Results	Conclusions
van Calcar (2009)	11 PKU patients 4 female, 7 male Aged 11–31 years; 8 AwPKU	AA-MF	Usual patient AA formula	Cross-over	8 days (4 days AA, 4 days CGMP)	acceptability, safety, plasma AA, BUN, insulin and measures of protein utilization	- the CGMP-MF was preferred to the AA-MF by 10/11 patients - no adverse reactions to the CGMP diet reported - similar Phe levels on both diets - postprandial Phe was significantly higher than fasted Phe on AA-MF, not CGMP-MF - higher plasma insulin with CGMP-MF compared to AA-MF - significantly lower BUN with CGMP-MF compared to AA-MF	CGMP, supplemented with limiting AA, is a safe and highly acceptable alternative to L-AA as the primary protein source in the dietary management of PKU. As an intact protein source, CGMP improves protein retention and Phe utilization compared with L-AA.
		CGMP-MF	CGMP & His, Leu, Met, Trp					
Lim (2007)	49 PKU patients Aged 12-42 years	Various foods and beverages made with CGMP	The nitrogen concentration of CGMP and AA beverages was similar.	Randomised sensory evaluations	One-off sensory tasting	Sensory evaluations (appearance, odour, taste, texture)	Compared to the AA-based beverage and low protein crackers, the CGMP beverage and crackers were scored as more acceptable in terms of appearance, odour and taste	Palatable, low-Phe foods and beverages can be made with CGMP to provide an alternative Phe protein source in the dietary management of PKU.
		AA-based chocolate beverage						
		Low protein cracker						

Author (year)	Study sample	Study product(s)	Composition	Design	Duration	End Points	Results	Conclusions
Ney (2008)	Adult with classical PKU Male Aged 29 years	AA-MF	Usual patient AA formula	Case report	15 weeks (3 weeks AA-MF, 10 weeks CGMP-MF, 2 weeks AA-MF) Patient was provided with weighed portions of food with precisely controlled Phe content for the first 6 weeks.	Plasma Phe, Tyr and other AA (during the first 6, well-controlled, weeks) Chemistry panel analyses: electrolytes, albumin, pre-albumin, and liver function	- Safety and acceptability of CGMP-MF - 10% reduction in plasma Phe levels (significant) - no significant differences in plasma Tyr levels - significant increases in plasma LNAA - improved distribution of dietary protein throughout the day compared with the AA-MF - no adverse effects of consumption of CGMP-MF (based on physical examinations and chemistry panel analyses - patient enjoyed the CGMP-MF and felt more alert than with his usual AA-MF	CGMP appears to provide a safe dietary source of LNAA for this patient Incorporation CGMP-MF into the PKU diet improves the taste, variety and convenience of the diet, which may lead to improved dietary adherence, metabolic control and ultimately QoL for individuals with PKU.
		CGMP-MF						

Author (year)	Study sample	Study product(s)	Composition	Design	Duration	End Points	Results	Conclusions
Ney (2016)	30 ET PKU patients (n=20 classical) 18 female, 12 male Aged 15-49 years; 25 AwPKU	AA-MF	A variety of available (on prescription) AA formula	Cross-over (randomised)	3 weeks	Fasting plasma AA profiles, blood Phe, food records and neuropsycho-logical tests: - Beck Anxiety Inventory (BAI) - Behaviour Rating Inventory of Executive Function (BRIEF)	<u>CGMP-MF vs. AA-MF:</u> - frequency MF intake was higher with CGMP-MF - CGMP-MF were rated as more acceptable - improved GI symptoms and less hunger reported on CGMP-MF - no significant increase in plasma Phe on CGMP-MF (despite increased Phe intake) - significant decrease in plasma Phe on AA-MF - no significant differences Phe between MF (similar Phe control) - no significant differences in BAI and BRIEF scores	CGMP-based MF provide a safe and acceptable option for the dietary management of PKU. Greater acceptability and fewer side effects CGMP-MF vs. AA-MF may enhance dietary adherence.
		CGMP-MF	A variety of available (on prescription) CGMP-based formula					

Author (year)	Study sample	Study product(s)	Composition	Design	Duration	End Points	Results	Conclusions
Ney (2017)	30 ET PKU patients (n=20 classical) 18 female, 12 male Aged 15-49 years; 25 AwPKU	AA-MF	A variety of available (on prescription) AA formula	Cross-over (randomised)	3 weeks	Metabolomic analysis of plasma (n=18) and urine (n=9) samples. Catecholamines and 6-sulfatoxymelatonin in 24-h urine samples Neuropsychological tests (see Ney et al. (2016) and Stroup et al. (2017c)).	<u>CGMP-MF vs. AA-MF:</u> - Tyr and Trp intake ~50% higher with AA-MF - AA-MF were consumed in larger quantities, less frequently throughout the day - Performance on cognitive tests and Tyr/Trp-derived neurotransmitter concentrations did not differ. - On AA-MF: higher levels of microbiome-compounds synthesized from Tyr & Trp (in the kynurenine pathway).	- Tyr from AA-MF lower bioavailability, partly due to greater degradation by intestinal microbes. - Research is needed to understand how metabolism of Trp via the kynurenine pathway and changes in intestinal microbiota affect individuals with PKU.
		CGMP-MF	A variety of available (on prescription) CGMP-based formula					

Author (year)	Study sample	Study product(s)	Composition	Design	Duration	End Points	Results	Conclusions
Pinto (2017)	11 PKU patients 8 female, 3 male (n=1 HPA, n=4 mild, n=6 classical PKU) Mean age 27 (±10) years; n=9 AwPKU	AA-MF	Usual patient AA formula	Cross-over (non-randomised)	~2 years (time on AA 13±5 months; time on CGMP 13±7 months)	Anthropometry, body composition, blood pressure, biochemical markers (vitamins, minerals, lipids, CHO and protein status), nutritional intake assessment (24h recall), metabolic control (Phe, Tyr, Phe:Tyr)	Similar PE intake from CGMP (0.85 g/kg) and AA (0.75g/kg). <u>After introduction CGMP-MF:</u> - no difference blood Phe - blood Tyr increased - Phe:Tyr decreased - anthropometry and body composition as well as blood pressure measures did not change Haemoglobin A1C decreased - all other biomarkers remained unchanged - lower overall energy intake (not significant)	- Partial CGMP contribution to total protein substitute intake did not affect nutritional status or body composition. - Blood Phe control was not adversely affected despite increase in dietary Phe from CGMP. - Observed increase in Tyr was surprising as Tyr intake decreased. - Increased blood Tyr and decreased Phe:Tyr may be beneficial for cognitive functioning. - Compared to L-AA, CGMP may have a more satiating effect due to its slower absorption.
		CGMP-MF	Glytactin Bettermilk/RTD (ready to drink)					

Author (year)	Study sample	Study product(s)	Composition	Design	Duration	End Points	Results	Conclusions
Proserpio et al. (2018)	86 PKU patients 41 female, 45 male (n=6 LD) Age range 8-55 years	4 AA-MF (5g PE) 4 CGMP-MF (5g PE)	<u>Neutral:</u> 16.5g MetaX powder (+100 mL water) 7.1g CHO, 2.3g fat, 5g protein Glytactin RTD (100mL) 9g CHO, 1.4g fat, 5g protein Chocolate, strawberry and tomato flavours were prepared by adding 2g flavouring powder	Randomised sensory evaluations	One-off sensory tasting	<u>Liking (VAS):</u> extremely disliked (1) -extremely liked (10) <u>Check all that apply (CATA):</u> 27 sensory attributes including appearance, odour, taste, flavour and texture terms	- CGMP-MF obtained higher liking scores compared to AA-MF - Age had a significant effect on liking: younger patients provided higher scores than older patients for both MF - Patients with poor adherence (based on Phe levels and EU guidelines) gave significantly lower liking scores for both MF - Patients with poor adherence gave significantly higher liking scores to CGMP-MF than AA-MF	Different foods and beverages with CGMP could be developed to improve dietary treatment adherence of individuals with PKU from school age onwards.

Author (year)	Study sample	Study product(s)	Composition	Design	Duration	End Points	Results	Conclusions
Stroup (2017a)	8 PKU patients 4 female, 4 male (n=4 classical) Mean age 27.25 years (range 16-35); n=6 AwPKU	AA-MF	Usual patient AA formula	Cross-over (not randomised)	1-3 weeks	Potential renal acid load (PRAL) Bone mineral density (BMD) Total energy, macronutrient, micronutrients, and AA intake	- AA provided 1.5–2.5-fold higher PRAL and resulted in 3- fold greater renal net acid excretion compared to CGMP. - Dietary protein, calcium & magnesium intake were similar - CGMP significantly reduced urinary excretion of calcium by 40% and magnesium by 30%. - Urinary calcium with AA negatively correlated with L1–L4 BMD.	Compared to CGMP-MF, AA-MF increased dietary acid load, subsequently increasing urinary calcium and magnesium excretion, and likely contributing to skeletal fragility in PKU.
		CGMP-MF	CGMP (~70%) & Arg, His, Leu, Trp,and Tyr (~30%)					
Stroup (2017b)	30 ET PKU patients (n=20 classical) 18 female, 12 male Aged 15-49 years; 25 AwPKU)	AA-MF	A variety of available (on prescription) AA formula	Cross-over (randomised)	3 weeks	Fasting venepunctures, medical food logs, 3-day food records, metabolomic analysis of plasma and urine.	- Low-Phe diet in combination with either MF was *'adequate'* - without micronutrient supplementation of MF, >70% of PKU would have inadequate intakes for 11 micronutrients - despite micronutrient supplementation of MF inadequate intakes of potassium (93%) and choline (>40%) and excessive intakes of sodium (>63%) and folic acid (>27%) were observed - PKU had excessive sugar intake (27% of total energy)	Nutrient status was similar for both MF. More research related to micronutrient supplementation of MF for the dietary management of PKU is needed.
		CGMP-MF	A variety of available (on prescription) CGMP-based formula.					

Author (year)	Study sample	Study product(s)	Composition	Design	Duration	End Points	Results	Conclusions
Stroup (2017c)	30 ET PKU patients (n=20 classical) 18 female, 12 male Aged 15-49 years; 25 AwPKU	AA-MF	A variety of available (on prescription) AA formula	Cross-over (randomised)	3 weeks	Cognitive tests: - Delis-Kaplan Executive Function System: Verbal and design Fluency - CANTAB: Stockings of Cambridge, Spatial Span & Spatial Working Memory	CGMP-MF vs. AA-MF: - no significant differences in performance on Delis-Kaplan Executive Function System or any of the CANTAB tests.	N/A *(data paper)*
		CGMP-MF	A variety of available (on prescription) CGMP-based formula					
Stroup (2018a)	15 ET PKU patients (subset from Ney et al. (2016)) (n=8 classical PKU) 9 female, 6 male Aged 15-50 years	AA-MF	A variety of available (on prescription) AA formula	Cross-over (randomised)	1-3 weeks	BMD and body composition PRAL Nutritional intake (3-day food records)	- male participants significantly lower total body BMD (z-scores) - total femur z-scores negatively associated with AA-MF intake - males tended to consume more PE (not significant) - males and females similar urinary excretion of renal net acid, magnesium and sulphate - males had a higher urinary excretion of calcium (not significant) - females had a greater % body fat	Male PKU patients may have lower BMD compared to females, which could be related to higher AA-MF intake and excretion of calcium.
		CGMP-MF	A variety of available (on prescription) CGMP-based formula					

Author (year)	Study sample	Study product(s)	Composition	Design	Duration	End Points	Results	Conclusions
Stroup (2018b)	25 AwPKU (subset from Ney et al. (2016)) Aged 18-49 years 143 controls	AA-MF	A variety of available (on prescription) AA formula	Cross-over (randomised)	3 weeks	Fatty acid profiles of red blood cells (RBC). Metabolomics of plasma and urine for 9-10 PKU and 15 controls.	- RBC fatty acid profiles not different on AA-MF and CGMP-MF - PKU had significantly higher levels of n-6:n-3 fatty acids and lower DHA and EPA on AA-MF compared to controls - higher urinary excretion of TMAO on AA-MF than CGMP-MF - PKU had significantly lower plasma deoxy-carnitine (suggesting reduced carnitine biosynthesis)	- Supplementation with DHA is needed in PKU. - Carnitine from AA-MF showed reduced bioavailability, partly due to bacterial degradation to TMAO; bioavailability was greater with prebiotic CGMP-MF
		CGMP-MF	A variety of available (on prescription) CGMP-based formula					
Zaki (2016)	10 PKU patients 4 female, 6 male Median age 6.73 years (range 4-16; interquartile range 5.02-11.79)	AA-MF	0% CGMP, 100% AA	Prospective, self-controlled clinical trial	18 weeks (two 9 week phases)	Phe, Tyr, Phe:Tyr, other AA, liver function tests (ALP, AST, ALT, albumin, bilirubin, GGT), BUN, creatine and CBC Satiety and palatability (subjective)	- Aspartic acid and citrulline levels significantly lower in CGMP-AA MF phase - No significant differences in Phe, Phe:Tyr, other AA and other laboratory data between study phases. - All patients preferred CGMP-AA MF over AA-MF (better taste and satiety).	CGMP may be used to replace 50% of protein intake to improve nutritional value and palatability of the diet.
		CGMP-AA MF	50% CGMP, 50% AA					

Key: (L-)AA: Amino Acids; ALT: Alanine transaminase; ALP: Alkaline phosphatase; Arg: Arginine, AST: Aspartate transaminase; BCAA: Branched Chain Amino Acids; BMD: Bone mineral density; BMI: Body Mass Index; BUN: Blood urea nitrogen; CBC: Complete Blood Count; CCK: Cholecystokinin; CGMP: Casein Glycomacropeptide; GGT: Gamma-Glutamyl Transferase; GLP-1: Glucagon-like peptide-1; His: Histidine; Ile: Isoleucine; MF: Medical Food; Leu: Leucine; PE: Protein Equivalent; Phe: Phenylalanine; Phe:Tyr: Phe/Tyr ratio; PPY: Peptide tyrosine-tyrosine; PRAL: Potential renal acid load; SIDM: Society for Inherited Metabolic Disorders; TMFO: trimethylamine N-oxide (compound that is synthesized by bacteria from carnitine); Trp: Tryptophan; Tyr: Tyrosine; Val: Valine; VAS: Visual Analogue Scale

6.1.4.2.2 Metabolic control

Furthermore, better acceptability and fewer side effects of protein substitutes may increase adherence to dietary management of PKU, which in turn could lead to improved Phe control and, ultimately, to improved nutritional management (MacLeod et al., 2010; van Calcar et al., 2009) and status, QoL and cognitive functioning of individuals with PKU. Short-term, self-reported, adherence to CGMP-based protein substitutes tends to be good: Browne et al. (2018) observed a self-reported adherence of 96% during a 31 day intervention in children and adults with PKU and Ney et al. (2016) reported higher frequency of intake of CGMP-based protein substitutes compared to AA-based protein substitutes in AwPKU over a 3-week period. However, the impact of the use of CGMP-based protein substitutes on Phe control is not yet clear. Two 4-day interventions with a CGMP-based diet in AwPKU did not show significant changes in Phe-levels (MacLeod et al., 2010; van Calcar et al., 2009). The authors argued that this was possibly due to the relatively short length of intervention. However, Daly et al. (2017) observed a decline in blood Phe control in children after a 6 month intervention with a CGMP-based protein substitute. Phe levels remained within the target range. The authors concluded that the observed increase in Phe levels was most likely the result of residual Phe in CGMP. In contrast, despite an increase in dietary Phe from CGMP, Pinto et al. (2017) and Browne et al. (2018) observed no adverse effect on blood Phe control in their studies. Moreover, in both studies, an increase in blood Tyr was observed. Pinto et al. (2017) also found a decreased Phe:Tyr ratio.

6.1.4.2.3 Cognitive function

Previous research on the association between Tyr levels and Phe:Tyr ratio and cognitive functioning in PKU has suggested that low lifetime Phe:Tyr ratios may be beneficial for EF (Luciana et al., 2001; Sharman et al., 2009). However, Ney et al. (2017) observed no difference in cognitive performance in AwPKU after 3 weeks on CGMP-based or AA-based protein substitutes.

6.1.4.2.4 Bone and dental health

In addition to proposed benefits described above, Stroup, Sawin et al. (2017) observed lower dietary acid load and a decrease in urinary calcium and magnesium excretion in AwPKU after 3 weeks on CGMP-based protein substitutes compared to 3 weeks on AA-based protein substitutes. Hence, the use of CGMP-based protein substitutes instead of AA-based protein substitutes may also be beneficial to bone health. Finally, it has been suggested that CGMP-based protein substitutes may have a positive effect on dental health and bad breath compared to AA-based protein substitutes. CGMP-based protein substitutes are less acidic (more neutral pH), are proposed to have an antibacterial effect, and contain a lower level of sulphur-containing AA compared to the (conventional) AA-based protein substitutes (Brody, 2000).

6.2 Study aims

Many adolescent and adult PKU patients are reluctant to adhere to their Phe-free protein substitutes and to maintain a strict low-protein diet once they reach adulthood (Schulz & Bremer, 1995). However, these patients are often either wary of, or unused to, naturally protein-rich foods, especially those of animal origin. As a result they tend to follow a self-selected, unmeasured, un-supplemented low-protein diet which is vegan or vegetarian and limited in food variety (Feillet & Agostoni, 2010; van Spronsen & Burgard, 2008).

Many of these ‘semi-adherent’ patients do not fully adhere to diet because they dislike the distinctive taste and aftertaste of AA-based protein substitutes. The CGMP-AA Protein Substitute has been developed specifically for this group of patients. It offers an alternative protein source that is more palatable and has been flavoured with a more adult palate in mind. As experience and research dictates that many AwPKU who attempt to resume the strict PKU diet fail, the purpose of the CGMP-AA Protein Substitute was to nutritionally complete the diet of semi-adherent ET AwPKU, rather than to return these individuals to strict dietary treatment. Hence, the aim of the study reported in this chapter was to provide a protein substitute that this group of patients

would be able to take on top of their self-restricted diet and to determine if this improved their dietary practices.

Products based on CGMP for the dietary management of PKU are widely available but were not available within the UK at the start of this research. Although there is data to support the use of products based on CGMP in the dietary management of PKU there is a lack of longer-term data. The current research is a pilot study which primarily assessed the acceptability, palatability and nutritional adequacy of CGMP-AA Protein Substitute in semi-adherent ET AwPKU. Adherence to CGMP-AA Protein Substitute and effects of consumption of the protein substitute for 12(±1) weeks on nutritional status, QoL/wellbeing and cognitive functioning were examined.

6.3 Method

6.3.1 Design

Study 3, part 2 was a 12-week intervention with a newly developed protein substitute (CGMP-AA Protein Substitute; see 6.3.3) in the semi-adherent ET AwPKU who participated in Study 3, part 1 (see Chapter 5). Nutritional status, QoL/wellbeing and cognitive functioning, which were assessed at baseline (see Chapter 5), were re-assessed 6(±1) and 12(±1) weeks after introduction of CGMP-AA Protein Substitue (see Figure 6.1).

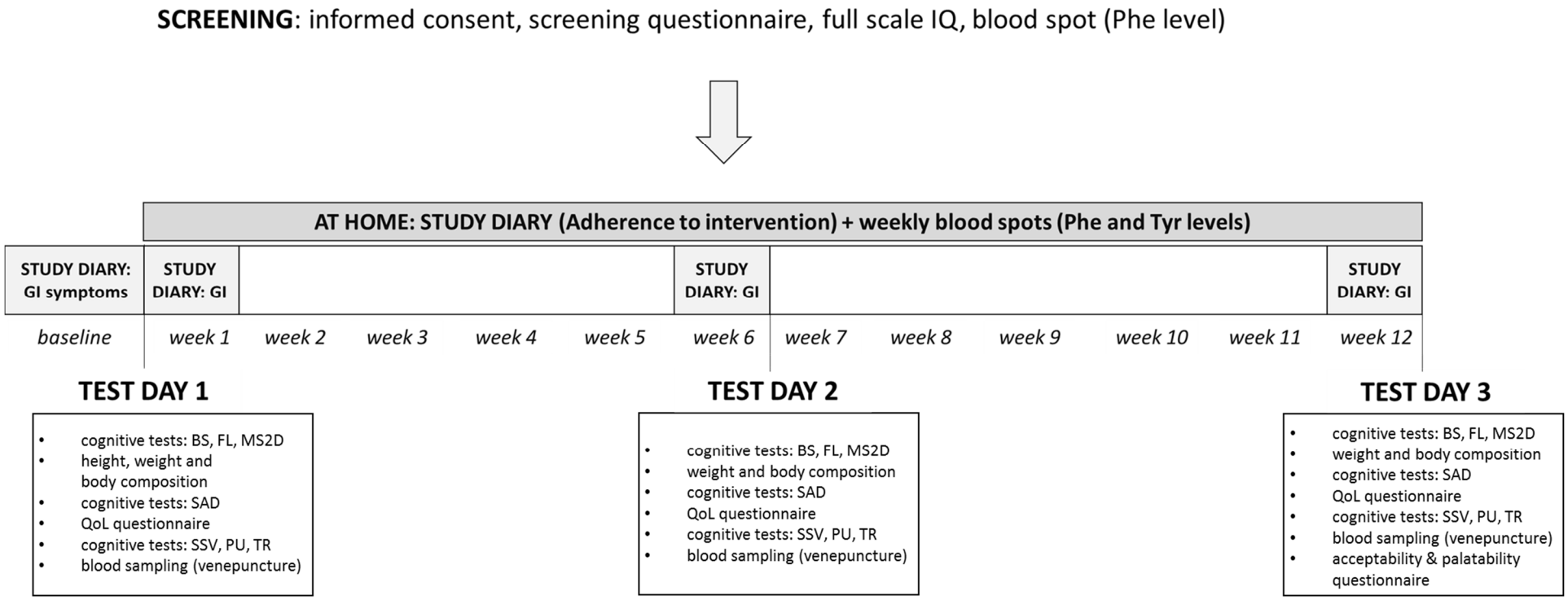


Figure 6.1 Study procedure flow diagram for Study 3, part 2

Key: BS: baseline speed; FL: flanker; GI: gastrointestinal; MS2D: memory search 2-dimensional objects; Phe: phenylalanine; PU: pursuit; SAD: sustained attention dots; SSV: set shifting visual; TR: tracking; Tyr: tyrosine

6.3.2 Participants

Ten semi-adherent ET AwPKU who had completed Study 3, part 1 were enrolled onto this study. A full description of inclusion and exclusion criteria can be found in Chapter 5 (see Tables 5.3 and 5.4).

6.3.3 Anthropometric measures

Participants' weight and body composition (BMI and %body fat) were re-assessed at test days 2 (T2; after 6±1 weeks) and 3 (T3; after 12±1 weeks) using the Tanita SC-240 bio-impedance scale (Tanita Europe B.V., Amsterdam, the Netherlands).

6.3.4 Nutritional status

6.3.4.1 Venous blood measures

A venous blood sample (~19mL whole blood) was obtained on all test days via venepuncture. Samples were analysed for a full AA profile, protein status (albumin, pre-albumin and transferring), CRP and a range of micronutrients and related measures (e.g. enzymes, hormones). The procedure for sample handling, storage and analysis has been described previously (see Section 5.3.4.1). To limit the influence of small inter-batch differences in reagents used to perform blood assays, samples from the same participant were always analysed at the same time.

6.3.4.2 Dried blood spots (DBS)

In addition to the DBS obtained at screening (see Section 5.3.4.2), participants were asked to perform weekly finger pricks for 12(±1) weeks to obtain DBS samples for the analysis of blood Phe and Tyr. Participants were provided with pre-labelled filter papers, pre-addressed envelopes with postage and lancets. Participants were verbally instructed on correct blood sampling procedures during their first test session and were provided with written instructions to take home (see Appendix JJ).

6.3.5 Cognitive performance testing

Cognitive performance was re-assessed at the second (T2) and third (T3) test sessions, using the same selected ANT tasks that were used at baseline (T1). A description of the selected test battery and individual tasks can be found in Chapter 5 (see Table 5.6). Where available, different parallel versions of tasks were used across the different test days.

6.3.6 Questionnaires

6.3.6.1 PKU Quality of Life Questionnaire (PKU-QoLQ)

Participants' QoL was re-assessed at each test day using the PKU-QoLQ questionnaire. More information about the construct and scoring of this questionnaire can be found in Section 5.3.7.1.

6.3.6.2 Acceptability and palatability questionnaire

On the final test day (T3), participants were asked to complete a questionnaire regarding the acceptability and palatability of the study product (see Appendix KK). Participants were asked to provide information about their habits regarding the use of the protein substitute (e.g. where they would take the product) and to rate several product attributes (e.g. taste, texture, ease of use). Furthermore, participants were asked to give feedback on the protein substitute.

6.3.7 Study diary

The study diary, assessing adherence to and acceptability of CGMP-AA Protein Substitute was available electronically (Qualtrics©) and required separate entries for each day, which enabled the investigator to keep track of participants completing the study diary in real time. However, participants with limited access to a computer were provided with a paper version of the study diary (see Appendix MM).

A blank section was provided for each day of the study diaries for participants to record any adverse events (AE) or other information they wished to report.

6.3.7.1 Adherence

All semi-adherent ET AwPKU were required to record their intake of CGMP-AA Protein Substitute in the daily study diary. They were asked to provide information on number of sachets prescribed, number of sachets taken, time of day taken and reasons for not taking the required amount, when applicable. Participants were instructed to be honest and as accurate as possible when completing their study diary. They were explained that the aim of the research was to develop a protein substitute that works for them and, hence the importance of reporting of any issues they encountered with regards to adherence to the protein substitutes was emphasised.

6.3.7.2 Acceptability: gastrointestinal (GI) symptoms

In the week leading up to the first test day (week 0), the first week after the introduction of the study product (week 1) and the weeks leading up to the second and third test days (weeks 6 and 12, respectively), participants completed questions on gastrointestinal (GI) wellbeing within the study diary. Participants reported bowel movements using the Bristol Stool Form Scale (Lewis & Heaton, 1997) and scored eleven GI symptoms (subjective ratings for wind, constipation, diarrhoea, feeling bloated, feeling sluggish, stomach pain/cramp, bowel pain/cramp, heartburn, burps, nausea and vomiting) 5-point Likert scale (1=none; 2=minimal; 3=moderate; 4=a lot/very; 5=extreme).

6.3.7.3 Protein intake: 3 day food diary

To assess protein intake at the end of the trial, participants were asked to complete a 3 day food diary (ideally on 2 weekdays and 1 weekend day) in the week leading up to their final test day. Participants were given verbal instructions on how to fill out the 3 day food diary. Furthermore, at the front of the diary (paper version) or at the beginning

of each separate diary entry (electronic version), detailed information on how they should record food and drink consumption using household measures was provided. Study diaries were reviewed by the HARU research dietitian, who analysed the dietary records using nutritional analysis software (Windiets, Research Version, 2010). Foods were analysed using different databases/sources, including UK food tables. Moreover, for specialised low-protein foods and foods not listed in any of the databases, manufacturer's nutritional information from food packets provided by participants or company websites was entered into the Windiets supplementary database and used as appropriate.

6.3.8 Study product

The study product that was provided to the participants for the duration of the intervention (~12 weeks), was a protein substitute specifically developed for semi-adherent ET AwPKU ((≥16 years of age): CGMP-AA Protein Substitute. CGMP-AA Protein Substitute is a Food for Special Medical Purposes (FSMP) and was developed by Vitaflo (International) Ltd. It is a coffee flavoured, powdered, low-Phe supplement containing CGMP-isolate, AA, CHO, fat, vitamins, minerals and DHA. Table 6.5 provides an overview of the nutritional information of CGMP-AA Protein Substitute per 100g and per one sachet (31g). Each sachet provided 15 grams protein equivalent (15g PE).

Each participant's intake was reviewed by a specialized metabolic dietitian to ensure their nutritional needs were met, but all participants were recommended to take two sachets of CGMP-AA Protein Substitute daily. The study product was taken orally. Participants were provided with an information brochure containing information regarding storage and preparation of CGMP-AA Protein Substitute.

Table 6.5 Nutritional composition of CGMP-AA Protein Substitute

		per 100g	per 31g sachet
Energy	kcal	311	96
Protein	g	48.4	15
Phenylalanine	mg	129	40
Carbohydrate	g	19.2	6
Fat	g	4.5	1.4
of which DHA	mg	400	124
Vitamins			
Vitamin A	µg RE	560	174
Vitamin D	µg	26	8.1
Vitamin E	mg αTE	13	4.0
Vitamin C	mg	90	28
Vitamin K	µg	56	17
Thiamin	mg	1.4	0.43
Riboflavin	mg	1.4	0.43
Niacin	mg	8.3	2.6
Niacin equivalents	mg NE	20.3	6.3
Vitamin B6	mg	2.4	0.74
Folic Acid	µg	250	77
Vitamin B12	µg	3.8	1.2
Biotin	µg	32	9.9
Pantothenic acid	mg	4.4	1.4
Choline	mg	480	149
Minerals			
Sodium	mg	910	282
	mmol	39.1	12.1
Potassium	mg	1000	310
	mmol	25	7.8
Chloride	mg	19	5.9
	mmol	0.5	0.2
Calcium	mg	2100	651
Phosphorus	mg	1400	434
Magnesium	mg	300	93

		per 100g	per 31g sachet
Trace Elements			
Iron	mg	28	8.7
Copper	mg	1.5	0.47
Zinc	mg	22	6.8
Manganese	mg	0.7	0.22
Iodine	µg	200	62
Molybdenum	µg	54	17
Selenium	µg	63	19
Chromium	µg	14	4.3
Amino Acids			
L-Alanine	g	2.50	0.78
L-Arginine	g	1.42	0.44
L-Aspartic Acid	g	3.56	1.10
L-Cystine	g	0.05	0.02
L-Glutamine	g	8.18	2.54
Glycine	g	0.42	0.13
L-Histidine	g	1.23	0.38
L-Isoleucine	g	4.30	1.33
L-Leucine	g	8.38	2.60
L-Lysine	g	2.45	0.76
L-Methionine	g	1.16	0.36
L-Phenylalanine	g	0.13	0.04
L-Proline	g	4.85	1.50
L-Serine	g	3.05	0.95
L-Threonine	g	6.93	2.15
L-Tryptophan	g	0.72	0.22
L-Tyrosine	g	5.42	1.68
L-Valine	g	3.47	1.08

Following the baseline visit, each participant was provided with two weeks supply of the study product. Following this, with the consent of the participant, a delivery at home service was set up by Vitaflo. Vitaflo kept participants' contact information safe, secure and confidential and this information was only used for supplying the study product. If it was considered appropriate for the participant to continue taking CGMP-AA Protein Substitute after completing all test sessions, Vitaflo continued supply of the product free of charge and will keep doing so until it becomes available on Advisory Committee on Borderline Substances (ACBS) prescription.

6.3.9 Procedure

Semi-adherent ET AwPKU were required to complete the daily study diary with regards to GI symptoms experienced during the week leading up to their first test session (to obtain a baseline measure). Upon completion of the first test session (see Chapter 5), the semi-adherent ET AwPKU were provided with CGMP-AA Protein Substitute, a study diary for the intervention, and supplies to complete and return weekly DBS (e.g. lancets, filter papers, pre-labelled envelopes). After 6(±1) and 12(±1) weeks, participants returned for their second and third test days, which followed the same protocol as the first test session (see Figure 6.1). On the third test day, participants were asked to complete a short questionnaire on the palatability and ease of use of CGMP-AA Protein Substitute (see Appendix KK). Participants were contacted weekly to check how they were getting on and reminded to complete their study diaries and take blood samples for the DBS.

6.3.10 Ethical considerations

This research was part of the clinical trial reported in Chapter 5 and was approved by the Yorkshire & The Humber - Sheffield REC (16/YH/0273, 15/08/2016), Leeds Teaching Hospitals NHS Trust R&D (20/07/2016) and Salford Royal NHS Foundation Trust R&D (17/10/2016). Because CGMP-AA Protein Substitute is a FSMP, not medication, the research was not classed as a clinical trial for the investigation of a medicinal product (CTIMP).

This research was conducted in accordance with the ethical principles expressed in the Declaration of Helsinki (World Medical Association, 2013) and the principles of GCP (National Institute for Health Research, 2016).

The informed consent of each participant was obtained in writing prior to commencement of the study (see Appendix R). At screening, participants were given written and verbal information about the purpose of the study, all procedures involved, and what was required of them during participation (see Appendix P). Furthermore, with their consent, participants were contacted regularly as a form of follow-up. Patients were asked about adverse events (AE) at these points of contact as well as at the start at each test session. Any AE were recorded in a log (see Appendix LL) and in the case of serious or recurring AE the investigator would contact the study sponsor and metabolic team immediately to evaluate the situation so that appropriate actions could be taken (e.g. discontinuing a patient in the research, treatment, electively stopping the trial). Finally, participants' travel costs were reimbursed.

6.3.11 Statistical analysis

The study diary data from Qualtrics© were exported to Excel. Cognitive test data were extracted from ANT and entered intro Excel. Furthermore, subjective data and results from blood assays were entered into Excel. All data were checked for accuracy prior to analysis with SPSS Version 22. Results are reported using means, standard deviations (SD) and 95% confidence intervals (95% CI). Differences in nutritional status and

measures of QoL/wellbeing across test days were explored using ANOVAs with time (number of weeks relative to baseline; for analysis of DBS) or session (3 levels: test day 1, 2 or 3) as the within subjects factor. Cognitive performance in terms of speed (msec) and accuracy (n errors) on selected ANT tasks was explored using ANOVAs with session (3 levels: test day 1, 2 or 3) as within subjects factor. RT of correct responses (msec) and number of errors (n) on the MS2D task were subject to a 3 (session: test day 1, 2 or 3) x 2 (type of stimulus: low (1 target) vs. high (3 targets) working memory load) ANOVA. Similarly, differences speed of correct responses (msec) and number of errors on part 2 of the FL task and part 3 of the SSV task were assessed using 3 (session: test day 1, 2 or 3) x 2 (type of stimulus: compatible trials vs. incompatible trials) ANOVAs. To assess differences in sustained attention, MST (RT) and number of errors (n) during the first and last 10 series (120 trials) on the SAD task were subject to a 3 (session: test day 1, 2 or 3) x 2 (time: first 120 trials vs. last 120 trials). Motor skills under lower (TR) and higher (PU) controlled processing demands was explored by subjecting the mean deviation of the moving target (accuracy of movement) and the standard deviation (stability of movement) of the trajectory that was followed on both tasks to a 3 (session: test day 1, 2 or 3) x 2 (task: TR vs. PU) ANOVA. Where applicable, post-hoc comparisons were made using the Tukey-Kramer method.

6.4 Results

6.4.1 Participant characteristics

Participant characteristics of the semi-adherent ET AwPKU enrolled in the intervention with CGMP-AA Protein Substitute are summarised in Table 5.7 in Chapter 5 (Section 5.4.1). All participants included in the baseline comparison reported in Chapter 5 completed the 12 week trial with CGMP-AA Protein Substitute. However, one participant missed their second test session and one participant did not attend their final test session. Both participants were asked to complete and return the test day

questionnaires to limit the amount of missing data, which one of them did. Moreover, not all study diaries and DBS were returned.

6.4.1.1 Adverse events (AE)

No serious or recurring AE related to CGMP-AA Protein Substitute were reported. However, one participant broke their wrist between their first and second test session (participant 9) and another participant broke their ankle between their second and third test day (participant 8; this participant did not return for their final test day). These AE are unlikely to be related to the study product as both participants had a history of broken bones.

6.4.2 Acceptability of CGMP-AA Protein Substitute

6.4.2.1 Study diary: GI wellbeing

No AE in relation to consumption of CGMP-AA Protein Substitute were reported on the Bristol Stool Form Scale. Figure 6.2 shows mean self-reported GI symptoms in week 0 (the week leading up to T1), week 1 (the first week using CGMP-AA Protein Substitute) and weeks 6 and 12 (the weeks leading up to T2 and T3). On an individual level, some participants experienced minimal to moderate wind, but overall CGMP-AA Protein substitute did not seem to negatively affect GI symptoms of participants on the trial. One participant with inflammatory bowel disease (IBD) reported her IBD symptoms decreased throughout the trial.

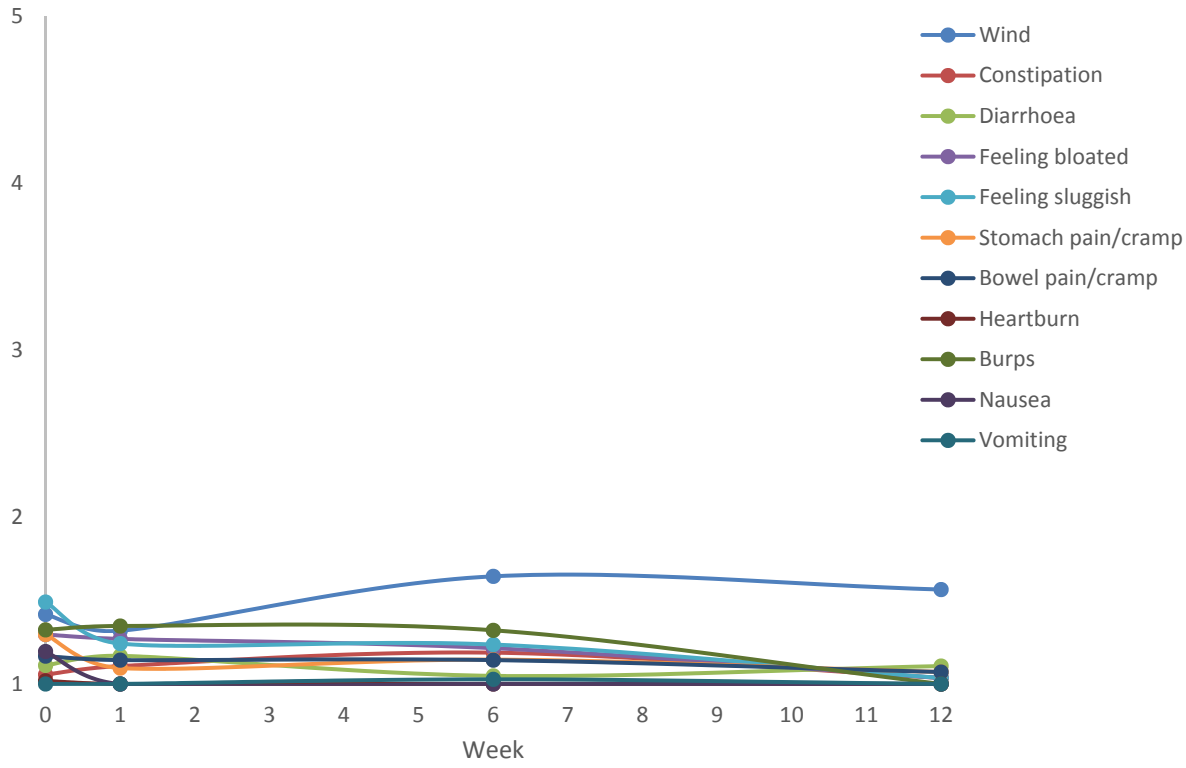


Figure 6.2 Mean self-reported GI symptoms at baseline (week 0) and during week 1, 6, and 12 (1=none; 2=minimal; 3=moderate; 4=a lot/very; 5=extreme)

Notes: Study diary data missing for participants 4 (only completed baseline + first 4 weeks) and 8 (only completed first 6 weeks).

6.4.2.2 Feedback: acceptability questionnaire

Mean ratings of attributes of CGMP-AA Protein Substitute are shown in Figure 6.3 (for individual ratings, see Appendix V). Participants especially liked the taste of the study product. Furthermore, participants reported they found taking the CGMP-AA Protein Substitute for the duration of the trial quite easy and thought the convenience of the study product was acceptable (see Figure 6.3). In addition, seven of the semi-adherent ET AwPKU found CGMP-AA Protein Substitute easier to use than their normal or previous protein substitutes, two reported the ease of use was similar and one found their normal substitutes easier to use because they were ready to drink and had a better texture.

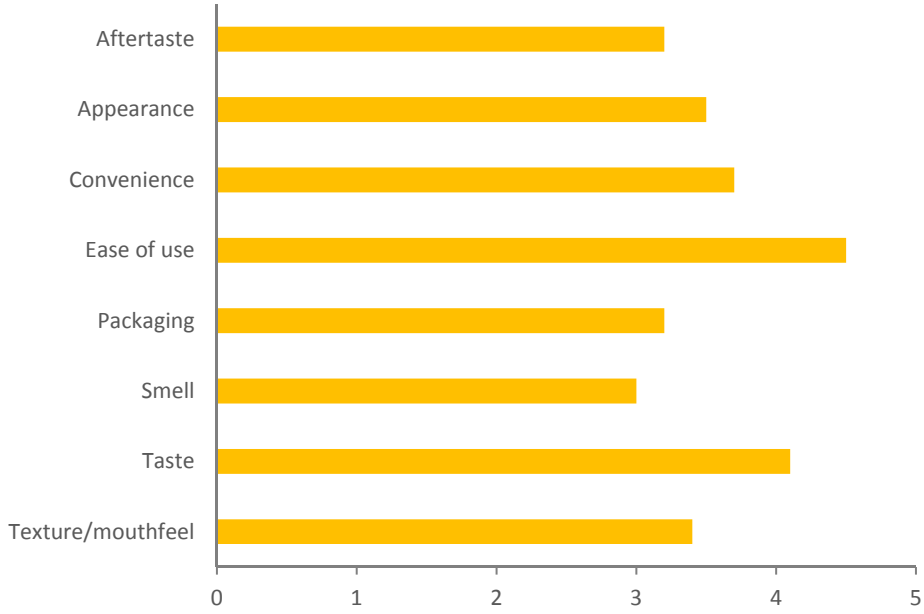


Figure 6.3 Mean ratings of attributes of CGMP-AA Protein Substitute

Key: for convenience: 1=very inconvenient; 2=inconvenient; 3=OK; 4=convenient; 5=very convenient; For ease of use/taking it: 1=very difficult; 2=difficult; 3=OK; 4=easy; 5=very easy; For all other attributes: 1=I really did not like it!!; 2=I did not like it!; 3=Neither liked or disliked it; 4=I liked it!; 5=I loved it!!)

Appendix W shows the additional qualitative feedback provided in free-text responses to the acceptability questionnaire. Participants particularly liked the taste and the absence of a "horrible bitter" aftertaste. They commented that they liked that the flavour was aimed at a more mature palate, although not everyone was a fan of the coffee flavour. Furthermore, participants liked the fact that they were only required to take CGMP-AA Protein Substitute twice a day (morning and evening) and commented that this "made it a lot easier to adhere to". In addition, several of the semi-adherent ET AwPKU mentioned taking CGMP-AA Protein Substitute did not upset their stomach or make them feel sick or bloated and patients reported having more energy and an improved mood compared to the start of the trial (see Appendix W). One patient said the study product "helped improve my control of PKU". The main issues with CGMP-AA Protein Substitute were problems with texture, i.e. the protein substitute was sometimes notes as being a bit "bitty" as a result of issues with dissolving the powdered sachets. One of the participants also mentioned they did not find the powdered sachets

convenient. Several (n=2) of the participants said it would be good if CGMP-AA Protein Substitute could be RTD. In addition, they said it would be nice to have a variety of flavours.

6.4.2.3 Continued product usage

One participant, who particularly struggled with the texture (i.e. issues with dissolving the powder; lumps) and inconvenience (not RTD) of CGMP-AA Protein Substitute discontinued taking the protein substitute upon completion of their final test day. All other participants chose to continue taking CGMP-AA Protein Substitute after the trial.

6.4.3 Adherence to CGMP-AA Protein Substitute (study diary)

Figure 6.4 provides an overview of average self-reported weekly adherence to CGMP-AA Protein Substitute during the trial. Average self-reported adherence between T1 and T2 was similar to adherence reported between T2 and T3 (see Table 6.6).

Overall average self-reported adherence was high (81.32%). However, one participants' average self-reported adherence was below 67% (20g PE) and two participants did not return 50% or more of their study diary. Two participants reported (near) 100% adherence throughout the trial (participants 5 and 9). Main reasons for in-adherence were related to lifestyle and failure to plan accordingly: participant 10 forgot to take their substitute during a week away with work; participant 2 reported she temporarily stopped taking the CGMP-AA Protein Substitute because they were too overwhelmed with other things happening in their life; participant 3 was on holiday in week 7. Participant 1 struggled with the texture of the substitute.

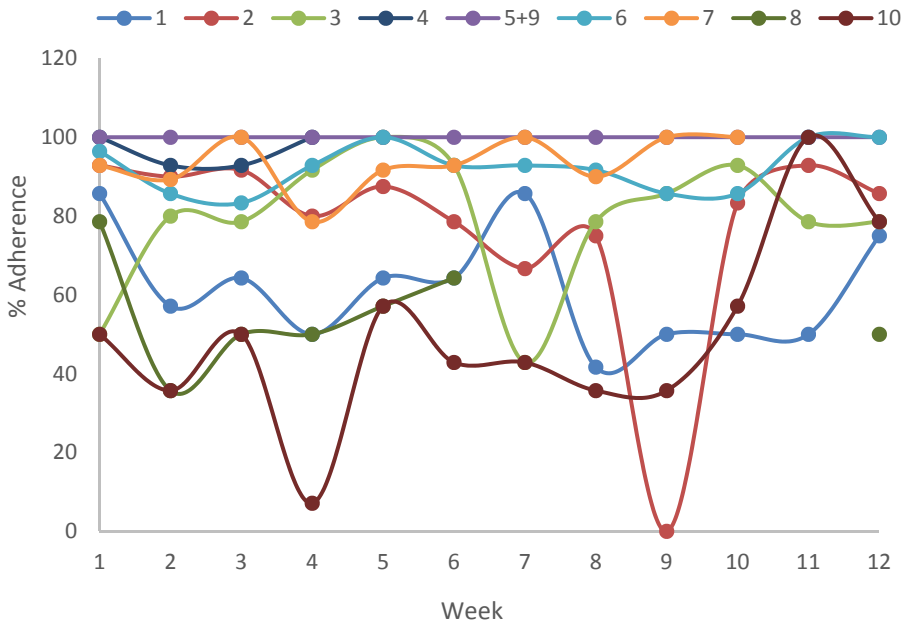


Figure 6.4 Average weekly self-reported adherence (%) to CGMP-AA Protein Substitute for all semi-adherent ET AwPKU on the trial

As is illustrated by Figure 6.4, (self-reported) adherence to CGMP-AA Protein Substitute varied widely amongst participants. To better explore the effects of CGMP-AA Protein Substitutes, results related to nutritional status, QoL and cognitive performance are reported for the whole sample and for participants with an overall average adherence of ≥67% (20g PE).

Table 6.6 Average self-reported adherence between T1 and T2, and T2 and T3

	Average self-reported adherence (%)		
Participant	**T1-T2**	**T2-T3**	**Overall (T1-T3)**
1	64.29	58.73	61.51
2	86.77	67.26	77.01
3	82.18	76.19	79.19
4	96.43	n/a	n/a
5	100.00	100.00	100.00
6	91.87	92.66	92.26
7	90.87	97.50	94.19
8	55.95	n/a	n/a
9	100.00	96.43	98.21
10	40.48	58.33	49.40
Average (whole sample)	80.84	80.76	81.32
Average (subsample)	88.01	88.34	90.14

Note: n/a = study diary not sufficiently completed/not returned; highlighted rows show participants with average adherence ≥67%

Figure 6.5 shows the overall average adherence (%) to CGMP-AA Protein Substitute for the whole sample and the subsample with an overall average adherence of ≥67% (n=6).

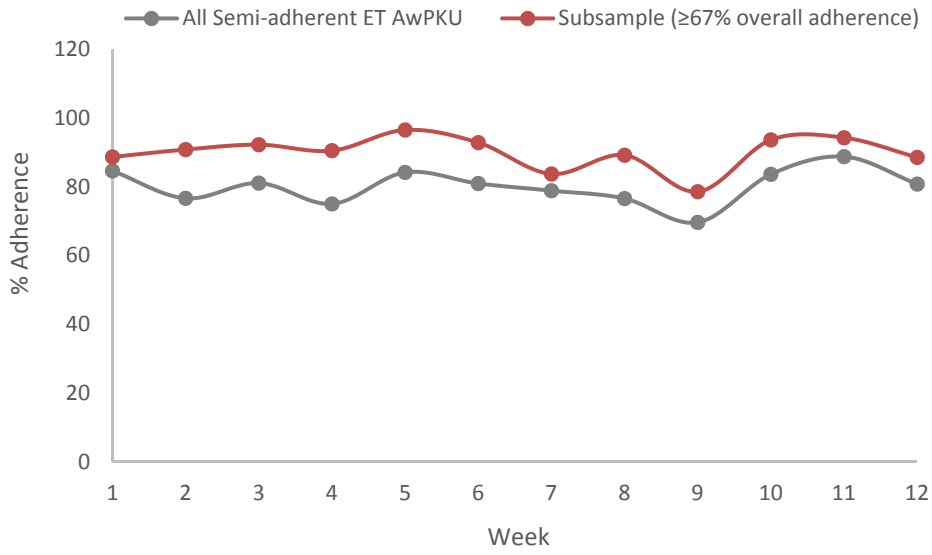


Figure 6.5 Overall average weekly self-reported adherence (%) to CGMP-AA Protein Substitute for all semi-adherent ET AwPKU on the trial and a subsample (those with ≥67% overall adherence)

Table 6.7 provides an overview of participant characteristics (obtained at screening) of the participants with an overall self-reported adherence to CGMP-AA Protein Substitute of ≥67% and <67%. Those with lower overall average adherence to the study product and procotol (2 participants did not return sufficient study diary data to calculate overall adherence to CGMP-AA Protein Substitute), were significantly younger in age. Furthermore, they (non-significantly(higher Phe and Tyr levels at the time of screening and a higher full scale IQ (see Table 6.7).

Table 6.7 Participant characteristics (obtained at screening) of semi-adherent ET AwPKU with a self-reported overall adherence to CGMP-AA Protein Substitute of ≥/<67%

		Overall average adherence		
		≥67%	**<67%**	
n (female)		6 (4)	4 (2)	*X2*(1)=.28, *p*=.60
age (years)	*mean (SD)*	37.40 (10.39)	24.84 (5.15)	*t*(8)=-2.21, *p*=.06
	range	24.21-53.84	19.51-29.39	
Phe (µmol/L)	*mean (SD)*	1115 (119.45)	1213.25 (211.45)	*t*(8)=.61, *p*=.56
	range	1039-1278	1027-1489	
Tyr (µmol/L)	*mean (SD)*	49.00 (42.00)	67.25 (42.03)	*t*(8)=.67, *p*=.52
	range	19-117	30-108	
Phe:Tyr	*mean (SD)*	38.49 (21.85)	27.27 (19.84)	*t*(8)=-.82, *p*=.43
	range	8.96-61.29	9.51-46.53	
Protein intake	*mean (SD)*	0.66 (0.07)	0.66 (0.05)	*t*(8)=-.06, *p*=.95
(g/kg/day)	*range*	0.59-0.74	0.59-0.69	
IQ	*mean (SD)*	116.58 (16.97)	128.33 (8.14)	*t*(8)=1.109, *p*=.30
	range	92-136	119-134	

6.4.4 Protein intake (3 day food diary)

Five participants returned their 3 day food diaries at the end of the 12(±1) week intervention. Natural dietary protein intake, intake from protein substitutes (MF in the table) and total protein at baseline (T1) and end of study (T3) are displayed in Table 6.8.

Table 6.8 Protein intake at baseline (T1) and end of study (T3)

Ppt	Baseline (T1)					End of study (T3)				
	Natural protein	MF	Total protein	Body weight	Protein intake	Natural protein	MF	Total protein	Body weight	Protein intake
	g	*g*	*g*	*kg*	*g/kg*	*g*	*g*	*g*	*kg*	*g/kg*
1	30	20	50	85.1	0.59	46	15	61	83.3	0.73
2	46	0	46	68.2	0.67	20.5	30	50.5	71.8	0.70
3	42	0	42	70.9	0.59	36	15	51	65.8	0.81
4	52	0	52	83.2	0.63				83.8	
5	28	40	68	98.5	0.69				98.5	
6	40	30	70	94.4	0.74	53	30	83	94.3	0.88
7	15	40	55	93.6	0.59	33	70	103	89.6	1.15
81	15	30	45	68	0.66				.	
9	30	20	50	67.2	0.74				66.2	
10	20	30	50	72	0.69				71.1	

Notes: [1] Missed final test session (T3); **Key**: MF: Medical food; Ppt: participant

Three of the five participants who returned their 3-day food diaries had a satisfactory protein intake (>0.75g/kg/day) at the end of the 12 week trial. The remaining two semi-adherent ET AwPKU had a total protein intake just below the RDI (0.70 and 0.73 g/kg/day).

6.4.5 Nutritional status

Table 6.9 gives an overview of mean (SD, 95% CI) concentrations of (micro)nutrients, assessed in blood samples from semi-adherent ET AwPKU (see Appendix X for results of the subsample of semi-adherent ET AwPKU with an average self-reported adherence to CGMP-AA Protein Substitute of ≥67%). The reference range for each measure is provided. Nutritional status at baseline and changes in nutritional status throughout the trial varied between participants.

Results are missing for three participants at T2 (n=2 unable to obtain blood sample, n=1 did not attend test session) and two participants at T3 (n=1 unable to obtain blood sample, n=1 did not attend test session). No measures of nutritional status are missing in the subsample (n=6).

Table 6.9 Mean (SD, 95% CI) measures of nutritional status of semi-adherent ET AwPKU at each test session (n=10)

	Reference range (µmol/L)	T1			T2			T3			
		mean	SD	95% CI	mean	SD	95% CI	mean	SD	95% CI	*F*, *p* and η_p^2 (session)
Albumin† (g/L)	35-50	44.10	5.63	40.08-48.12	40.57	3.36	37.46-43.68	40.13	4.88	36.04-44.21	*F*(2,22)= 1.82, *p*=.19, η_p^2=.14
Pre-albumin† (g/L)	0.20-0.50	0.26	0.04	0.23-0.29	0.28	0.05	0.24-0.33	0.30	0.02	0.28-0.32	*F*(2,21)=2.87, *p*=.08, η_p^2=.22
Transferrin† (g/L)	2.00-3.20	2.52	0.37	2.26	2.79	2.57	0.71	1.91	3.23	2.22	*F*(2,21)=1.09, *p*=.36, η_p^2=.09
Alkaline Phosphatase† (U/L)	30-130	78.30	13.55	68.61-87.99	71.71	19.22	53.93-89.49	59.75*	12.44	49.35-70.15	*F*(2,22)=3.43, *p*=.05, η_p^2=.24
Calcium† (mmol/L)	2.20-2.60	2.32	0.17	2.20-2.44	2.20	0.20	2.02-2.39	2.29	0.26	2.07-2.51	*F*(2,22)=.66, *p*=.53, η_p^2=.06
Copper‡ (µmol/L)	11-22	17.00	5.78	12.86-21.14	18.74	9.43	10.02-27.46	18.36	6.27	13.13-23.60	*F*(2,22)=.15, *p*=.87, η_p^2=.01
Homocysteine‡ (µmol/L)	<18	9.89	3.41	7.27-12.51	10.50	3.39	6.94-14.06	9.29	3.09	6.42-12.15	*F*(2,19)=.22, *p*=.81, η_p^2=.02
Iron† (µmol/L)	14-31 (♂)	21.68	11.27	3.74-39.61	14.40	2.97	-12.28-41.08	18.83	6.75	2.07-35.59	*F*(2,6)=.44, *p*=.66, η_p^2=.13
	11-29 (♀)	15.23	4.85	10.14-20.33	13.34	3.61	8.85-17.83	17.86	3.15	13.95-21.77	*F*(2,13)=1.60, *p*=.24, η_p^2=.20
Phosphate† (mmol/L)	0.80-1.50	1.06	0.14	0.96-1.16	1.08	0.12	0.96-1.19	0.95	0.20	0.79-1.11	*F*(2,22)=1.57, *p*=.23, η_p^2=.13
Parathyroid hormone‡ (PTH) (pmol/L)	1.50-7.60	3.96	1.33	2.93-4.98	5.04	1.95	3.24-6.85	4.51	2.66	2.29-6.74	*F*(2,21)=.57, *p*=.58, η_p^2=.05
Selenium‡ (µmol/L)	0.80-2.00	0.92	0.13	0.83-1.01	0.99	0.20	0.80-1.17	1.04	0.18	0.89-1.19	*F*(2,22)=1.16, *p*=.33, η_p^2=.10
Vitamin A† (µmol/L)	1.05-3.39	2.00	0.60	1.37-2.63	1.74	0.62	0.97-2.51	2.63	0.87	1.72-3.55	*F*(2,21)=2.83, *p*=.08, η_p^2=.21
Vitamin B12† (ng/L)	211-911	403.56	134.89	299.87-507.24	475.17	208.68	256.17-694.16	452.71	185.00	281.62-623.81	*F*(2,19)=.34, *p*=.72, η_p^2=.04
Vitamin D† (nmol/L)	>75	**57.47**	37.97	28.28-86.65	**73.08**	45.30	25.54-120.62	**71.54**	36.52	37.76-105.32	*F*(2,19)=.37, *p*=.69, η_p^2=.04
Vitamin E† (µmol/L)	12-42	22.73	6.84	15.56-29.91	19.54	5.58	12.61-26.47	25.53	5.97	19.27-31.80	*F*(2,14)=1.28, *p*=.31, η_p^2=.15
Zinc‡ (µmol/L)	9.80-17.90	10.33	3.36	7.92-12.74	9.39	2.30	7.26-11.52	9.76	1.56	8.45-11.07	*F*(2,22)=.28, *p*=.76, η_p^2=.03

Notes: †serum; ‡plasma; *p<.05; **p<.01; ***p<.001 (Tukey post hoc; compared to T1); highlighting shows reference ranges and (group) deficiencies

6.4.5.1 Protein status: albumin, pre-albumin, and transferrin

Analysis revealed no main effects of session on plasma levels of albumin, pre-albumin or transferrin (see Table 6.9 and Appendix X).

Table 6.10 Overview of number of observed deficiencies in measures of protein status in semi-adherent ET AwPKU at T1 (n=10) and T3 (n=8)

	T1 (n=10)	T3 (n=8)			
	Deficiencies (n)	Deficiencies (n)			No longer deficient (n)
		Enduring	*New*	*Total*	
Albumin	1	0	1	1	1
Pre-albumin	0[1]	n/a	0	0	n/a
Transferrin	1	0	2	2	1

Notes: [1] level at T1 unknown for n=1

6.4.5.2 Micronutrients and related measures

6.4.5.2.1 C-reactive protein (CRP)

Two participants had elevated CRP levels at the time of each test session. The participant that had a broken wrist at the time of the second test session had a particularly high concurrent CRP level. Furthermore, CRP was elevated in one additional participant at T2 and in another participant at T1. These participants had both reported to have been ill during the week leading up to these test sessions.

There were no significant main effects of session on any of the nutritional measures (see Table 6.9 and Appendix X). Alkaline phosphatase levels reduced over time but the effect of session just failed to reach significance (p=0.051). Although overall vitamin D levels seemed to increase during the 12 week trial, mean vitamin D levels of the whole sample were below the reference range at all three test sessions. Mean vitamin D levels of the subsample with ≥67% self-reported adherence to CGMP-AA Protein Substitute were within reference range at T2 and T3, albeit at the lower end (see Appendix X).

Table 6.11 provides an overview of frequency of observed deficiencies at the start (baseline; T1) and end (T3) of the 12(±1) week trial. In addition to results reported in the table, on participant's homocysteine levels were slightly elevated at T1, but they were within the reference range at T3. Moreover, three participants had copper levels above

the reference range at T3, whereas only one of these participants had elevated levels at T1. Finally, the participant with an elevated iron level at baseline (T1) had a serum iron concentration within the reference range at the end of the trial (T3).

Table 6.11 Overview of number of observed deficiencies in micronutrients and related measures in semi-adherent ET AwPKU at T1 (n=10) and T3 (n=8)

	T1 (n=10)	T3 (n=8)			
	Deficiencies (n)	Deficiencies (n)			No longer deficient (n)
		Enduring	*New*	*Total*	
Alkaline Phosphatase	n/a	n/a	n/a	n/a	n/a
Calcium	2[1]	0	1	1	1
Copper	1	1	n/a	1	1
Homocysteine	0	n/a	n/a	0	n/a
Iron	4[3]	1	n/a	1	1
Phosphate	0	n/a	2	2	n/a
PTH	0	n/a	1	1	n/a
Selenium	2	0	n/a	0	2
Vitamin A	1[2]	n/a	2	2	n/a
Vitamin B12	0	n/a	n/a	0	n/a
Vitamin D	7[3]	5	1	6	n/a
Vitamin E	1[2]	n/a	n/a	n/a	n/a
Zinc	5[1]	4	1	5	0

Notes: [1] level at T3 unknown for n=1; [2] level unknown at T3; [3] level at T3 unknown for n=2

6.4.5.3 Full amino acid profile

Table 6.12 gives an overview of mean (SD, 95% CI) concentrations of (micro)nutrients, assessed in blood samples from semi-adherent ET AwPKU (see Appendix Y for results of the subsample). As a group, participants showed deficiencies in His, Lys, Asp, Glu, Orn and Pro across all test sessions. However, average His and Lys concentrations appeared to be slightly improved at T3 compared to T1. Finally, average Tyr levels were below the reference range at T1 and T3. Results from the full AA profile assays at all test days as well as results from the weekly DBS, which were analysed for Phe and Tyr levels, are described in more detail below (see Table 6.12).

Table 6.12 Mean (SD, 95% CI) amino acid concentrations of semi-adherent ET AwPKU at each test session (n=10)

	Reference range (µmol/L)	T1			T2			T3			
		mean	SD	95% CI	mean	SD	95% CI	mean	SD	95% CI	***F*, *p* and η_p^2 (session)**
Alanine (Ala)	248-778	263.33	76.06	204.87-321.80	267.14	67.96	204.29-330.00	258.13	35.41	228.52-287.73	*F*(2,22)=.05, p=.96, η_p^2<.001
Arginine (Arg)	30-198	43.33	15.45	31.46-55.21	38.86	9.70	29.88-47.83	44.13	12.36	33.79-54.46	*F*(2,22)=.35, p=.71, η_p^2=.03
Asparagine (Asn)	35-128	38.67	11.14	30.11-47.23	45.14	12.36	33.71-56.58	43.75	7.23	37.71-49.79	*F*(2,22)=.68, p=.52 η_p^2=.06
Aspartic acid (Asp)	45-125	**9.30**	7.97	3.60-15.00	**11.40**	3.78	6.70-16.10	**11.57**	3.46	8.37-14.77	*F*(2,22)=.004, p=1.00, η_p^2<.001
Citrulline (Cit)	13-52	31.56	9.32	24.40-38.72	33.00	8.76	24.90-41.10	34.00	7.41	27.81-40.19	*F*(2,22)=.16, p=.86, η_p^2=.01
Cysteine (Cys)	17-124	40.67	8.70	33.98-47.36	36.71	12.01	25.61-47.82	44.75	7.52	38.47-51.03	*F*(2,22)=1.41, p=.27, η_p^2=.11
Glutamate (Glu)	46-428	**39.22**	17.20	26.00-52.45	**41.43**	25.77	17.59-65.27	**35.75**	14.87	23.32-48.18	*F*(2,22)=.19, p=.83, η_p^2=.02
Glutamine (Gln)	270-1159	433.00	99.21	356.74-509.26	458.86	102.23	364.31-553.41	472.13	94.64	393.01-551.24	*F*(2,22)=.37, p=.69, η_p^2=.03
Glycine (Gly)	185-552	244.89	99.25	168.60-321.18	248.86	129.46	129.13-368.59	248.13	108.21	157.66-338.59	*F*(2,22)=.009, p=.99, η_p^2<.001
Histidine (His)†	81-193	**65.22**	14.70	53.92-76.52	**65.14**	12.29	53.77-76.51	**73.38**	10.78	64.36-82.39	*F*(2,22)=1.13, p=.34, η_p^2=.09
Isoleucine (Iso)†	35-127	49.22	15.62	37.22-61.23	50.00	22.34	29.34-70.66	47.63	13.76	36.12-59.13	*F*(2,22)=.04, p=.96, η_p^2<.001
Leucine (Leu)†	80-229	102.00	21.21	85.69-118.31	101.00	33.72	69.81-132.19	103.13	25.30	81.97-124.28	*F*(2,22)=.01, p=.99, η_p^2<.001
Lysine (Lys)†	165-378	**135.67**	30.78	112.01-159.33	**126.00**	33.47	95.05-156.95	**147.13**	29.66	122.33-171.92	*F*(2,22)=.90, p=.42, η_p^2=.08
Methionine (Met)†	9-52	13.22	4.18	10.01-16.43	13.71	4.31	9.73-17.70	14.25	3.41	11.40-17.10	*F*(2,22)=.05, p=.95, η_p^2<.001
Ornithine (Orn)	117-279	**41.33**	17.30	28.04-54.63	**36.29**	17.48	20.12-52.45	**42.00**	8.88	34.58-49.42	*F*(2,22)=.34, p=.72, η_p^2=.03
Phenylalanine (Phe)†	120-7001	**1114.00**	198.12	972.27-1255.73	**1102.00**	172.81	942.18-1261.82	**1257.00**	241.47	1055.12-1458.88	*F*(2,22)=1.34, p=.27, η_p^2=.11
Proline (Pro)	123-451	**112.33**	36.70	84.12-140.54	**109.86**	49.15	64.40-155.31	**106.63**	24.80	85.90-127.35	*F*(2,21)=.05, p=.95, η_p^2=..005
Serine (Ser)	68-256	89.22	23.55	71.12-107.33	92.43	35.88	59.24-125.62	85.25	23.22	65.83-104.67	*F*(2,22)=.14, p=.87, η_p^2=.01
Taurine (Tau)	80-344	87.44	70.47	33.28-141.61	88.57	36.78	54.56-122.59	80.88	19.84	64.29-97.46	*F*(2,22)=.05, p=.95, η_p^2<.001
Threonine (Thr)†	60-231	88.11	26.31	67.89-108.33	134.86*	41.60	96.38-173.33	116.38	27.28	93.57-139.18	***F*(2,22)=5.01, p=.02, η_p^2=.31**
Tyrosine (Tyr)‡	57-110	**47.00**	20.56	31.20-62.80	57.14	33.87	25.82-88.47	**48.88**	19.19	32.83-64.92	*F*(2,22)=.45, p=.64, η_p^2=.04
Valine (Val)†	117-359	212.56	45.54	177.55-247.56	196.86	63.51	138.12-255.59	195.38	26.51	173.21-217.54	*F*(2,22)=.21, p=.82, η_p^2=.02

Notes: [1] recommended reference range for AwPKU in the UK prior to implementation of new European guidelines (van Wegberg et al., 2017); †Essential Amino Acid; ‡Conditionally Essential Amino Acid (in PKU); *p<.05; **p<.01; ***p<.001 (Tukey post hoc; compared to T1); highlighting shows reference ranges and (group) deficiencies

6.4.5.3.1 Phenylalanine, tyrosine and phenylalanine/tyrosine ratio

Participants 1, 2, 3, 5, 6 and 7 returned >50% of their DBS (most returned >80%). Analysis revealed no main effect of time (weeks relative to baseline) on levels of Phe or Tyr or participants' Phe:Tyr ratio.

Overall, plasma Phe levels were slightly higher at T3 compared to T1. No main effect of session was observed for either the whole sample (see Table 6.12) or the subsample (Appendix Y). As expected, all participants had Phe levels above 700 μmol/L throughout the trial. Furthermore, analysis revealed no main effect of session on Tyr concentrations for all semi-adherent ET AwPKU (see Table 6.12) or the subsample (Appendix Y). Only two of the semi-adherent ET AwPKU had Tyr levels within reference ranges at T1. One of these participants was deficient in Tyr at T2, but their levels were within target range at the end of the intervention (T3). The other participant's blood could not be obtained at T2 and T3. Tyr levels of three participants, who were previously (T1) found to be deficient in Tyr, were within target range at T2, but one of these participants was found to be deficient in Tyr at T3 (one participant's bloods could not be obtained at T3). Of the remaining five participants who had deficient Tyr levels at T1, four were still deficient at T3 (one participant did not attend their final test session). There was no main effect of session on Phe:Tyr ratio.

6.4.5.3.2 (Other) essential amino acids

There was a main effect of session on levels of Thr (see Table 6.12), such that average Thr levels were significantly increased at T2 compared to T1 (p=.01). Mean Thr levels were also higher at T3 than T1 (see Table 6.12), but post hoc comparisons revealed no significant differences between sessions. None of the semi-adherent ET AwPKU showed deficiencies in Thr at the time of any of the test sessions.

No significant main effects of session on levels of His, Iso, Leu, Lys, Met or Val were observed (see Table 6.12 for whole sample and Appendix Y for the subsample).

Although, in some participants, concentrations of EAA tended to improve, the majority of these participants still showed deficiencies in one or more EAA at T3 (see

Table 6.13). Of the nine semi-adherent ET AwPKU who were deficient in His at baseline (T1), seven showed deficient His levels at T3 (n=2 unknown). The participant that was deficient in Met, Iso and Leu at baseline was no longer deficient in these EAA at T3. The other semi-adherent ET AwPKU with deficient Iso and Leu levels at T1 still showed deficiencies in both EAA at T3. Furthermore, of the eight participants who showed deficiencies in Lys at baseline, one had a concentration of Lys within the reference range at T3 (n=1 unknown). None of the semi-adherent ET AwPKU showed deficiencies in Val or Thr throughout the research.

Table 6.13 Overview of number of observed deficiencies in EAA in semi-adherent ET AwPKU at T1 (n=10) and T3 (n=8)

	T1 (n=10)	**T3 (n=8)**			
	Deficiencies (n)	**Deficiencies (n)**			**No longer deficient (n)**
		Enduring	***New***	***Total***	
Histidine (His)	9	7	n/a	7	n/a
Isoleucine (Iso)	2	1	n/a	1	1
Leucine (Leu)	2	1	n/a	1	1
Lysine (Lys)	8	6	n/a	6	1
Methionine (Met)	1	0	n/a	0	1
Threonine (Thr)	0	n/a	n/a	0	n/a
Valine (Val)	0	n/a	n/a	0	n/a

6.4.5.3.3 Non-essential amino acids

Analysis revealed no main effect of session on concentrations of any of the non-essential AA (see Table 6.12 for all participants and Appendix Y for results of the subsample). Table 6.14 provides an overview of the number of participants with deficient levels of all non-essential AA that were analysed as part of the full AA profile. Although some participants' levels improved during the trial, deficiencies were still observed, mainly in Asp, Glu, Orn and Pro.

Table 6.14 Overview of number of observed deficiencies in non-essential AA in semi-adherent ET AwPKU at T1 (n=10) and T3 (n=8)

	T1 (n=10)	T3 (n=8)			
	Deficiencies (n)	Deficiencies (n)			No longer deficient (n)
		Enduring	*New*	*Total*	
Alanine (Ala)	4	3	n/a	3	1
Arginine (Arg)	2	n/a	1	1	2
Asparagine (Asn)	2	n/a	1	1	2
Aspartic acid (Asp)	10	7	n/a	7	1
Citrulline (Cit)	0	n/a	n/a	0	n/a
Cysteine (Cys)	0	n/a	n/a	0	n/a
Glutamate (Glu)	6	4	1	5	2
Glutamine (Gln)	11	n/a	n/a	0	n/a
Glycine (Gly)	3	2	n/a	2	1
Ornithine (Orn)	10	8	n/a	8	n/a
Proline (Pro)	6	5	12	6	1
Serine (Ser)	2	1	n/a	1	1
Taurine (Tau)	8	4	n/a	4	2

[1] Gln level at T3 unknown; [2] Pro level at T1 unknown

6.4.6 Quality of Life (QoL)

One participant missed their second test session and did not complete the PKU-QoLQ that was sent out to them. Results of PKU-QoLQ samples for the whole sample (n=10) and subsample (n=6) are reported below.

6.4.6.1 Self-rated health and symptoms

Table 6.15 provides an overview of mean (SD, 95% CI) PKU-QoLQ scores for self-rated health and PKU-related symptoms for all semi-adherent ET AwPKU across all three test sessions. Mean (SD, 95% CI) PKU-QoLQ health and symptom scores for the subsample can be found in Appendix Z.

6.4.6.1.1 Self-rated health

Overall self-rated health improved after introduction of CGMP-AA Protein Substitute, but analysis did not reveal a main effect of session (see Table 6.15). Furthermore, observed changes in self-reported health in the subsample were not significant (Appendix Z).

6.4.6.1.2 PKU-related symptoms

Analysis revealed a significant main effect of session on PKU-QoLQ scores for anxiety, lack of concentration, sadness, slow thinking, tiredness, and trembling hands (see Table 6.15). Post-hoc analysis revealed that, at the second test session, scores for anxiety ($p=.02$), lack of concentration ($p=.03$), sadness ($p=.03$), slow thinking ($p=.02$), tiredness ($p=.03$), and trembling hands ($p=.03$) had significantly improved compared to the first test session. PKU-QoLQ scores for these symptoms were also better at T3 compared to T1, but observed differences were only significant for scores of anxiety ($p=.005$), lack of concentration ($p=.04$), slow thinking ($p=.009$), and tiredness ($p=.04$). Self-reported ratings of moodiness tended to improve after introduction of CGMP-AA Protein Substitute, but the effect of session failed to reach significance (see Table 6.16). Furthermore, self-reported scores for aggressiveness, headaches, irritability, and stomach aches improved slightly after the first test session (T1), but no main effect of session was observed for any of these PKU-related symptom scores (see Table 6.15). Most symptom scores tended to improve from T1 to T2, but would then increase again slightly between T2 and T3 (e.g. scores for sadness). Only scores for anxiety continued to improve after T2.

Table 6.15 Mean (SD, 95% CI) self-reported health and symptom PKU-QoLQ scores for each test session (n=10)

	T1			T2			T3			T3 vs. T11			
	Mean	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	**+**	**-**	**=**	***F*, *p* and η_p^2 (session)**
Self-rated health	62.50	6.72	47.30-77.70	44.44	5.56	31.63-57.26	47.22	5.01	35.67-58.77	6	1	3	*F*(2,26)=2.92, *p*=.07, ηp2=.18
Aggressiveness	15.00	6.67	-0.08-30.08	5.56	5.56	-7.26-18.37	13.89	8.45	-5.59-33.37	2	1	7	*F*(2,26)=.51, *p*=.61, η_p^2=.04
Anxiety	65.00	10.00	42.38-87.62	**30.56***	6.94	14.54-46.57	**19.44****	5.56	6.63-32.26	8	1	1	***F*(2,26)=6.92, *p*=.004, η_p^2=.35**
Headaches	37.50	10.70	13.29-61.71	19.44	8.10	0.77-38.12	19.44	8.10	0.77-38.12	6	1	3	*F*(2,26)=1.55, *p*=.23, η_p^2=.11
Irritability	50.00	9.13	29.35-70.65	16.67	4.17	7.06-26.28	27.78	5.01	16.23-39.33	6	1	3	*F*(2,26)=.51, *p*=.61, η_p^2=.04
Lack of concentration	55.00	8.16	36.53-73.47	**27.78***	6.51	12.76-42.80	**30.56***	6.94	14.54-46.57	7	1	2	***F*(2,26)=4.63, *p*=.02, η_p^2=.26**
Moodiness	57.50	7.50	40.53-74.47	33.33	7.22	16.69-49.98	25.00	8.33	5.78-44.22	6	1	3	*F*(2,26)=2.73, *p*=.08, η_p^2=.17
Sadness	47.50	4.49	37.35-57.65	**22.22***	5.83	7.20-37.24	25.00	7.22	8.36-41.64	6	1	3	***F*(2,26)=3.86, *p*=.03, η_p^2=.23**
Slow thinking	45.00	8.98	24.70-65.30	**16.67***	4.17	7.06-26.28	**16.67****	5.89	3.08-30.25	6	0	4	***F*(2,26)=6.48, *p*=.005, η_p^2=.33**
Stomach aches	17.50	9.17	-3.24-38.24	11.11	7.35	-5.84-28.06	13.89	8.45	-5.59-33.37	2	2	6	*F*(2,26)=.19, *p*=.83, η_p^2=.01
Tiredness	80.00	6.24	65.89-94.11	**52.78***	6.51	37.76-67.80	**55.56***	9.11	34.55-76.56	5	0	5	***F*(2,26)=4.63, *p*=.02, η_p^2=.26**
Trembling hands	45.00	11.90	24.70-65.30	**16.67***	4.17	7.06-26.28	16.67	6.94	3.08-30.25	5	1	4	***F*(2,26)=3.65, *p*=.04, η_p^2=.22**

Notes: [1] + improved; - worsened; = unchanged; ? unknown; *p<.05; **p<.01; ***p<.001 (Tukey post hoc; compared to T1)

Analysis of the subsample of semi-adherent ET AwPKU who had an average self-reported adherence to CGMP-AA Protein Substitute of at least 67%, revealed a significant main effect of session on PKU-QoLQ scores for anxiety ($F(2,15)=16.95$, $p<.001$, $\eta_p^2=.69$), lack of concentration ($F(2,15)=4.59$, $p=.03$, $\eta_p^2=.38$), sadness ($F(2,15)=6.45$, $p=.01$, $\eta_p^2=.46$), tiredness ($F(2,15)=4.29$, $p=.03$, $\eta_p^2=.36$), and trembling hands ($F(2,15)=4.57$, $p=.03$, $\eta_p^2=.38$), but not slow thinking (see Appendix Z). Additionally, a significant main effect of session on scores for headaches ($F(2,15)=6.89$, $p=.008$, $\eta_p^2=.48$) and moodiness ($F(2,15)=17.50$, $p<.001$, $\eta_p^2=.70$) was observed in the subsample of semi-adherent ET AwPKU (see Appendix Z). Post-hoc analysis revealed that, at the second test session, scores for anxiety ($p=.002$), headaches ($p=.01$), lack of concentration ($p=.03$), moodiness ($p<.001$), sadness ($p=.01$), tiredness ($p=.04$), and trembling hands ($p=.03$) had significantly improved compared to the first test session. PKU-QoLQ scores for these symptoms were also better at T3 compared to T1, but observed differences were only significant for scores of anxiety ($p<.001$), headaches ($p=.02$), and moodiness ($p=.001$). Similar to results observed in the whole sample, analysis of the subsample revealed no significant main effect of session on PKU-QoLQ scores for aggressiveness, headaches, irritability, and stomach aches (see Appendix Z).

6.4.6.2 Perceived impact of PKU

Table 6.16 provides an overview of mean (SD, 95% CI) PKU-QoLQ scores of perceived emotional (self-esteem, unfairness, worries about the future/future children) and social (impact on relationships with family/partner, difficulties making friends, feeling embarrassed or left out) impact of all semi-adherent ET AwPKU across all three test sessions. Mean (SD, 95% CI) PKU-QoLQ impact scores for the subsample can be found in Appendix AA.

Table 6.16 Mean (SD, 95% CI) self-reported PKU-QoLQ impact scores for each test session (n=10)

	T1			T2			T3			T3 vs. T11			
	Mean	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	**+**	**-**	**=**	***F*, *p* and η_p^2 (session)**
Emotional impact	50.50	7.51	33.52-67.48	40.63	8.63	20.22-61.03	46.67	6.97	30.59-62.74	5	5	0	$F(2,25)=.44$, $p=.65$, $\eta_p^2=.03$
Social impact	30.00	6.84	14.52-45.48	**5.79***	2.22	0.67-10.90	18.52	6.37	3.83-33.21	6	2	2	**$F(2,26)=4.81$, $p=.02$, $\eta_p^2=.27$**

Notes: [1] + improved; - worsened; = unchanged; ? unknown; *p<.05; **p<.01; ***p<.001 (Tukey post hoc; compared to T1)

There was a significant main effect of session on perceived social impact of having PKU (see Table 6.16). Post hoc comparisons revealed that perceived impact was significantly decreased at T2 compared to T1 (p=.012) but there was no significant difference at the end of the 12(±1) week intervention (T2) compared to baseline (T1). No significant main effect of session on perceived emotional impact was observed (see Table 6.16). Analysis of the subsample did not reveal a significant main effect of session on either emotional or social impact scores (Appendix AA).

6.4.7 Cognitive performance

Table 6.17 shows mean (SD, 95% CI) speed (msec) on the BS task as well as speed of correct responses (msec) and number of errors (n) for all types of trials on both parts of the FL task on each test day (see Appendix CC for BS and FL task performance of the subsample).

6.4.7.1 Baseline Speed (BS)

There was no main effect of session on speed on the BS task (see Table 6.17 and see Appendix CC for results of the subsample).

6.4.7.2 Flanker (FL)

Repeated measures analysis did not reveal a significant main effect of session on speed or number of errors on the second part of the task (see Table 1 in Appendix BB). There was a significant main effect of type of stimulus ($F(1,25)=21.95$, $p<.001$, $\eta_p^2=.47$) and a session*type of stimulus interaction ($F(2,25)=4.71$, $p=.02$, $\eta_p^2=.27$) on speed of correct responses. When data were pooled across sessions, participants were slower on incompatible versus compatible trials, but this difference was not present at T2. In addition, there was a significant main effect of type of stimulus on number of errors ($F(1,25)=8.15$, $p=.009$, $\eta_p^2=.25$), such that semi-adherent ET AwPKU were less accurate on the incompatible versus the compatible trials (see Table 6.17).

Table 6.17 Mean (SD, 95% CI) speed of correct responses (msec) and number of errors (n) on the BS and FL tasks for all test sessions

	T1			T2			T3			
	mean	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	***F*, *p* and η_p^2 (session)**
Baseline Speed (BS)										
Speed (msec)	265.50	43.59	234.32-296.68	264.38	45.47	226.36-302.39	268.89	37.36	240.17-297.61	$F(2,25)=.03$, $p=.97$, $\eta_p^2<.01$
Flanker (FL)										
Part 1 - Compatible										
Speed (msec)	445.30	86.08	383.72-506.88	411.44	77.47	351.90-470.99	415.13	88.76	340.92-489.33	$F(2,24)=.46$, $p=.64$, $\eta_p^2=.04$
Errors (n)	0.60	0.97	-0.09-1.29	1.00	1.66	-0.27-2.27	0.63	0.74	0.00-1.25	$F(2,24)=.32$, $p=.73$, $\eta_p^2=.03$
Part 1 - Neutral										
Speed (msec)	465.90	90.02	401.50-530.30	430.00	91.27	359.85-500.15	430.88	60.98	379.89-481.86	$F(2,24)=.58$, $p=.57$, $\eta_p^2=.05$
Errors (n)	1.40	1.26	0.50-2.30	0.67	0.71	0.12-1.21	0.63	0.74	0.00-1.25	$F(2,24)=1.93$, $p=.17$, $\eta_p^2=.14$
Part 2 - Compatible										
Speed (msec)	485.80	97.51	416.05-555.55	500.11	106.65	418.13-582.09	447.25	62.68	394.84-499.66	$F(2,25)=.06$, $p=.94$, $\eta_p^2<.01$
Errors (n)	1.90	1.85	0.57-3.23	1.11	1.17	0.21-2.01	1.67	2.69	-0.40-3.74	$F(2,25)=.38$, $p=.69$, $\eta_p^2=.03$
Part 2 - Incompatible										
Speed (msec)	542.70	141.91	441.19-644.21	502.67	104.68	422.20-583.13	540.89	125.51	444.41-637.36	$F(2,25)=.30$, $p=.75$, $\eta_p^2=.02$
Errors (n)	3.30	3.83	0.56-6.04	2.67	2.60	0.67-4.66	1.89	2.32	0.11-3.67	$F(2,25)=.52$, $p=.60$, $\eta_p^2=.04$

Analysis of the subsample only revealed a significant main effect of type of stimulus on speed of correct responses ($F(1,14)=12.35$, $p=.003$, $\eta_p^2=.47$). No other significant main effects or interactions were observed (see Table 1 in Appendix HH).

6.4.7.3 Memory Search 2-Dimensional Objects (MS2D)

Mean (SD, 95% CI) RTs and number of hits, correct responses, false alarms and misses of semi-adherent ET AwPKU on both parts of the MS2D task during all three test days are shown in Table 6.18 (see Appendix DD for results of the subsample). Repeated measures analysis revealed no main effect of session (see Table 2, Appendix BB), but there was a main effect of type of stimulus on speed ($F(1,25)=352.53$, $p<.001$, $\eta_p^2=.93$) and number of errors ($F(1,25)=23.13$, $p<.001$, $\eta_p^2=.48$), such that participants were slower and made more errors under a high (3 targets) compared to low (1 target) working memory load (see Table 6.18). No significant session*type of stimulus interactions for speed (msec) or accuracy (n errors) were observed (see Table 2, Appendix BB).

Analysis of the subsample revealed similar results, with significant main effects of type of stimulus on speed ($F(1,15)=361.59$, $p<.001$, $\eta_p^2=.96$) and number of errors ($F(1,15)=12.93$, $p=.003$, $\eta_p^2=.17$), but no further significant main effects or interactions (see Table 2 in Appendix HH).

Table 6.18 Mean (SD, 95% CI) outcome measures on both parts of the MS2D task for all test sessions

	T1			T2			T3			
	mean	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	***F*, *p* and η_p^2 (session)**
Part 1										
Hits (n)	23.30	1.06	22.54-24.06	22.78	1.48	21.64-23.92	23.56	0.73	23.00-24.11	$F(2,25)=1.11$, $p=.35$, $\eta_p^2=.08$
Speed (msec)	554.30	116.41	471.03-637.57	546.33	156.05	426.38-666.29	617.67	158.09	496.14-739.19	$F(2,25)=.67$, $p=.52$, $\eta_p^2=.05$
Correct (n)	23.30	0.95	22.62-23.98	23.67	0.50	23.28-24.05	23.22	0.44	22.88-23.56	$F(2,25)=1.10$, $p=.35$, $\eta_p^2=.08$
Speed (msec)	671.30	225.96	509.66-832.94	638.67	264.69	435.20-842.13	704.78	267.20	499.39-910.17	$F(2,25)=.16$, $p=.86$, $\eta_p^2=.01$
Misses (n)	0.70	1.06	-0.06-1.46	1.22	1.48	0.08-2.36	0.44	0.73	-0.11-1.00	$F(2,25)=1.11$, $p=.35$, $\eta_p^2=.08$
Speed (msec)	521.00	162.52	262.39-779.61	601.60	372.24	139.40-1063.80	478.67	20.26	428.35-528.99	$F(2,9)=.22$, $p=.81$, $\eta_p^2=.05$
False alarms (n)	0.70	0.95	0.02-1.38	0.33	0.50	-0.05-0.72	0.78	0.44	0.44-1.12	$F(2,25)=1.10$, $p=.35$, $\eta_p^2=.08$
Speed (msec)	472.50	112.98	292.73-652.27	772.33	353.64	-106.17-1650.83	664.29	157.98	518.18-810.39	$F(2,11)=2.11$, $p=.17$, $\eta_p^2=.28$
Part 2										
Hits (n)	18.00	4.08	15.08-20.92	19.78	3.03	17.45-22.11	19.56	4.53	16.07-23.04	$F(2,25)=.58$, $p=.57$, $\eta_p^2=.05$
Speed (msec)	2219.20	619.00	1776.40-2662.00	1657.56	486.55	1283.56-2031.55	1923.11	508.13	1532.53-2313.69	$F(2,25)=2.53$, $p=.10$, $\eta_p^2=.17$
Correct (n)	21.50	4.90	17.99-25.01	22.00	1.41	20.91-23.09	23.44	1.01	22.67-24.22	$F(2,25)=.99$, $p=.39$, $\eta_p^2=.07$
Speed (msec)	2942.60	690.19	2448.87-3436.33	2438.89	530.53	2031.09-2846.69	2716.00	765.17	2127.84-3304.16	$F(2,25)=1.34$, $p=.28$, $\eta_p^2=.10$
Misses (n)	6.00	4.08	3.08-8.92	5.11	3.14	2.70-7.52	4.44	4.53	0.96-7.93	$F(2,25)=.37$, $p=.70$, $\eta_p^2=.03$
Speed (msec)	2740.67	844.65	2091.41-3389.92	2269.11	611.92	1798.75-2739.47	2486.29	1099.55	1469.37-3503.20	$F(2,22)=.69$, $p=.51$, $\eta_p^2=.06$
False alarms (n)	2.50	4.90	-1.01-6.01	1.11	1.54	-0.07-2.29	0.56	1.01	-0.22-1.33	$F(2,25)=.99$, $p=.39$, $\eta_p^2=.07$
Speed (msec)	2981.00	1424.61	1212.12-4749.88	2723.00	1188.85	831.28-4614.72	2397.67	1061.70	-239.74-5035.07	$F(2,9)=.20$, $p=.82$, $\eta_p^2=.04$

6.4.7.4 Sustained Attention Dots (SAD)

MST (the mean RT of a set number of series of 12 trials; msec) and number of errors (n) for all 50 series (600 trials), the first 10 series (120 trials), and the last 10 series (120 trials) are shown in Table 6.19 (see Appendix EE for SAD task performance of the subsample).

6.4.7.4.1 Inhibition

There was no main effect of session on MST or number of errors (the inhibition outcome) of SAD task (see Table 6.19 and Appendix EE).

6.4.7.4.2 Sustained attention

There was no main effect of session on MST and number of errors on the sustained attention outcome on the SAD task (see Table 3, Appendix BB). There was a significant effect of time on MST ($F(1,25)=14.63$, $p=.001$, $\eta_p^2=.37$) and number of errors ($F(1,25)=5.08$, $p=.03$, $\eta_p^2=.17$), such that participants were significantly slower towards the end of the task (last 10 series vs. first 10 series; see Table 6.19). There was no interaction between session and time on either outcome measure (see Table 3, Appendix BB). Analysis of the subsample only showed a significant main effect of time on speed of correct responses ($F(1,15)=10.54$, $p=.005$, $\eta_p^2=.41$). No other significant main effects or interactions were observed (see Table 3 in Appendix HH).

Table 6.19 Mean (SD, 95% CI) Mean Series Time (MST; msec) and number of errors (n) on the SAD task for all test sessions

	T1			T2			T3			
	Mean	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	***F*, *p* and η_p^2 (session)**
Inhibition										
MST (msec)	9.98	2.35	8.30-11.66	9.18	2.61	7.18-11.19	9.24	2.25	7.52-10.97	$F(2,25)=.33$, $p=.73$, $\eta_p^2=.03$
Errors (n)	34.80	21.26	19.59-50.01	32.56	28.63	10.55-54.56	27.78	28.21	6.10-49.46	$F(2,25)=.18$, $p=.84$, $\eta_p^2=.01$
Sustained attention										
MST (msec) - first 10 series	9.48	1.93	8.10-10.86	9.06	2.21	7.36-10.77	8.96	2.15	7.30-10.61	$F(2,25)=.17$, $p=.85$, $\eta_p^2=.01$
Errors (n) - first 10 series	5.90	4.95	2.36-9.44	5.22	3.60	2.46-7.99	5.00	5.36	0.88-9.12	$F(2,25)=.10$, $p=.91$, $\eta_p^2<.01$
MST (msec) - last 10 series	11.52	3.42	9.07-13.96	10.37	3.04	8.03-12.70	10.79	3.01	8.47-13.11	$F(2,25)=.32$, $p=.73$, $\eta_p^2=.03$
Errors (n) - last 10 series	7.40	5.36	3.57-11.23	6.78	4.99	2.94-10.62	7.00	5.92	2.45-11.55	$F(2,25)=.03$, $p=.97$, $\eta_p^2<.01$

6.4.7.5 Set Shifting Visual (SSV)

Table 6.20 shows mean (SD, 95% CI) speed of correct responses (msec) and number of errors (n) for each type of trial for all three parts of the SSV task (see Appendix FF for results of the subsample). Repeated measures analysis did not reveal a significant main effect of session on speed (msec) or number of errors (n) on part 3 of the task (see Table 4, Appendix BB). There was a significant main effect of type of stimulus on speed of correct responses ($F(1,25)=12.13$, $p=.002$, $\eta_p^2=.33$), such that the semi-adherent ET AwPKU were slower on the incompatible versus the compatible trials (see Table 6.20). No significant session*type of stimulus interactions were observed (see Table 4, Appendix BB).

Analysis of the subsample revealed a main effect of session on speed of correct responses on compatible trials on the 3rd part of the SSV task ($F(2,15)=3.80$, $p=.046$, $\eta_p^2=.34$), such that participants were faster at T2 and T3 compared to T1. This effect was not observed for the whole group (see Table 6.20). In addition, repeated measures analysis of the subsample showed a significant main effect of type of stimulus on speed ($F(1,15)=14.83$, $p=.002$, $\eta_p^2=.50$), but not number of errors on part 3 of the SSV task. The semi-adherent ET AwPKU were faster on the compatible versus the incompatible trials (see Appendix FF). No further significant main effects or interactions were observed (see Table 4 in Appendix HH).

Table 6.20 Mean (SD, 95% CI) speed of correct responses (msec) and number of errors (n) on the SSV task for all test sessions

	T1			T2			T3			
	mean	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	***F*, *p* and η_p^2 (session)**
Part 1 - Compatible										
Speed (msec)	436.6	105.32	361.26-511.94	423.56	132.01	322.08-525.03	436.33	111.49	350.63-522.03	$F(2,25)=.04$, $p=.96$, $\eta_p^2<.01$
Errors (n)	0.7	1.06	-0.06-1.46	0.89	1.05	0.08-1.70	0.44	0.53	0.04-0.85	$F(2,25)=.53$, $p=.60$, $\eta_p^2=.04$
Part 2 - Incompatible										
Speed (msec)	817.9	425.63	513.42-1122.38	766.78	375.43	478.19-1055.36	802.33	314.58	560.52-1044.14	$F(2,25)=.05$, $p=.96$, $\eta_p^2<.01$
Errors (n)	1.7	1.64	0.53-2.87	1.67	0.71	1.12-2.21	1.67	1.66	0.39-2.94	$F(2,25)=.002$, $p=1.00$, $\eta_p^2<.001$
Part 3 - Compatible										
Speed (msec)	1028.1	304.31	810.41-1245.79	801.11	218.92	632.83-969.39	853.78	221.62	683.43-1024.13	$F(2,25)=2.11$, $p=.14$, $\eta_p^2=.14$
Errors (n)	1.5	1.43	0.47-2.53	1.56	1.51	0.40-2.72	2.11	2.20	0.42-3.81	$F(2,25)=.35$, $p=.71$, $\eta_p^2=.03$
Part 3 - Incompatible										
Speed (msec)	1123.7	435.37	812.26-1435.14	989.33	419.32	667.02-1311.65	1033.78	328.22	781.49-1286.07	$F(2,25)=.28$, $p=.76$, $\eta_p^2=.02$
Errors (n)	2.7	2.83	0.68-4.72	1.89	1.62	0.65-3.13	2.67	1.73	1.34-4.00	$F(2,25)=.41$, $p=.67$, $\eta_p^2=.03$

6.4.7.6 Motor skills: tracking (TR) and pursuit (PU)

Mean (SD, 95% CI) accuracy (mm; mean deviation of the trajectory that was followed) and the stability (mm; standard deviation of the trajectory that was followed) on the TR and PU tasks is shown in Table 6.21 (see Appendix GG for results of the subsample). Repeated measures analysis revealed no main effects of session (see Table 5, Appendix BB), but there was a main effect of task (TR vs. PU) on both the accuracy ($F(1,24)=100.28$, $p<.001$, $\eta_p^2=.81$) and stability ($F(1,24)=12.94$, $p=.001$, $\eta_p^2=.35$) of movement, such that semi-adherent ET AwPKU had better accuracy and stability on the TR compared to the PU task (see Table 6.21). No significant group*task interactions for the accuracy and stability of movement were observed (see Table 5, Appendix BB).

Analysis of the subsample also revealed significant main effects of task on the accuracy ($F(1,15)=170.96$, $p<.001$, $\eta_p^2=.83$) and stability ($F(1,15)=10.22$, $p=.006$, $\eta_p^2=.41$) of movement. No further significant main effects or interactions were observed (see Table 5 in Appendix HH).

Table 6.21 Mean (SD, 95% CI) accuracy (mm) and stability (mm) on the TR and PU tasks for all test sessions

	T1			T2			T3			
	mean	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	***F*, *p* and η_p^2 (session)**
Tracking (TR)										
Accuracy[1] (mm)	0.87	0.97	0.17-1.56	0.98	0.78	0.33--1.63	1.31	1.27	0.33-2.29	$F(2,24)=.46$, $p=.64$, $\eta_p^2=.04$
Stability[2] (mm)	1.56	0.92	0.90-2.22	1.46	0.43	1.10-1.82	2.06	0.84	1.41-2.71	$F(2,24)=1.49$, $p=.25$, $\eta_p^2=.11$
Pursuit (PU)										
Accuracy[1] (mm)	3.86	0.64	3.40-4.31	3.92	0.77	3.27-4.56	3.94	1.05	3.13-4.74	$F(2,25)=.05$, $p=.95$, $\eta_p^2<.01$
Stability[2] (mm)	2.55	0.81	1.97-3.14	2.25	0.47	1.85-2.64	2.95	1.62	1.70-4.19	$F(2,25)=1.14$, $p=.34$, $\eta_p^2=.08$

[1] Accuracy (mm): mean deviation of the trajectory that was followed; [2] Stability (mm): standard deviation of the trajectory that was followed

6.5 Discussion

The primary aim of this intervention study, was to nutritionally improve the diet of a group of semi-adherent ET AwPKU, who had an insufficient protein intake at baseline, using CGMP-AA Protein Substitute. The acceptability of and adherence to the study product was also assessed. In addition to this, the study aimed to examine whether supplementing a self-restricted low-protein diet with CGMP-AA Protein Substitute had any effects on nutritional status, QoL/wellbeing and cognitive functioning over the course of 12 weeks.

6.5.1 Summary of results

6.5.1.1 Acceptability of CGMP-AA Protein substitute

The acceptability of CGMP-AA Protein Substitute was similar to the acceptability of other CGMP-based protein substitutes, with the majority of participants reporting they preferred CGMP-AA Protein Substitute over their normal or previous protein substitutes (Daly, Evans, Chahal, Santra, & MacDonald, 2017; Lim et al., 2007; Ney et al., 2009, 2016; Proserpio et al., 2018). Participants particularly enjoyed the taste and (lack of) aftertaste of the study product. However, some of the participants reported issues with the texture ("bitty") and smell (only upon separating). Furthermore, participants reported that only taking the protein substitute twice daily made it easier to adhere to. As discussed previously, patients with PKU are usually advised to spread the intake of their protein substitutes across the day to avoid rapid increases in Phe levels and for better absorption and utilisation of nutrients provided by the substitutes (van Wegberg et al., 2017). However, previous research has shown that many patients only take their protein substitutes twice daily, against dietary advice (MacDonald, 2000). Taking protein substitutes in the morning and evening only is preferred over not taking them at all. Advising patients to take them twice daily might improve overall adherence to the diet in those struggling to follow the general advice (i.e. take them more frequently).

In addition to good acceptability of the study product, patients did not report any notable adverse effects of CGMP-AA Protein Substitute on GI health. In fact, one semi-

adherent ET AwPKU with IBD commented that their symptoms had decreased upon introduction of CGMP-AA Protein Substitute. In addition, several of the participants found that the study product did not upset their stomach or make them feel sick and bloated, which were issues they had with their usual AA-based protein substitutes. This could be related to the fact that CGMP-based protein substitutes are less acidic than AA-based protein substitutes. Moreover, research suggests CGMP may have prebiotic properties (Ney et al., 2017; Sawin et al., 2015; Stroup et al., 2018) and could mediate intestinal inflammation via the gut microbiota (Basson, Lam, & Cominelli, 2017; Hvas et al., 2016; Hvas, Bendix, Dige, Dahlerup, & Agnholt, 2018; Sawin et al., 2015).

Finally, nine of the semi-adherent ET AwPKU (90%) continued taking CGMP-AA Protein Substitute after the intervention, which reflects the acceptability of the product.

6.5.1.2 Adherence to CGMP-AA Protein Substitute

Adherence to the study product and study protocol (e.g. completing and returning the study diary and DBS) differed per participant. Overall average self-reported adherence to CGMP-AA Protein Substitute throughout the trial was approximately 81%. However, two participants had a self-reported adherence below 67% and two participants only completed the first 4-6 weeks of their study diary. The remaining six participants had an overall self-reported adherence of >90%. This is slightly lower than the self-reported adherence to a different CGMP-based protein substitute (96%) reported by Browne et al. (2018), but the patients included in their study sample were all adherent to their AA-based protein substitutes prior to participation and the intervention was short-term (4 weeks). Furthermore, average self-reported adherence between T1 and T2 was similar to that reported between T2 and T3, suggesting that the semi-adherent ET AwPKU with good adherence to CGMP-AA Protein Substitute are able to maintain adequate adherence over a longer period of time. Main issues with adherence reported by the semi-adherent ET AwPKU included life events (e.g. week away with work, holiday, weekend trip and forgetting to take substitute) and – mainly for less adherent participants – the texture, taste, and inconvenience of CGMP-AA Protein Substitute. The availability of more (variety of) flavours as well as an RTD option might improve the

adherence of patients reporting issues with the taste, texture and convenience of CGMP-AA Protein Substitute as well as help maintain longer-term adherence.

6.5.1.3 Protein intake

Compared to baseline, total protein intake at the end of the intervention was increased in all participants who returned their 3 day food diaries (n=5). Moreover, based on the minimum RDI (British Nutrition Foundation, 2018), three of those five participants had a satisfactory protein intake (>0.75g/kg/day) at the end of the 12 week trial. The remaining two semi-adherent ET AwPKU had a total protein intake just below the RDI (0.70 and 0.73 g/kg/day). Despite an observed increase in total protein intake and a self-reported adherence of ≥67% (at least 20g PE/day) in four of these participants, total protein intake at T3 was lower than expected in all but one participant. It seems that the semi-adherent ET AwPKU changed their dietary protein intake upon introduction of CGMP-AA Protein Substitute despite being instructed not to. The recently published European guidelines for the management of PKU (van Wegberg et al., 2017) recommend that total protein intake in AwPKU should be higher[1] to account for the reduced availability of synthetic AA compared to AA from whole protein, and to optimise blood-Phe levels. However, it has been suggested that CGMP-based protein substitutes have a greater bioavailability compared to AA-based protein substitutes (Ney et al., 2017). It is unclear what this means for optimal protein intake in ET AwPKU, especially in those following a self-restricted diet, in whom a decreased percentage of total protein comes from protein substitutes. Despite the observed increase in total protein intake during the trial and good acceptability of and adherence to CGMP-AA Protein Substitute in the majority of patients, intake was still unsatisfactory in light of the European guidelines. In addition, the required total protein intake for optimal health and wellbeing in ET AwPKU might increase as patients age: it has been shown that optimal protein intake

[1] For AwPKU: 1g/kg/day corrected with an additional 40% of L-AA from AA-based protein substitutes

for healthy older adults is twice as high as the minimum RDI for adults (Wolfe, Miller, & Miller, 2008).

6.5.1.4 Nutritional status

Despite the additional Phe supplied by CGMP-AA Protein Substitute, no significant increase in Phe levels was observed. Although this is in line with the majority of previous studies with CGMP, who reported metabolic control in their study samples was not adversely affected by consumption of CGMP (Browne et al., 2018; Ney et al., 2016; Pinto et al., 2017; Zaki et al., 2016), this might also be due to a compensatory decrease in intake of natural protein. In addition, Pinto et al. (2017) reported a significant increase in blood Tyr, which was not observed here. However, results showed that Thr levels were significantly increased 6(±1) weeks after introduction of CGMP-AA Protein Substitute, reflecting good adherence during these first 6(±1) weeks. Whilst self-reported adherence to CGMP-AA Protein Substitute between T2 and T3 was similar to that between T1 and T2, Thr levels at T3, although still higher compared to baseline (n.s.), had decreased compared to T2, suggesting decreased adherence in some of the participants in the second half of the trial.

Group deficits in vitamin D, Tyr, His, Lys and several non-essential AA observed at baseline were still observed at T3. However, outcomes of nutritional status were not available for all participants at T2 and T3, which makes it difficult to interpret overall group results. Furthermore, looking at results at an individual level, it is clear that some of the semi-adherent ET AwPKU altered their diet during the trial, which will have affected the results. Participant 7 is a good example (see Appendix II). This participant had a satisfactory zinc, calcium and protein (albumin and transferrin) status at baseline, but was deficient in these measures at the end of the trial, despite excellent self-reported adherence to CGMP-AA Protein Substitute (94.19%) and protein intake (1.15 g/kg/day) at the end of the trial. These results suggest that this participant had cut down on consumption of meat and dairy products upon introduction of CGMP-AA Protein Substitute.

Changes in the dietary practice of several of the participants on the trial has make it difficult to assess the effects of supplementing a self-restricted low-protein diet with CGMP-AA Protein Substitute on nutritional status. However, the study does suggest that CGMP-AA Protein Substitute might be a good stepping stone to returning to diet for ET AwPKU who are following a self-restricted low-protein diet and struggle to adhere to currently available protein substitutes.

6.5.1.5 Quality of Life (QoL)

The clearest effects of the intervention reported in this chapter were observed on QoL-related measures. At baseline (Chapter 5), self-reported PKU-QoLQ scores for lack of concentration, moodiness, sadness, tiredness and social and emotional impact were significantly higher for semi-adherent ET AwPKU compared to adherent ET AwPKU. As discussed previously (Chapter 5), these differences could be due to decreased levels of dopamine, norepinephrine and serotonin, and related impairments in sleep, as a result of increased Phe levels. In addition, semi-adherent ET AwPKU tended to report higher levels of anxiety, headaches and slow thinking than adherent ET AwPKU, but these observed differences failed to reach significance. Although Phe levels nor nutritional status did not significantly change throughout the intervention with CGMP-AA Protein Substitute, results revealed significant improvements in self-reported PKU-QoLQ scores of anxiety, lack of concentration, sadness, slow thinking, tiredness and social impact, but not emotional impact at T2. With the exception of scores of sadness and social impact, PKU-QoLQ scores remained significantly improved at T3. In addition, scores for moodiness and headaches significantly improved in the subsample with an average self-reported adherence of ≥67%. Another possible explanation for the differences observed between adherent and semi-adherent ET AwPKU as discussed in Chapter 5 was an insufficient intake of protein, and potentially a lower energy intake and higher intake of fat, as observed in other studies (Das et al., 2013; Hochuli et al., 2017; Modan-Moses et al., 2007). An increased total protein intake and, consequently, possible an increased intake of energy and (micro)nutrients related to consumption of CGMP-AA Protein Substitute may therefore have contributed to changes observed in scores of tiredness,

lack of concentration and slow thinking. Moreover, scores of anxiety, sadness, moodiness and social impact may have improved as a result of increased perceived support due to participation in the research.

6.5.1.6 Cognitive performance

With the exception speed on the SSV task, no significant differences in cognitive performance were observed throughout the trial. This is not surprising as there were no significant differences in metabolic control or status of micronutrients and other measures (e.g. homocysteine) related to cognitive functioning. Furthermore, as discussed in Chapter 5, cognitive performance varied within the group of semi-adherent ET AwPKU at baseline.

6.5.2 Limitations and future research

In addition to limitations discussed in Chapter 5, main limitations of the current intervention include the use of self-report measures, issues with adherence to the study protocol and missing data in the small study sample. The use of self-report measures introduces the possibility of social desirability bias and participants might have over-reported their adherence to CGMP-AA Protein Substitute. Another issue with self-report over a longer period of time, is that participants might complete their study or food diaries the evening before their next test session. In the current study, the study and food diaries were available electronically, which allowed the investigator to keep track of entries in real time and, when necessary, remind participants to complete new entries. However, participants with limited access to a computer were provided with a paper version of the study diary. As discussed in Chapter 2, the majority of methods to assess adherence to the dietary management of PKU are self-report, apart from DBS. The use of product count (Prince et al., 1997) was considered when developing the study protocol, but this method was rejected, as it is susceptible to social desirability bias too.

Furthermore, the initial high adherence to CGMP-AA Protein Subsitute could be the result of being part of the research and might not reflect longer-term adherence to the

protein substitute. Observed effects on wellbeing of the semi-adherent ET AwPKU could be due to the increased support as a result of participating in the research. Although the current intervention in ET AwPKU with a CGMP-based protein substitute was longer than previous interventions, longer term follow-up is needed to assess continued adherence and associated effects on wellbeing. Another methodological consideration for the design of future research with CGMP-based protein substitutes is the use of cross-over trials which include a study-arm with AA-based protein substitutes. A cross-over design was considered for the current study, but, as the target population of this research was already reluctant to adhere to their current protein substitutes, there were concerns that the semi-adherent ET AwPKU would not want to switch to the AA-arm after having been on the CGMP-AA arm. Furthermore, a cross-over design would not have been feasible here due to time constraints. However, a cross-over design would give a better insight with regards to the differences in adherence to both types of protein substitutes as well as differences in outcomes related to nutritional status and wellbeing over a prolonged period of time. Moreover, it would provide better insight as to whether the adherence to CGMP-AA Protein Substitute (both self-reported and reflected in Thr levels) and observed effects on wellbeing were a result of participating in the current research, the novel CGMP-based protein substitute, or both.

A further limitation of the research is the missing data for nutritional status and self-reported adherence to the study product. Missing bloods were due to participants not attending one of their test sessions, the investigator and metabolic team being unable to obtain a blood sample during a test session or participants not returning DBS. Furthermore, some participants did not return (some of) their study diaries.

Moreover, some of the participants changed their dietary practices despite being instructed not to. This, in combination with missing blood samples, makes it difficult to assess the effects of supplementing a self-restricted low-protein diet with CGMP-AA Protein Substitute on nutritional status. Similar future research could include more frequent assessments of dietary intake to correct for the observed compensation in natural protein intake allow adjustment of the dose of provided protein substitutes accordingly. Furthermore, as discussed in Chapter 5, assessment of dietary intake

should focus on other macronutrients (besides protein) as well as intake of other key nutrients and total energy.

Finally, ET AwPKU who are self-restricting their protein intake but are no longer taking any protein substitutes are likely to be at an increased risk of developing nutritional deficiencies than those still consuming a limited amount of protein substitutes (see Chapter 5). These patients are likely to be (more) reluctant to try new protein substitutes, even if these have been shown to be more palatable. Future research should investigate the acceptability of CGMP-AA Protein Substitute in this group of patients.

6.5.3 Implications and conclusions

Semi-adherent ET AwPKU, following a more relaxed low-protein diet, are often reluctant to adhere to their protein substitutes. Some patients do not see the need to take their protein substitutes, but the majority struggle with the taste and texture of the protein substitutes that were available at the start of this research. CGMP-AA Protein Substitute was found to be very acceptable by the vast majority of semi-adherent ET AwPKU on the trial and might be a good stepping stone to returning to diet. Although self-reported adherence to CGMP-AA Protein Substitute was consistent throughout the course of the trial, results related to QoL suggest adherence may have dropped after T2. This might be related to taste or product fatigue. The availability of a larger variety of flavours as well as a RTD option may contribute to maintained adherence to CGMP-AA Protein Substitute.

Finally, although there were no clear changes in nutritional status or cognitive function, introduction of CGMP-AA Protein Substitute to the dietary management of semi-adherent ET AwPKU significantly improved several self-reported aspects of their QoL/wellbeing, suggesting that it can play a valuable role in the dietary management of semi-adherent PKU and/or PKU wishing to return to diet.

Chapter 7 General discussion

This thesis has examined cognitive function and QoL/wellbeing in ET AwPKU, along with a consideration of the factors which relate to adherence to the dietary management of this rare metabolic disease. The systematic review (Chapter 3) and Studies 2 and 3 presented in this thesis (Chapters 4, 5, and 6) have demonstrated that there are subtle impairments in cognitive function in ET AwPKU but that these vary depending on the characteristics of the sample studied, the cognitive domains assessed and the design of the study. This chapter explores the key findings of the empirical research presented in this thesis and its relationship to the literature in order to draw clear conclusions about the impact of PKU on cognitive function and the impact of dietary management of ET AwPKU who do not adhere to the prescribed diet.

The research presented in this thesis also considered nutritional status of ET AwPKU in the empirical chapters. The factors which affect adherence to the protein substitutes, which are an integral part of the PKU diet, were examined in the survey (Study 1, Chapter 2) which indicated that there were personal and protein substitute related predictors of (non)adherence. These factors were taken into account in the intervention study which examined the potential of a novel protein substitute containing CGMP (Chapter 6) to improve (supplement) the diet of semi-adherent ET AwPKU. This study examined acceptability of and adherence to the protein substitute, as well as effects on nutritional status, QoL/wellbeing and cognitive function.

7.1 Cognitive functioning in ET AwPKU

Samples of ET AwPKU are highly heterogeneous (e.g. different genotypes, treatment histories and levels of adherence), self-selecting and sample sizes are generally small. Moreover, research in ET AwPKU to date has used a wide variety of cognitive tests spanning a range of cognitive domains and differing in sensitivity. Because of this, findings of research on cognitive functioning of ET AwPKU across cognitive domains have been somewhat inconsistent.

Impairments in cognitive performance have been observed more frequently on measures of speed than accuracy, and this may be the result of a speed-accuracy trade-off due to slower or more cautious executive decision-making processes. Compared to controls and normative data, impairments in cognitive performance of ET AwPKU have been most consistently reported on tasks of sustained attention, working memory and motor skills. Furthermore, there is some evidence of deficits in performance on tasks of attentional capacity, verbal fluency, complex language skills, complex EF and inhibitory control. Deficits seem to be more pronounced on tasks which have a higher cognitive load. In addition, results from the exploratory study reported in Chapter 4 suggest that ET AwPKU may have subtle deficits in hippocampal function as reflected by episodic memory performance on a pattern separation task. Limited longitudinal reasearch has indicated that cognitive performance remains stable or improves over extended periods beyond childhood. The long-term cognitive outcomes of ECT AwPKU, as well as the cognitive trajectory of E(C)T AwPKU, however, remain unclear.

7.2 Quality of Life (QoL) and wellbeing in ET AwPKU

Limited research in PKU has assessed HRQoL AwPKU. With the exception of HRQoL related to cognitive functioning (Bosch et al., 2007; Demirdas et al., 2013; Huijbregts et al., 2018),none of the studies have reported any differences in (generic) HRQoL between AwPKU and controls or data from a reference population (Bosch et al., 2007, 2015; Cotugno et al., 2011; Demirdas et al., 2013; Huijbregts et al., 2018; E. Simon et al., 2008). Despite the absence of clear evidence for impaired HRQoL in adolescents and adults with PKU, research has consistently reported increased internalizing disorders such as depressive mood, anxiety, low self-esteem, social withdrawal and decreased positive emotions in PKU (Brumm et al., 2010; Gentile et al., 2010; Huijbregts et al., 2018; Smith et al., 2000).

7.3 Are deficits in cognitive functioning and mood in ET AwPKU related to elevated phenylalanine levels?

7.3.1 Associations between metabolic control and cognitive functioning in ET AwPKU

The systematic review (Hofman, Champ, Lawton, Henderson, & Dye, 2018) reported in Chapter 3 revealed associations between cognitive performance across cognitive domains and metabolic control throughout life, but research has produced inconsistent findings. The online study (Chapter 4) did not find any correlations between metabolic control and cognitive test performance across a range of cognitive domains but objective measures of metabolic control, typically Phe levels, could not be obtained and so were self-reported. These may have been subject to social desirability bias. This is important because some, but not all, research in ET AwPKU has shown differences in performance on tasks assessing cognitive functioning between patients with poor or good metabolic control at the time of testing. In the study of adherent and semi-adherent ET AwPKU (Chapter 5) where concurrent levels of Phe and Tyr were available, no significant differences in performance on cognitive tasks assessing processing speed, inhibition, flexibility, working memory, sustained attention and motor skills were observed between ET AwPKU with significantly worse metabolic control at the time of testing and those whose metabolic control was good. The large inter-individual variance in Phe levels reported by previous research and the limitations of measures of metabolic control discussed in Chapter 3, combined with relatively small sample sizes in studies of ET AwPKU, suggest that the correlations observed in previous studies may not be reliable. Moreover, discrepancies in analysis and reporting of published studies make it difficult to compare study outcomes and obtain a clear picture of the impact of metabolic control on cognition in ET AwPKU. Clearly, an assessment of the long-term effects of poor metabolic control on cognitive function in AwPKU is also important but this is precluded by inconsistencies in the literature and was not possible to assess in the studies reported in this thesis.

Nevertheless, it is clear from the systematic review that elevated Phe levels can negatively affect cognitive functioning in ET AwPKU and it has been suggested that this may even accelerate cognitive decline (see Section 4.5.1). In addition fluctuations in Phe throughout life and altered Phe:Tyr ratios may be associated with observed cognitive deficits and require further attention. There was considerable heterogeneity in the metabolic control of the ET AwPKU in the comparative study of adherent and semi-adherent ET Aw PKU (Chapter 5) and in this small sample, very few differences in cognitive performance were observed between those with good (adherent ET AwPKU) and poor (semi-adherent ET AwPKU) metabolic control.

The question remains whether cognitive deficits observed in ET AwPKU are associated with poor metabolic control alone or whether other factors, such as nutrient deficiencies that impact on brain functioning, contribute to the pathophysiology and cognitive deficits observed in these patients. Factors that could contribute to disturbances in brain function and structure are discussed below and summarized in Figure 7.1.

7.3.2 Associations between metabolic control and mood in ET AwPKU

Results from Study 3 (Chapter 5) indicated that semi-adherent ET AwPKU, who had significantly higher concurrent Phe levels, reported significantly more severe mood-related symptoms (moodiness and sadness). This is consistent with ten Hoedt et al. (2011) who observed significantly lower mood states during a 4-week Phe loading period in ET AwPKU compared to 4 weeks during which they took a placebo. In contrast, Das et al. (2013) reported no significant differences in mood between AwPKU with good and poor metabolic control. However, Das et al. (2013) used a generic tool to assess mood which may not have been sufficiently sensitive to detect changes in subjective state related to PKU.

Differences in mood and cognitive functioning of ET AwPKU and observed associations with metabolic control might be due to individual differences in vulnerability to high levels of Phe (van Vliet et al., 2018), and this requires further research.

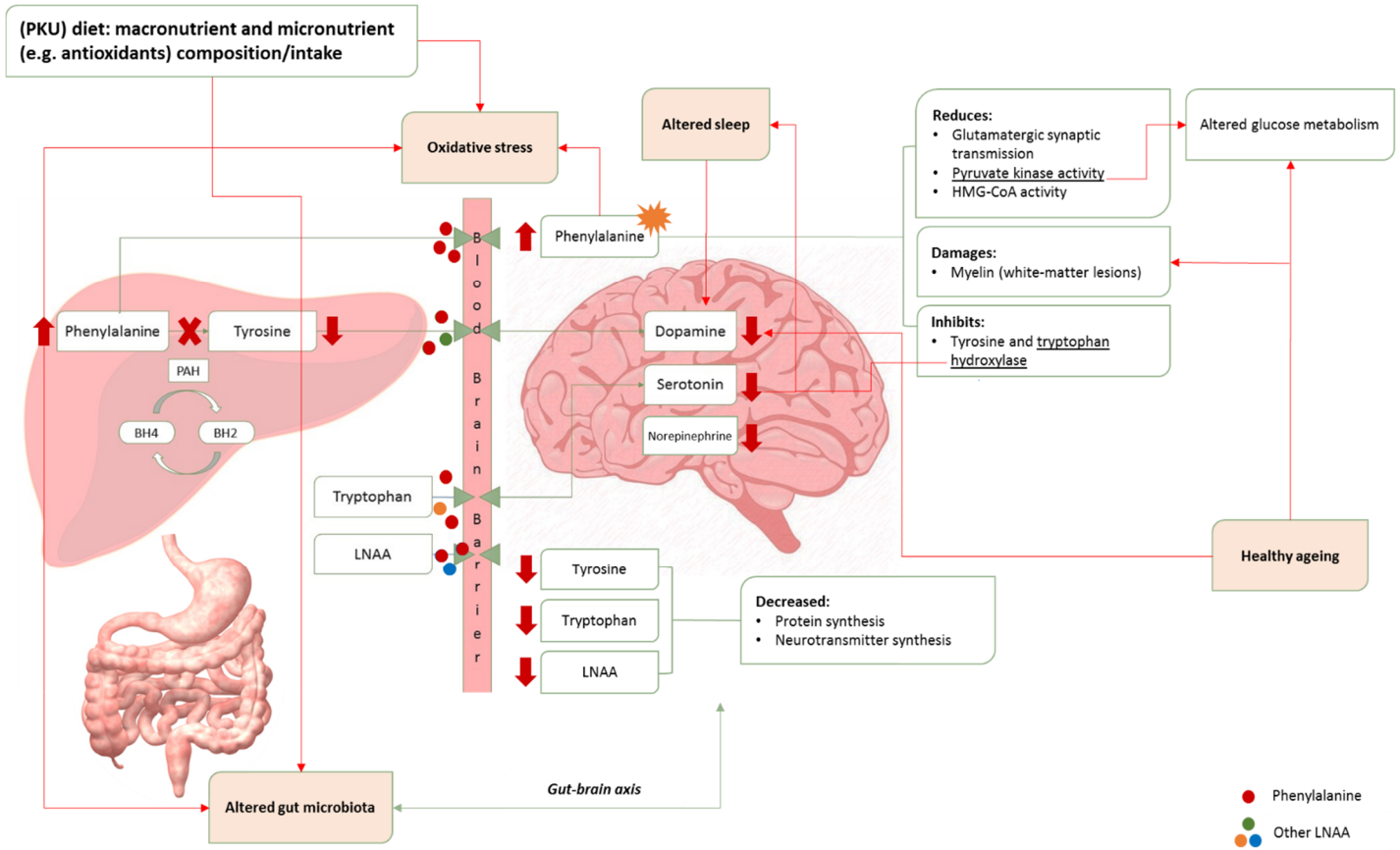


Figure 7.1 Mechanisms which may contribute to disturbances in brain structure and function in PKU, and similarities with pathophysiology observed in altered sleep and cognitive ageing

7.3.3 Disturbances in brain function and structure associated with phenylalanine

7.3.3.1 Neurotransmitter metabolism and white matter integrity

The two main theories of the mechanisms by which Phe causes disturbances in the pathophysiology of the PKU brain and affects mood and cognitive functioning of individuals with PKU, centre upon the effects of increased Phe concentrations and decreased levels of other LNAAs in the brain (see also Section 1.2 and Figure 1.2). Elevated levels of blood Phe, resulting from a disturbed Phe metabolism (i.e. decreased or no PAH activity), are thought to saturate LNAA-transporters at the BBB. Increased Phe brain levels can result in neurotoxicity, affecting white matter integrity, synaptic functioning, cerebral glucose metabolism and cholesterol synthesis. Furthermore, decreased brain levels of other LNAA (e.g. Tyr, Trp) lead to decreased protein and neurotransmitter synthesis.

7.3.3.2 Oxidative stress

There is also evidence to suggest that oxidative stress plays a role in the pathophysiology of PKU (Bruinenberg, 2017). Oxidative stress is an imbalance between the production and removal of reactive oxygen species[1], resulting from an accumulation of reactive oxygen species and/or a decreased antioxidant capacity in cell tissues. The brain is especially susceptible to oxidative stress due to its relatively poor antioxidant capacity and its high oxygen consumption (Halliwell & Gutteridge, 2007). The accumulation of Phe and its metabolites has the potential to increase ROS production (Fernandes et al., 2010; Kienzle Hagen et al., 2002), which could result in oxidative damage to proteins, lipids, CHO, and DNA (Simon et al., 2013). Furthermore, antioxidant capacity in PKU has found to be decreased as a result of a lower activity of antioxidant enzymes (Mazumder, Paul, & Borah, 2013; Rosa et al., 2012).

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[1] Highly reactive metabolites of oxygen (O_2)

7.3.3.3 Sleep

As discussed in Chapter 5, it has recently been suggested that sleep may be disturbed in PKU (Bruinenberg et al., 2017). Important modulators of sleep are dopamine, norepinephrine, melatonin and serotonin (Eban-Rothschild et al., 2016; Holst et al., 2016), which are known to be affected in PKU (see Section 1.2). Moreover, altered sleep has been linked to disturbances in mood (Meerlo et al., 2015; Short & Louca, 2015) as well as cognitive functioning (Banks, 2007; Couyoumdjian et al., 2010).

Impairments in mood and cognitive functioning associated with altered sleep are comparable to the cognitive deficits observed in ET AwPKU. One of the theories (Alhola & Polo-Kantola, 2007) about the mechanism of effects of disturbed sleep on cognitive function suggests that sleep deprivation has selective effects on the PFC and consequently, impairs cognitive domains that depend on PFC functioning (Babkoff, Zukerman, Fostick, & Ben-Artzi, 2005). A meta-analysis of the impact of short-term sleep deprivation on cognitive performance found that sleep disturbances negatively impact cognition across various cognitive domains including processing speed, attention and EF such as working memory (Lim & Dinges, 2010). Moreover, sleep problems have been linked to increased feelings of depression, anxiety and stress (Meerlo et al., 2015; Short & Louca, 2015). In addition to increased self-reported moodiness and sadness, Study 3 also found more issues with self-reported tiredness in semi-adherent compared to adherent ET AwPKU. Similarly, the significantly lower mood states during a 4-week loading period with Phe observed in ET AwPKU were accompanied with significantly more fatigue in these participants (ten Hoedt, de Sonneville, et al., 2011). Reported disturbances in the mood of ET AwPKU in both studies may have been modulated by sleep disturbances.

7.3.4 Other potential contributors to cognitive functioning and mood in ET AwPKU

7.3.4.1 Nutritional status

Chapter 5 highlighted the importance of various nutrients in maintaining optimal brain function. Nutrients such as vitamin D, vitamin B6, and omega-3 fatty acids can intervene in the synthesis and release of dopamine and serotonin (Kaneko et al., 2015; Shabbir et al., 2013). Furthermore, various nutrients are involved in the maintenance of white matter integrity (i.e. myelination and myelin recovery). In addition to disturbances in cholesterol and protein synthesis, which are affected by high levels of Phe, deficiencies in iron and B12 have been linked to the process of myelination (Agamanolis et al., 1976; Badaracco, Siri, & Pasquini, 2010; Kirksey, Morre, & Wasynczuk, 1990). Finally, deficiencies in antioxidants (e.g. selenium, zinc) could contribute to oxidative stress observed in PKU (see Section 7.3.3.2). Although protein substitutes should, in theory, ensure adequate intake of important (micro)nutrients, deficiencies in several of the nutrients of importance for optimal brain function have been reported in AwPKU (see Section 5.1.1 and (Montoya Parra et al., 2018)). Observed deficiencies are likely to be related to poor adherence to the dietary management, especially with regards to protein substitutes, of PKU. Deficiencies in several of the nutrients involved in brain function (e.g. vitamin D and zinc) were also observed at an individual level in both adherent and semi-adherent ET AwPKU who participated in the research reported in Chapter 5. Apart from having significantly higher levels of homocysteine, semi-adherent ET AwPKU did not differ significantly in nutritional status compared to adherent ET AwPKU, despite having poorer adherence to protein substitutes. It is possible that the limited intake of protein substitutes by some of the semi-adherent ET AwPKU in combination with a (albeit insufficient) increase in consumption of foods which are higher in protein, provided sufficient nutrients in these patients. ET AwPKU who are self-restricting their protein intake but are no longer taking any protein substitutes are likely to be at a greater risk of developing nutritional deficiencies than the semi-adherent ET AwPKU studied in Study 3 and future research should assess the consequences of this dietary practice on nutritional status, cognitive functioning and QoL/wellbeing of these

patients. Providing (additional) supplementation with selected nutrients or a combination of these might improve mood and cognitive functioning of ET AwPKU.

7.3.4.2 Gut-brain axis

There is limited research that suggests the gut microbiome of PKU patients differs to that of healthy controls and that this difference may be modulated by increased Phe levels and/or the nutritional composition of the PKU diet (de Oliveira et al., 2016). Moreover, limited research has suggested consumption of CGMP-based protein substitutes promotes a more favourable gut microbiome compared to protein substitutes based on L-AA alone (see Section 6.1.4), which implies that the consumption of AA-based protein substitutes contributes to altered gut microbiota. The gut microbiome has been shown to influence brain function and physiology via the gut-brain axis[2], through which it can modulate cognitive functioning as well as depression and anxiety (Basson et al., 2017).

7.3.5 Is cognitive ageing accelerated in ET AwPKU?

Age-related disruptions in the metabolism of dopamine (Erixon-Lindroth et al., 2005; Kaasinen & Rinne, 2002) and white matter integrity (Gunning-Dixon, Brickman, Cheng, & Alexopoulos, 2009; Madden, Bennett, & Song, 2009) are similar to those observed in PKU (see Figure 7.1). Furthermore, in a pilot study, Wasserstein, Snyderman, Sansaricq, & Buchsbaum (2006) observed altered cerebral glucose metabolism in several areas of the brain (including the PFC) in ET AwPKU (aged 23-35 years) compared to healthy controls. Furthermore, they found that older patients were more likely to have altered glucose mechanism than younger patients. Similar alterations in cerebral glucose mechanism have been observed in healthy older adults (50+ years of age) (Moeller et al., 1996; Willis et al., 2002). The effect of ageing on cerebral glucose metabolism in patients with PKU requires further study (Wasserstein et al., 2006).

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[2] biochemical signalling that takes place between the GI tract and the central nervous system

Similarities between the pathophysiology of PKU and healthy older adults has led to the postulation that individuals with PKU might be more vulnerable to the effects of ageing (Romani et al., 2018; Wasserstein et al., 2006). Interestingly, cognitive performance of ET AwPKU (aged 20-50 years) seems to follow the same pattern that has been observed in age-related cognitive decline in healthy older adults (50+ years of age). Research by Romani et al. (2018), as well as results reported in Chapter 4 of this thesis, found impairments in speed, but not accuracy, in performance of ET AwPKU on a range of cognitive tasks. Moreover, ET AwPKU were significantly slower than healthy controls on tasks that required decision making, but not reaction time on simple single response tasks in both studies. Exploration of data collected using a pattern separation task (Chapter 4) suggest that ET AwPKU may show issues in their ability to discriminate closely similar pictures from pictures they were shown previously (pattern separation) at a younger age than healthy controls. However, no evidence for impaired pattern separation was found in this study and more research is needed to assess whether pattern separation is actually impaired in ET AwPKU. It is important to note that these studies were cross-sectional and, therefore, reported deficits in cognitive performance may be due to individual differences and do not necessarily reflect the trajectory of cognitive ageing in PKU. Adequate dietary intake of nutrients, such as B-vitamins, antioxidants and omega-3 fatty acids may help protect against the cognitive decline associated with normal ageing (Katz & Friedman, 2008), and the same may be true in PKU.

7.4 Adherence to dietary management in ET AwPKU

It has been well-documented that adherence to the strict and complex dietary management of PKU tends to decrease with age (see Section 1.4.3). It is clear from the survey reported in Chapter 3 as well as the empirical research reported in Chapters 5 and 6 that dietary practices and adherence to the dietary management vary widely amongst ET AwPKU. Moreover, results from the survey illustrate that predicting and, therefore, managing adherence to the dietary management of PKU is complex and involves many factors (also see Section 2.1.2). As the majority of the (albeit limited) research on predictors of/barriers to dietary adherence in (young) AwPKU is rather

dated, it is important to continue to update our understanding of barriers to adherence as eating behaviour changes in relation to social norms and opportunities and the availability, variety and palatability of low-protein foods and protein substitutes improves. Results from the survey (Chapter 3) were in line with previous research and demonstrate that attributes of protein substitutes, especially aftertaste and convenience, contribute to poorer dietary adherence in ET AwPKU. In addition, it was shown that prior discontinuation of the PKU diet was a significant predictor of adherence to protein substitutes. Despite assessing a multitude of possible factors, other internal (e.g. behavioural) and external (e.g. social) factors, not measured in Study 1, contribute to difficulties with adherence to the PKU diet. At present, little is known about the effects of PKU on the health of ageing ET AwPKU and the extent to which dietary adherence could mitigate deleterious effects in older age. Hence, it is important to understand the barriers to continuous adherence to the dietary management of PKU and how these many change as ET AwPKU age.

7.4.1 Use of CGMP-AA Protein Substitute to improve (supplement) the diet of semi-adherent ET AwPKU

Previous research has demonstrated that protein substitutes based on a mixture of CGMP and AA are rated as more acceptable than AA-based protein substitutes, not only in terms of taste but also in terms of appearance and odour (Lim et al., 2007; Ney et al., 2009, 2016; Proserpio et al., 2018). Chapter 6 explored the acceptability of a CGMP-based protein substitute in ET AwPKU who were following a self-restricted diet in combination with poor adherence to their prescribed protein substitutes, with the aim of nutritionally improving their diet.

CGMP-AA Protein Substitute was found to be very acceptable by the vast majority of semi-adherent ET AwPKU on the trial and nine of the participants (90%) continued using the protein substitute after the intervention.

Despite excellent self-reported adherence to CGMP-AA Protein Substitute in the majority of the semi-adherent ET AwPKU who returned all their study diaries (overall average self-reported adherence >80%), no significant changes in nutritional status were

observed. Looking at individual results, it was clear some of the participants changed their dietary habits (i.e. cut down on intake of high-protein foods such as meat and dairy products) upon introduction of CGMP-AA Protein Substitute, despite being instructed not to. This made it impossible to assess the nutritional adequacy of CGMP-AA Protein Substitute in supplementing the diet of ET AwPKU who are semi-adherent.

However, despite the absence of clear changes in nutritional status, there were subtle differences in cognitive performance on selected tasks over time in those who showed greatest adherence (sub-group analysis) to the dietary protein substitutes in the 12 week intervention study compared to those with lower adherence.

The clearest effects of CGMP-AA Protein Substitute in this study was the improvement in Qol/wellbeing outcomes, particularly the reduction in anxiety which was maintained throughout the intervention. These findings are important, given that anxiety related disorders are the second most frequently reported psychiatric complication in PKU (Koch et al., 2002). It is possible that improvements in mental health could facilitate adherence to the PKU and lead to potential long-term benefits to nutritional status and cognitive function.

7.4.2 Resumption of dietary management in ET AwPKU and the potential role for CGMP-AA Protein Substitute as a stepping-stone to return to diet

Research about resumption to diet is limited in AwPKU, but suggests that over 50% of AwPKU who return to diet fail to maintain adequate adherence over a prolonged period of time. This is not surprising as the behaviour change involved in returning to diet is multifaceted and trying to implement multiple behaviour changes at once can be challenging. Moreover, returning to the PKU diet require planning and organisation, as well as inhibition, which may be impaired in inviduals with poor metabolic control (see Section 6.1.3 and Chapter 3). It is clear from responses to the survey reported in Chapter 2 that past off-diet behaviour is a predictor of poor adherence to protein substitutes, and the same is probably true for adherence to the strict low protein diet. Developing a

liking for a wider variety of more palatable foods, is likely to increase the temptation to stray from the diet and increase issues with the (after)taste of AA-based protein substitutes (see Section 6.1.3).

Due to these barriers to successful resumption to the dietary management of PKU, the aim of the intervention with CGMP-AA Protein Substitute was to nutritionally complete the diet of semi-adherent ET AwPKU. Therefore, participants in the 12-week intervention were instructed to supplement their usual diet with CGMP-AA Protein Substitute and not to change their 'normal' dietary habits. Nonetheless, it was clear from individual changes in nutritional status, that some of the semi-adherent ET AwPKU changed their dietary practices by lowering their dietary protein intake, suggesting CGMP-AA Protein Substitute could potentially be used as a stepping stone to return to the PKU diet.

7.5 Strengths of the research

Chapter 3 (Hofman et al., 2018) provided a systematic review of cognitive functioning in ET AwPKU, which was previously lacking in the literature. In addition to providing an overview of cognitive performance across cognitive domains and reported associations with metabolic control, it highlighted inconsistencies in methodology, analysis and reporting of psychological research in ET AwPKU, which require attention in future research. Chapter 4 explored the suitability of remote (online) cognitive testing to access larger samples of ET AwPKU. The implication of using such a tool in cognitive research in this patient group is that it might improve recruitment by limiting the burden (e.g. time, travel) of participation in research. Furthermore, it might be a useful tool to permit the continuous longer-term follow-up of ET AwPKU as patients age which will enable greater understanding of the trajectory of cognition and age-related cognitive decline in PKU. The results of the study presented in Chapter 4, which employed remote cognitive tests, are similar to recent research, suggesting that this is an acceptable method to assess cognitive function in PKU research. Furthermore, although the CogTrack™ system did not cover all possible cognitive domains which are relevant to PKU, the study

reported in Chapter 4 was the first to assess performance of ET AwPKU on a pattern separation task.

Furthermore, the studies reported in Chapters 4, 5 and 6 all employed a cognitive test battery comprised of cognitive tasks designed to measure core aspects of cognitive functioning. Most research in ET AwPKU has employed a variety of separate cognitive tasks, and only a limited amount of research has used test batteries such as ANT, CANTAB and CogTrack™. Additionally, Chapters 5 and 6 included a PKU-specific QoL measure which has been shown to be sensitive to subtle QoL issues.

Chapter 5 allowed the improved characterization of ET AwPKU with poor adherence (based on total protein intake and Phe levels) compared to previous psychological research. It was the first explorative study to examine outcomes on QoL and cognitive functioning in semi-adherent ET AwPKU who represent a large yet understudied sub-group of PKU patients.

Chapter 6 reported on the first study to explore the acceptability of a CGMP-based protein substitute in a group of patients reluctant to take their usual protein substitutes; This was the first study to explore nutritional completion of diet in those with poor adherence (instead of resumption of the dietary treatment in off-diet PKU) and effects on nutritional status, QoL, and cognitive function. This study also represents one of few longer-term (>4 weeks) dietary interventions with a CGMP-based protein substitute.

7.6 Limitations of the research

ET AwPKU who participated in the studies presented in this thesis are likely to be a self-selected sample who are more concerned about their dietary management, which could positively bias findings. Nutritional deficiencies and consequent issues with QoL/wellbeing, cognitive functioning are likely to be more prevalent and/or severe in those who are less engaged with their dietary management and research.

Furthermore, studies on cognitive performance in ET AwPKU often include a relatively small (<50 AwPKU) number of highly heterogeneous (see Section 3.2.4.1) participants

and, therefore, are likely to be underpowered. An issue affecting sample size in the study reported in Chapter 4 is the drop-out rate: initial recruitment of ET AwPKU was high, but due to high drop-out rates between registering for the research and completing both test sessions (~75% compared to ~40% of healthy controls), only 28 ET AwPKU completed the research. This high drop-out rate could be due to the fact participants were recruited through social media instead of specialised metabolic centres, and patients may be more likely to engage in research that has an affiliation through a trusted source (Rare Barometer, 2018) or involves face to face contact and provision of feedback. Moreover, the research presented in Chapter 4 involved multiple (two, albeit it short; ~20 minutes) test sessions, increasing the likelihood of dropout (between sessions).

A further issue that affects sample size in research in PKU is that, due to the low prevalence of the disorder, countries have few specialist centres involved in the management of and research into PKU. Furthermore, patients tend to live in scattered locations across a country, and usually have to travel (relatively) far to get to these specialist centres (Rath et al., 2017). Significant barriers to participating in research in rare metabolic disorders include time spent travelling to and from test sessions and, in some cases, time taken off work (Amengual, Adams, Mink, & Augustine, 2016). In addition, the frequency of DNAs ("did not attend") in the metabolic unit at SFRT was high, limiting the pool of potential participants even further.

The use of self-report measures creates another (albeit inevitable) limitation of the research reported in the study chapters. Use of such measures introduces the possibility of social desirability bias (e.g. participants might have over-reported their adherence to CGMP-AA Protein Substitute) and, in the case of the study diaries, some data might not have been reported in real time (i.e. completed the evening before a test day instead of continuously throughout the previous 6 weeks). However, the majority of methods to assess adherence to the dietary management of PKU are self-report, apart from DBS.

Another limitation of the research reported in Chapters 5 and 6 is that, other than total protein intake, the dietary intake of the patients was not assessed. More information about the dietary practices of individual patients would allow for more comprehensive

interpretation of 1) the observed 'lack' of differences in nutritional status between adherent ET AwPKU and patients following seemingly nutritionally incomplete diets as well as 2) deficiencies observed at an individual level. Moreover, despite lack of clear nutritional deficiencies in the semi-adherent ET AwPKU compared to the adherent ET AwPKU, nutrient intake may have been deficient (Hochuli et al., 2017) and prolonged suboptimal intake of certain nutrients have detrimental long-term consequences for several aspects of the health of these patients.

Finally, the research reported in this thesis did not take other potentially confounders of cognitive functioning and psychological wellbeing (e.g. sleep and nutrients such as omega-3 fatty acids, cholesterol and folate) into account.

7.7 Implication and recommendations for future research

Despite preventing severe neurological impairments in patients with PKU, (conventional) dietary treatment is still associated with suboptimal cognitive outcomes in adulthood and potentially even accelerated cognitive ageing. It is likely that this is due to poor adherence to diet in a large number of PKU patients given that around 50% are estimated to be semi-adherent to diet (see 1.4.3.1, Chapter 1). There is a clear link between elevated Phe and cognitive deficits observed in ET AwPKU. However, the exact mechanism behind the negative effects of Phe are still unknown and recent findings suggest a potential modulating role for sleep in the development of the cognitive deficits observed in ET AwPKU. Another potential factor involved in the cognitive trajectory in ET AwPKU might be the microbiome, which could be affected by the composition of the low protein diet as well as the protein substitutes.

To gain a better understanding of mood and cognitive functioning and the development of cognitive deficits and disturbances in mood in ET AwPKU, future psychological research in ET AwPKU would benefit from 1) (inter)national multicentre-studies; 2) more homogeneous samples; 3) the inclusion of potential confounders (e.g. Phe fluctuations, Phe:Tyr ratio, nutritional intake/status, sleep, IQ, social support) which might help identify any factors that could protect ET AwPKU from observed cognitive deficits; 4)

improved attention to cognitive test selection and statistical analysis; and, finally, 5) more longitudinal research to investigate the trajectory of cognitive function and age-related cognitive decline, as well as long-term effects on QoL/wellbeing in ET AwPKU.

More frequent assessments of cognitive functioning and QoL/wellbeing beyond the age of 18 (currently the highest recommended age for a follow-up cognitive assessment in European Guidelines) should be considered as part of the routine follow-up of individuals with PKU. Assessing patient's cognitive functioning as part of routine care would allow for the assessment of patients who are usually reluctant to participate in research (often due to time constraints, see Section 7.6) and likely lead to larger datasets. Moreover, long-term follow-up of the same patients will provide more insight into the cognitive trajectory in PKU. The use of a remote system (like the one used in Chapter 4) to routinely assess cognitive performance in PKU would potentially limit the burden on both patients and their families (e.g. travel time, time taken off work) as well as metabolic centres (e.g. time and personnel).

7.8 Overview and conclusions

The five main aims of this thesis were set out in the Introduction (Chapter 1). These aims were addressed by the systematic review (Chapter 3) and the 3 experimental studies (Chapters 2, 4, 5 and 6) which shed light on the relationship between degree of adherence to the dietary management of PKU and nutritional status, Qol/wellbeing and cognitive function. Study 1 (Chapter 2) showed that poor adherence to the PKU diet is predicted by previous experience of off-diet behaviour and the negative attributes and low palatability of the prescribed protein substitutes (e.g. bitter taste and bitter aftertaste, plus inconvenience of use). It is clear from the systematic review, that despite early treatment, ET AwPKU show subtle deficits in cognitive function, particularly in sustained attention, working memory, and motor skills compared to matched healthy controls. Furthermore, Study 2 (Chapter 4) showed subtle deficits in episodic memory, an understudied cognitive domain in ET AwPKU. In addition, these deficits, which are evident in healthy ageing, were demonstrated at a younger age in ET Aw PKU compared to matched healthy controls, providing more evidence for the suggestion that cognitive

ageing might be accelerated in PKU. The comparison of adherent and semi-adherent ET AwPKU in Study 3 (part 1, Chapter 5) showed that semi-adherent ET AwPKU reported poorer Qol/wellbeing than adherent ET AwPKU. Finally, the 12-week dietary intervention (Study 3, part 2, Chapter 6) with a novel, more palatable, CGMP–based protein substitute, which was designed to nutritionally complete the diet of semi-adherent patients, showed clear benefits for their psychological wellbeing, especially reduced anxiety, which addresses one of the key psychiatric complications in this previously understudied group of PKU patients. Although the CGMP-based protein substitute had little effect on nutritional status and cognitive function over 12 weeks, the enhanced palatability and convenience could improve adherence and lead to improvements in nutritional status and possibly also cognitive function over a longer period. Hence, taken together, the results of the work reported in this thesis indicate the nutritional, psychological, cognitive and behavioural issues in living with PKU. It also highlights the potential to improve the nutritional status, Qol/wellbeing and cognitive function in ET AwPKU with poor dietary adherence. The development of novel protein substitutes with improved palatability and convenience of use are likely to help PKU patients to (continue to) adhere to dietary management.

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Appendices

Appendix A. Living with PKU survey (English)

PART 1 – FACTORS INFLUENCING DIETARY ADHERENCE I: ABOUT YOURSELF

To start, we would like to ask you to provide us with some general information about yourself. Please answer the following questions. The information in this questionnaire will remain strictly confidential and anonymous.

1. Are you Male ☐ Female ☐

2. What is your age? _____ years

If age is 17 or younger, participant will be redirected to the end of the survey explaining they have to be 18 years or over to complete the survey.

3. What is your nationality?
   - ☐ American
   - ☐ Australian
   - ☐ Canadian
   - ☐ British
   - ☐ Irish
   - ☐ New Zealand
   - ☐ Other, please specify _____

4. What is your country of residence?
   - ☐ Australia
   - ☐ Canada
   - ☐ Ireland
   - ☐ New Zealand
   - ☐ United Kingdom
   - ☐ United States of America
   - ☐ Other, please specify _____

5. Did you go to school in your current country of residence?*

Yes ☐ No ☐

** If YES, skip next question (6), if NO:*

6. In what country did you complete your education?

- ☐ Australia
- ☐ Canada
- ☐ Ireland
- ☐ New Zealand
- ☐ United Kingdom
- ☐ United States of America
- ☐ Other, please specify _____

7. What is your occupation? *

Employed	☐	Unemployed	☐
Retired	☐	Housewife/homemaker	☐
Student	☐		

** Answers "unemployed", "retired", "Housewife/homemaker" and "student' will be scored as occupational group E (question 9)*

If answer to question 7 was "employed" --> question 8.

8. Please indicate to which occupational group your current or most recent occupation belongs*

- ☐ Higher managerial, administrative or professional (e.g. established doctor, solicitor, director in large organization (200+ employees), top level civil servant/public service employee) *A*
- ☐ Intermediate managerial, administrative or professional (e.g. newly qualified (under 3 years) doctor, solicitor, director in small organization, middle manager in large organization, principal officer in civil service/local government) *B*
- ☐ Supervisory or clerical; junior managerial, administrative or professional (e.g. office worker, foreman with 25+ employees, salesperson etc) *C1*
- ☐ Skilled manual worker (e.g. nurse, midwife, skilled carpenter, plumber, painter, bricklayer, bus/ambulance driver, HGV driver, mechanic/AA patrolman etc) *C2*
- ☐ Semi-skilled or unskilled manual worker (e.g. manual workers, all apprentices to skilled trades, hairdresser, caretaker, shop assistant etc) *D*
- ☐ Full time carer of other household member *E*

** Answers represent different occupational groups (A-E)*

If answer to question 7 was "student" --> question 9.

9. Please specify your educational level*

**educational levels shown will be based on educational system of the country selected in question 5 (if answer to question 6 was YES) or 7 (if answer to question 6 was NO).*

10. What is you highest completed level of education?*

**educational levels shown will be based on educational system of the country selected in question 5 (if answer to question 6 was YES) or 7 (if answer to question 6 was NO).*

11. Have you ever repeated a year?/Were you ever held back a year?*

	No, never.	Yes, once.	Yes, twice or more.
Educational level	☐	☐	☐
Educational level	☐	☐	☐
Etc.	☐	☐	☐

**educational levels shown will be based on educational system of the country selected in question 5 (if answer to question 6 was YES) or 7 (if answer to question 6 was NO).*

12. Do you suffer from any other conditions apart from PKU? *

Yes ☐ No ☐

**If YES --> question 13. If NO --> question 14.*

13. Which condition(s)? *multiple answers possible*

- ☐ Anorexia
- ☐ Anxiety
- ☐ Asthma
- ☐ Depression
- ☐ Heart disease
- ☐ Type I diabetes
- ☐ Other, please specify _____

PART 2 - YOUR PKU DIET AND SUPPLEMENTS

The following questions are about your PKU diet and dietary supplements (protein substitutes), including your habits of dietary supplement consumption. We are interested in what you usually do even if this is not what your doctor or dietitian advised. The information in this questionnaire will remain strictly confidential and anonymous.

14. What is your dietary allowance? _____ exchanges/day OR _____ milligrams (mg) Phe/day OR _____ grams protein/day. *

**Open question with dropdown menu to select units (exchanges/mg Phe/grams protein).*

15. How often do you attend the metabolic clinic for a check-up? _____ times / year.

16. Do you take blood spots to monitor your phenylalanine levels?*

Yes ☐ No ☐

**If YES --> question 17. If NO -->question 18.*

17. How often do you take blood spots to monitor your phenylalanine levels?

- ☐ Once a month
- ☐ Once every 3 months
- ☐ Once every 6 months
- ☐ Once a year
- ☐ Other, please specify _____

18. Are you currently taking Kuvan©?*

Yes ☐ No ☐

**NOTE: Kuvan is not available in all countries –questions related to Kuvan will only appear for participants from countries where Kuvan is currently available.*

19. Are you taking dietary supplements? By supplement we mean the product or liquid (for example milk, powder) prescribed by your doctor. Your doctor might refer to this as your amino acid mixture. Please note: Kuvan is not a dietary supplement).*

Yes ☐ No ☐

**If yes --> question 25. If no --> question 24 (and skip questions 25-30).*

20. Why aren't you taking any supplements?

- ☐ I don't like taking them (select this option if you don't (always) take your dietary supplements but are still restricting protein / phenylalanine intake)
- ☐ I am completely off-diet (select this option if you are no longer taking any dietary supplements AND are following a 'normal' (not PKU) diet)
- ☐ I am enrolled in a clinical trial for a novel treatment for PKU (e.g. Peg-PAL)

- ☐ Other, please specify _____

21. How many dietary supplements do you take every day? _____ / day.

22. How many times a day do you generally take your dietary supplements? _____ times.

23. When do you generally take your dietary supplements (i.e. which times of day)? Please indicate how many dietary supplements you take at each selected time of day. *Multiple answers possible**

- ☐ Morning / Breakfast ___ *(number)*
- ☐ Afternoon / Lunch ___ *(number)*
- ☐ Dinner/Evening ___ *(number)*
- ☐ Other, please specify _____

**If multiple answers selected --> question 28. If single answer --> question 29.*

24. What's your preferred time to take your dietary supplements?

Thinking about your dietary supplements in general, please answer the following questions *(MMAS-8 (Morisky; validated scale for medical adherence, amended for dietary supplements in PKU) **

Question	Patient Answer (Yes () /No ())
1. Do you sometimes forget to take your dietary supplements?	
2. People sometimes miss taking their dietary supplements for reasons other than forgetting. Thinking over the past 2 weeks, were there any days when you did not take your dietary supplements?	
3. Have you ever cut back or stopped taking your dietary supplements without telling your doctor?	
4. When you travel or leave the house, do you sometimes forget to take your dietary supplements?	
5. Did you take all your dietary supplements yesterday?	
6. When you feel like your symptoms are under control, do you sometimes stop taking your dietary supplements?	
7. Have you ever felt distressed for strictly following your dietary supplements?	
8. How often do you have difficulty remembering to take all your dietary supplements? _ A. Never/rarely	

_ B. Once in a while
_ C. Sometimes
_ D. Usually
_ E. All the time

Scoring:

**If the answer to question 30.2 was YES --> question 31. If the answer to question 30.2 was NO --> question 32.*

25. What was the reason for skipping your dietary supplements (multiple answers possible)?
    - ☐ I don't like the taste of my supplements
    - ☐ I feel embarrassed taking my supplements in front of others
    - ☐ Wanting to fit in
    - ☐ Preparing my supplements is too much effort
    - ☐ Taking my supplements is too much effort
    - ☐ Other, please specify _____

If answers to questions 30.1-30.7 were NO & the answer to 30.8 was A:

26. What motivates you to follow your PKU diet and take your supplements?

_____

_____

_____

Otherwise:

27. What sort of thing would encourage you to be follow your PKU diet and you're your supplements?

    Open question

---

28. Please rank the following characteristics of (your) dietary supplements in order of importance (to you), 1 being most important, 7 being least important.
    - ☐ Appearance (packaging)
    - ☐ Convenience
    - ☐ Taste
    - ☐ Smell
    - ☐ After taste
    - ☐ Size
    - ☐ Variety
    - ☐ Texture (e.g. viscosity, lumps etc.)

Appendix B. Study 1: Participant Information Sheet

You have been invited to complete our online survey on living with PKU.
Before you decide if you want to participate or not, you should understand what this research will involve. Please read the following information carefully. If you have any questions please do not hesitate to contact the researchers (contact details below).

The aim of this survey is to get an idea of the lives and wellbeing of PKU patients worldwide. To this end, you will be asked to complete several questionnaires. The survey includes questions about diet and supplements, management of PKU, adherence to the PKU diet (i.e. how well you stick to your PKU diet), issues related to dietary treatment, and support from family/friends.

All data collected in this study will be confidential and stored separately from any identifying information you choose to provide. Only the researchers mentioned below will have access to your data but they will not be able to identify you.

Please answer each question as it applies to you and bear in mind there is no right or wrong answer, we are interested in your experience, not what you have been told to do.
You may withdraw from the survey at any point, without reason or consequence, by simply closing your browser. If you wish to withdraw your data once it has been submitted, you can do so by e-mailing the research team and stating your unique participant ID (which you will be asked to create at the start of the survey). Note that any data submitted before 29/02/2016 can only be withdrawn until that date, as all data gathered to that point will be will be analysed as part of an undergraduate project. If you complete the survey after 29/02/2016, you will have up to 31/12/2016 to withdraw your data.

This survey has been granted ethical approval by the School of Psychology Ethical Review Committee, University of Leeds, United Kingdom, reference number 15-0398 (07/01/2016).

Thank you in advance for your time, your responses and honesty are highly appreciated.

Research team:

Postgraduate researcher:
Denise Hofman
psdlh@leeds.ac.uk
+44 113 343 9190

Level 3 project student:
Joseph Dickinson
ps13jmrd@leeds.ac.uk

Supervision:

Dr Clare Lawton
c.l.lawton@leeds.ac.uk
+44 113 343 5741

Prof Louise Dye
l.dye@leeds.ac.uk
+44 113 343 5707

Appendix C. Study 1: Informed consent form

Consent agreement:

I understand that my participation is voluntary and that I am free to withdraw from the study, without giving any reason.

I understand that I can withdraw any submitted data by e-mailing the research team and providing my unique participant ID until 29/02/2016 (data submitted before 29/02/2016) or 31/12/2016 (data submitted after 29/02/2016).

I understand that the data I provide is to be used for no purpose other than research.

I understand that the data I provide will be kept safe and confidential and my data will be anonymised by removing all links to any potentially identifying information. This will be done before any analysis is performed.

I understand that the investigators may use the data anonymised collected for this project in future research but that the conditions on this form under which I have provided the data will still apply.

I understand that access to the anonymised data will be limited to researchers involved in the project and that data gathered on me during this research will not be made available to me.

I understand that my data will be stored in an anonymised form for a period of 10 years before being destroyed.

Consent:

- ☐ I have read the consent agreement and hereby fully and freely consent to my participation in this study.
- ☐ I do not wish to participate in this study (please leave the survey).

Appendix D. Study 1 and 2: Unique participant ID creation

Before you answer any questions, we would like to ask you to generate an unique participant ID. This participant ID is used so your answers will remain anonymised, but the researchers will be able to identify your data if you wish to withdraw.

Your participant ID is a seven figure code that is created by **the first two letters of your month of birth**, **the first two letters of your first name and the last 3 digits of your telephone number**.

For example, if you were born in **February**, your name was **John** and then your telephone number was 01133439190, your unique participant ID would be **FEJO190**.

Participant ID: _____

******Please make a note of your code as you will need to provide the research team with your participant ID if you wish to withdraw your answers from the study******

Contact details:

Postgraduate researcher:
Denise Hofman
psdlh@leeds.ac.uk
+44 113 343 9190 (office)
+44 113 343 5753 (research unit)

Level 3 project student:
Joseph Dickinson
ps13jmrd@leeds.ac.uk

Supervision:

Dr Clare Lawton
c.l.lawton@leeds.ac.uk
+44 113 343 5741

Prof Louise Dye
l.dye@leeds.ac.uk
+44 113 343 5707

Appendix E. Study 1: Discriminant function analysis

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	Sig.
Gender	.999	.059	1	64	.810
Age	.982	1.195	1	64	.278
Edu_highest	.951	3.280	1	64	.075
Occupational_status	1.000	.013	1	64	.909

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	Sig.
Classification	1.000	.005	1	58	.942
Allowance	.981	1.108	1	58	.297

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	Sig.
After_taste	.924	5.294	1	64	.025
Appearance	.998	.097	1	64	.757
Convenience	.921	5.481	1	64	.022
Smell	.990	.656	1	64	.421
Taste	.995	.351	1	64	.555
Texture	.999	.055	1	64	.816
Variety	.999	.085	1	64	.772
Quantity	.978	1.424	1	64	.237

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	Sig.
After_taste	.924	5.294	1	64	.025
Convenience	.921	5.481	1	64	.022
Edu_highest	.951	3.280	1	64	.075
Off_diet_past	.926	5.135	1	64	.027

Appendix F. Study 2: Survey metabolic control

What were your latest 3 phenylalanine blood levels (please also specify the unit, mg/dl OR µmol/L when known)?

1:

2:

3:

Appendix G. Study 2: Participant Information Sheet

We would like to invite you to take part in online research on cognitive functioning in adult PKU patients (2 sessions of ±15 minutes each).

The aim of this research is to investigate the link between adherence ("sticking to") the PKU diet and protein substitutes and cognitive outcomes (e.g. memory, attention) in adult (18+) PKU patients.

The research consists of **a short questionnaire** (±2 minutes) and a **few online (cognitive) tests** (±13 minutes). These tests are a series of computerised tests of mental performance. The tests will assess brain functions such as memory, reaction time, attention and problem solving skills. They are not designed to trick you. You just have to complete the tests to the best of your ability. You will have the opportunity to practise the tests and familiarise yourself with the procedures during the first online test session.

By participating in this research and completing both test sessions you have a **chance to win one of 50 Amazon vouchers worth £10 ($12.50) each**!

To sign up for the study we will ask you to provide a few details about yourself and create a unique participant ID.

This participant ID is used so your answers will remain anonymised, but the researchers will be able to identify your data if you wish to withdraw. All data collected in this study will be confidential and stored separately from any identifying information you choose to provide. Only the researchers mentioned below will have access to your data but they will not be able to identify you.

You may withdraw from the study at any point, without reason or consequence, by simply closing your browser. If you wish to withdraw your data once it has been submitted, you can do so by e-mailing the research team and stating your unique participant ID.

This research has been granted ethical approval by the School of Psychology Ethical Review Committee, University of Leeds, United Kingdom, reference number 17-0064 (08/02/2017).

Thank you in advance for your time, your responses are highly appreciated.

Contact details:
Postgraduate researcher:
Denise Hofman
psdlh@leeds.ac.uk
+44 113 343 9190 (office)
+44 113 343 5753 (research unit)

Supervision:
Dr Clare Lawton
c.l.lawton@leeds.ac.uk
+44 113 343 5741

Prof Louise Dye
l.dye@leeds.ac.uk
+44 113 343 5707

Appendix H. Study 2: Informed consent form

If you have read and understood the participant information (outlined on the previous page) and are willing to participate in this follow-up study, please read the consent agreement carefully and select the appropriate option below before continuing.

Consent agreement:

I understand that my participation is voluntary and that I am free to withdraw from the study, without giving any reason.

I understand that I can withdraw any submitted data by e-mailing the research team and providing my unique participant ID until 30/09/2017.

I understand that the data I provide is to be used for no purpose other than research.

I understand that the data I provide will be kept safe and confidential and my data will be anonymised by removing all links to any potentially identifying information. This will be done before any analysis is performed.

I understand that the investigators may use the data anonymised collected for this project in future research but that the conditions on this form under which I have provided the data will still apply.

I understand that access to the anonymised data will be limited to researchers involved in the project and that data gathered on me during this research will not be made available to me.

I understand that my data will be stored in an anonymised form for a period of 10 years before being destroyed.

Appendix I. Chapter 4: Study 2- SPSS Linear Mixed Models for CogTrack™ Pattern Separation and Simple Reaction Time Tasks

	Pattern Separation		Simple Reaction Time
	Speed (msec)[1]	Accuracy (% correct)	Speed (msec)
Main effect terms			
Group	F(1,100)=9.98, p=.002, η_p^2=.09	F(1,95)=.04, p=.85, η_p^2<.001	F(1,50)=.56, p=.46, η_p^2=.01
Gender	F(1,100)=.24, p=.62, η_p^2<.001	F(1,95)=1.01, p=.32, η_p^2=.01	F(1,50)=11.27, p=.002, η_p^2=.18
Type of Stimulus	F(1,100)=4.53, p=.04, η_p^2=.04	F(1,95)=.05 p=.83, η_p^2<.001	
Covariates			
Age	F(1,100)=1,21, p=.27, η_p^2=.01	F(1,95)=5.89, p=.02, η_p^2=.06	F(1,50)=21.22, p<.001, η_p^2=.30
Edu	F(1,100)=.83, p=.36, η_p^2<.01	F(1,95)= .03, p=.85, η_p^2<.001	
Interaction terms			
Group*Gender		F(1,95)=2.76, p=.10, η_p^2=.03	
Group*Type of Stimulus		F(1,95)=2.38, p=.13, η_p^2=.02	
Group*Age	F(1,100)=9.40, p=.003, η_p^2=.09	F(1,95)=6.32, p=.01, η_p^2=.06	F(1,50)=1.63, p=.21, η_p^2=.03
Group*Edu	F(1,100)=14.63, p<.001, η_p^2=.13	F(1,95)=2.14, p=.15, η_p^2=.02	
Type of Stimulus*Gender		F(1,95)=.06, p=.80, η_p^2<.01	
Type of Stimulus*Age		F(1,95)=1.36, p=.25, η_p^2=.01	
Type of Stimulus*Edu		F(1,95)=.16, p=.69, η_p^2<.01	
Group*Age*Edu	F(2,100)=7.20, p=.001, η_p^2=.13		

Notes: [1] Log-transformed reaction time data were used for analysis; where no F value is presented this interaction or covariate was not retained in the final model

Appendix J. Chapter 4: Study 2- SPSS Linear Mixed Models for CogTrack™ Choice Reaction Time and Digit Vigilance Tasks

	Choice Reaction Time (CRT)		Digit Vigilance (DV)		
	Speed (msec)[1]	Accuracy (% correct)	Speed (msec)	Accuracy (% correct)	False alarms (n)
Main effect terms					
Group	$F(1,45)=4.29$, $p=.04$, $\eta_p^2=.09$	$F(1,45)=.76$, $p=.39$, $\eta_p^2=.02$	$F(1,40)=6.13$, $p=.02$, $\eta_p^2=.13$	$F(1,47)=.69$, n.s.	$F(1,44)=.51$, $p=.48$, $\eta_p^2=.01$
Gender	$F(1,45)=4.47$, $p=.04$, $\eta_p^2=.09$	$F(1,45)=.01$, $p=.91$, $\eta_p^2<.001$	$F(1,40)=2.25$, $p=.14$, $\eta_p^2=.05$	$F(1,47)=1.65$, n.s.	$F(1,44)=.49$, $p=.49$, $\eta_p^2=.01$
Covariates					
Age	$F(1,45)=.03$, n.s.	$F(1,45)=.48$, $p=.49$, $\eta_p^2=.01$	$F(1,40)=.03$, $p=.88$, $\eta_p^2<.01$	$F(1,47)=3.26$, $p=.08$, $\eta_p^2=.07$	$F(1,44)=3.16$, $p=.08$, $\eta_p^2=.07$
Edu	$F(1,45)=.04$, n.s.	$F(1,45)=.46$, $p=.50$, $\eta_p^2=.01$	$F(1,40)=.62$, $p=.44$, $\eta_p^2=.02$	$F(1,47)=.15$, n.s.	$F(1,44)=.95$, $p=.33$, $\eta_p^2=.02$
Interaction terms					
Group*Gender		$F(1,45)=5.20$, $p=.03$, $\eta_p^2=.10$	$F(1,40)=1.28$, $p=.26$, $\eta_p^2=.03$	$F(1,47)=1.21$, n.s.	$F(1,44)=1.05$, $p=.31$, $\eta_p^2=.02$
Group*Age	$F(1,45)=5.32$, $p=.03$, $\eta_p^2=.11$	$F(1,45)=.34$, $p=.56$, $\eta_p^2<.01$	$F(1,40)=9.14$, $p=.004$, $\eta_p^2=.19$		
Group*Edu	$F(1,45)=5.92$, $p=.02$, $\eta_p^2=.12$	$F(1,45)=.001$, $p=.98$, $\eta_p^2<.001$	$F(1,40)=5.92$, $p=.02$, $\eta_p^2=.13$		$F(1,44)=1.33$, $p=.26$, $\eta_p^2=.03$
Gender*Age	$F(1,45)=2.53$, n.s.		$F(1,40)=2.44$, $p=.13$, $\eta_p^2=.06$		
Gender*Edu		$F(1,45)=.27$, $p=.61$, $\eta_p^2<.01$	$F(1,40)=2.04$, $p=.16$, $\eta_p^2=.05$		$F(1,44)=.41$, $p=.52$, $\eta_p^2<.01$
Group*Gender*Age			$F(1,40)=.70$, $p=.41$, $\eta_p^2=.02$		
Group*Gender*Edu		$F(1,45)=6.25$, $p=.02$, $\eta_p^2=.12$	$F(1,40)=1.16$, $p=.29$, $\eta_p^2=.03$		$F(1,44)=2.34$, $p=.12$, $\eta_p^2=.05$
Group*Age*Edu			$F(1,40)=7.91$, $p=.008$, $\eta_p^2=.17$		
Gender*Age*Edu	$F(2,45)=3.08$, $p=.06$, $\eta_p^2=.12$		$F(1,40)=2.39$, $p=.13$, $\eta_p^2=.06$		

Notes: [1] Log-transformed reaction time data were used for analysis; where no F value is presented this interaction or covariate was not retained in the final model

Appendix K. Chapter 4: Study 2- SPSS Linear Mixed Models for CogTrack™ Spatial Working Memory Task

	Spatial Working Memory	
	Speed (msec)[1]	Accuracy (% correct)
Main effect terms		
Group	F(1,99)=10.29, p=.002, η_p^2=.09	F(1,76)=.86, n.s.
Gender	F(1,99)=7.27, p=.008, η_p^2=.07	F(1,76)=1.08, n.s.
Stimulus	F(1,99)=5.99, p=.02, η_p^2=.06	F(1,76)=.42, n.s.
Covariates		
Age	F(1,99)=3.96, p=.049, η_p^2=.04	F(1,76)=.98, n.s.
Edu	F(1,99)=.007, p=.93, η_p^2<.001	F(1,76)=.79, n.s.
Interaction terms		
Group*Gender		F(1,76)=1.48, n.s.
Group*Stimulus		F(1,76)=.09, n.s.
Group*Age		F(1,76)=.87, n.s.
Group*Edu	F(1,99)=5.29, p=.02, η_p^2=.05	F(1,76)=.65, n.s.
Age*Edu		F(1,76)=.54, n.s.
Gender*Age	F(1,99)=8.27, p=.005, η_p^2=.08	F(1,76)=1.50, n.s.
Gender*Edu	F(1,99)=4.70, p=.03, η_p^2=.05	F(1,76)=.90, n.s.
Gender*Stimulus		F(1,76)=.38, n.s.
Stimulus*Age		F(1,76)=.63, n.s.
Stimulus*Edu		F(1,76)=.28, n.s.
Group*Gender*Stimulus		F(1,76)=.05, n.s.
Group*Gender*Age		F(1,76)=1.27, n.s.
Group*Gender*Edu		F(1,76)=1.22, n.s.
Group*Stimulus*Age		F(1,76)=.04, n.s.
Group*Stimulus*Edu		F(1,76)=.28, n.s.
Group*Age*Edu		F(1,76)=.81, n.s.
Gender*Stimulus*Age		F(1,76)=.58, n.s.
Gender*Stimulus*Edu		F(1,76)=.48, n.s.
Gender*Age*Edu	F(2,99)=3.45, p=.04, η_p^2=.07	F(1,76)=1.49, n.s.
Stimulus*Age*Edu		F(1,76)=.43, n.s.
Group*Gender*Stimulus*Age		F(1,76)=.01, n.s.
Group*Gender*Stimulus*Edu		F(1,76)=.09, n.s.
Group*Gender*Age*Edu		F(1,76)=.95, n.s.
Group*Stimulus*Age*Edu		F(1,76)=.17, n.s.
Gender*Stimulus*Age*Edu		F(1,76)=.66, n.s.
Group*Gender*Stimulus*Age*Edu		F(1,76)=.03, n.s.

Notes: [1] Log-transformed reaction time data were used for analysis; where no F value is presented this interaction or covariate was not retained in the final model

Appendix L. Chapter 4: Study 2- SPSS Linear Mixed Models for CogTrack™ Numeric Working Memory Task

	Numeric Working Memory	
	Speed (msec)[1]	Accuracy (% correct)
Main effect terms		
Group	F(1,100)=2.86, p=.09, η_p^2=.03	F(1,76)= 1.38, n.s.
Gender	F(1,100)=4.85, p=.03, η_p^2=.05	F(1,76)=1.72, n.s.
Stimulus	F(1,100)=1.58, n.s.	F(1,76)=2.84, n.s.
Covariates		
Age	F(1,100)=.81, n.s.	F(1,76)=.39, n.s.
Edu	F(1,100)=2.46, n.s.	F(1,76)=.21, n.s.
Interaction terms		
Group*Gender	F(1,100)=18.28, p<.001, η_p^2=.16	F(1,76)=.08, n.s.
Group*Stimulus		F(1,76)=1.66, n.s.
Group*Age	F(1,100)=2.17, n.s.	F(1,76)=1.19, n.s.
Group*Edu	F(1,100)=8.69, p=.004, η_p^2=.08	F(1,76)=.87, n.s.
Age*Edu		F(1,76)=.66, n.s.
Gender*Age		F(1,76)=.97, n.s.
Gender*Edu	F(1,100)=.07, n.s.	F(1,76)=2.63, n.s.
Gender*Stimulus		F(1,76)=3.10, p=.08, η_p^2=.04
Type of Stimulus*Age		F(1,76)=.36, n.s.
Stimulus*Edu		F(1,76)=1.95, n.s.
Group*Gender*Stimulus		F(1,76)=4.42, p=.04, η_p^2=.06
Group*Gender*Age		F(1,76)=.13, n.s.
Group*Gender*Edu		F(1,76)=.05, n.s.
Group*Stimulus*Age		F(1,76)=.72, n.s.
Group*Stimulus*Edu		F(1,76)=.72, n.s.
Group*Age*Edu		F(1,76)=.60, n.s.
Gender*Stimulus*Age		F(1,76)=1.50, n.s.
Gender*Stimulus*Edu		F(1,76)=2.42, n.s.
Gender*Age*Edu		F(1,76)=1.71, n.s.
Stimulus*Age*Edu		F(1,76)=.31, n.s.
Group*Gender*Stimulus*Age		F(1,76)=3.27, p=.08, η_p^2=.04
Group*Gender*Stimulus*Edu		F(1,76)=4.65, p=.07, η_p^2=.06
Group*Gender*Age*Edu		F(1,76)=.01, n.s.
Group*Stimulus*Age*Edu		F(1,76)=.18, n.s.
Gender*Stimulus*Age*Edu		F(1,76)= 1.18, n.s.
Group*Gender*Stimulus*Age*Edu		F(1,76)=3.46, p=.07, η_p^2=.04

Notes: [1] Log-transformed reaction time data were used for analysis; where no F value is presented this interaction or covariate was not retained in the final model

Appendix M. Chapter 4: Study 2- Correlations between self-reported metabolic control and cognitive performance on CogTrack™ tasks

		Recent Phe	Phe Control[1]
Simple Reaction Time (SRT)	Speed (msec)	-.027	-.105
Choice Reaction Time (CRT)	Accuracy (%)	-.178	-.143
	Speed (msec)	.141	.226
Digit Vigilance (DV)	Speed (msec)	-.055	.007
	Accuracy (%)	.000	-.012
	False alarms (n)	.072	.178
Spatial Working Memory (SWM)	**Original Stimuli**		
	Accuracy (%)	.318	.353
	Speed (msec)	.135	.125
	New Stimuli		
	Accuracy (%)	.309	.281
	Speed (msec)	.120	.283
	Speed of correct responses	.129	.248
Numeric Working Memory (NWM)	**Original Stimuli**		
	Accuracy (%)	.089	.211
	Speed (msec)	.111	.041
	New stimuli		
	Accuracy (%)	-.295	-.232
	Speed (msec)	.307	.214
	Speed of correct responses	.224	.140
Pattern Separation	Original Stimuli – Accuracy	.106	.142
	New Stimuli – Accuracy	-.102	-.103
	Original Stimuli – Speed	.096	.298
	New Stimuli – Speed	.217	.270
	Speed of correct responses	.174	.328

Notes: [1] average of 3 most recent known Phe levels

Appendix O. Chapter 5: Study 3, Part 1- Participant Information Sheet: Adherent Early Treated Adults with PKU (ET AwPKU)

Participant Information Sheet A – Control at baseline

Dietary adherence, nutritional status, quality of life and cognitive functioning of Phenylketonuria (PKU) patients

This information sheet should provide you enough information about the study in order to allow you to make an informed decision about participation. Before you decide whether or not you wish to take part in the study it is important that you understand why the study is being done and what it will involve for you. Please read the following information carefully and discuss it with others if you wish. Feel free to ask questions if anything is unclear or you would like further explanation (contact details can be found at the end of this Participant Information Sheet).

What is the research about?
You are invited to take part in a study looking at the differences in nutritional status, quality of life and cognitive function (e.g. memory, attention, reaction time, etc.) of adult PKU patients who are adherent to (i.e. stick to) both their protein-low diet and protein substitutes and adult PKU patients who have issues adhering to their prescribed protein substitutes. The study is a result of a collaboration between the University of Leeds, Leeds Teaching Hospitals NHS Trust, Mark Holland Metabolic Unit (Salford Royal Foundation Trust), Vitaflo (International) Ltd and Arla Food Ingredients.

Why have I been asked to take part?
You have been invited to take part in this study because you have Phenylketonuria and have been identified as a potential participant by your metabolic consultant (Dr Reena Sharma) as you meet the study criteria (aged 16 years or over, not pregnant, adherent to low-protein diet and protein substitutes).

Do I have to take part?
It is up to you to decide whether or not to take part. If you decide to take part you will be asked to sign a consent form when you come for a screening session. Once enrolled on the study **you are still free to withdraw at any time and without giving a reason**.

What will I have to do?
You can express your interest to take part in the study by contacting Denise Hofman (contact details at the end of this information sheet). The study procedures will be explained to you and you will be free to ask any questions (via phone, e-mail, face-to-face) which you may have about any aspect of the study. You will have at least one week from receiving this information sheet to decide whether you want to participate.

Screening visit
If you wish to participate you will be invited to a screening visit. This visit can either take place at the Barnes Clinical Trial Unit (Salford Royal Foundation Trust) or the Human Appetite Research Unit (School of Psychology, University of Leeds); whichever is easiest for you. During this visit you will have the opportunity to ask questions about the study. If you are still happy to participate in the research, you will then sign a consent form confirming you agree to participate in the study and complete a

recruitment questionnaire which asks you various details about yourself (e.g. contact details, age, PKU, education, health). We will obtain other information from your medical records with your permission (by signing the consent form) such as previous blood phenylalanine and tyrosine levels. This data will be shared, in anonymised form, with the research team at the University of Leeds for purposes of this research. After this, you will be asked to provide us with a completed blood spot (finger prick) and you will be asked to complete a few simple tasks to assess your full scale IQ. The screening visit will last a maximum of 1 hour.

Test day

If you meet all study criteria, you will be required to attend Barnes Clinical Trial Unit (SRFT) or the Human Appetite Research Unit (again, whichever you prefer) on a separate occasion for a testing session.

The testing session will last about 2 hours. During the session you will be asked to complete a questionnaire which assesses your quality of life (QoL). Furthermore your height, weight and body composition will be measured, you will be asked to complete the cognitive tests and, finally, we will obtain a venous blood sample (~20mL/4 tsp).

The results from the study will be used towards an educational qualification (PhD) by a member of the research team (Denise Hofman).

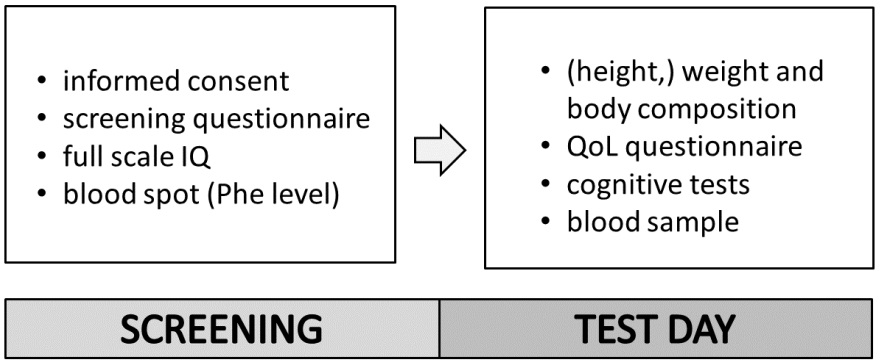


Figure 1 – Study flow-chart

How will my body composition be measured?

We use a technique called bioimpedance and this requires you to stand on a scale, dressed but in your bare feet. This machine measures the amount of fat and muscle you have in your body by passing a small electric current through your body and measuring the resistance. This is completely safe, provided that you do not have a cardiac pacemaker fitted, and you will not be able to feel anything.

What are the cognitive tests?

These are a series of computerised tests of mental performance. The tests will assess brain functions such as memory, reaction time, attention and problem solving skills. The tests are not designed to trick you. You just have to complete the tests to the best of your ability. You will have the opportunity to practise the tests and familiarise yourself with the procedures. A member of the study team will be in the room with you as part of the testing procedure so if you are unsure about anything you have to do as part of the test, you will be able to ask.

What will happen to the blood samples?

The blood spot collected during the screening visit will be analysed for phenylalanine and tyrosine levels at the metabolic lab at St. James's University Hospital, Leeds. The venous blood samples will be processed and stored in multiple micro tubes for the analysis of a full amino acid profile, protein status, vitamin and micronutrient markers of health and nutritional status. Samples will be transported to the Biochemical Genetics lab at St. James's University Hospital, where they will be stored until analysed. Analyses of the samples will take place in several specialist labs at St. James's University Hospital. All samples and data will be labelled by number only, and only the researchers collaborating on the project will have access to the data. We would like to store any unused blood samples as we expect to do genetic mutation testing on these samples in the future, to determine your PKU genotype. We will only do this with your written consent. If you do not want us to store your bloods for this purpose, any unused plasma or serum and all the red blood cells will be destroyed. It is important to point out that you will be free to withdraw and ask for your samples to be destroyed at any time without giving a reason.

What are the benefits of taking part?

Taking part in this research will contribute to the growing research in Phenylketonuria. It will help us identify any potential problems associated with non-adherence to the diet, as we will compare your results to results of age, gender and IQ matched PKU patients struggling to adhere to their protein substitutes.

What are the disadvantages of taking part?

The research will involve you visiting either the Barnes Clinical Trial Unit (SRFT) or the Human Appetite Research Unit (Leeds University) on 2 separate occasions. Where possible, time constraints incurred by taking part in the research will be kept to a minimum. The test and screening sessions can take place in the morning or afternoon to minimise inconvenience to you. Each session will last 2 hours.
The risks associated with the finger prick for the bloodspot and the venepuncture include discomfort, bruising and fainting. Study staff are fully trained in both techniques and first aid trained and will take every step to minimise any of the risks associated. Moreover, one of the trained research nurses will be present during blood sampling to further minimise any of the risks associated.

Who has reviewed this study?

All research is looked at by an independent group of people, called a Research Ethics Committee (REC), to protect your interests. This study has been reviewed and given a favourable opinion by the Yorkshire and the Humber – Sheffield REC.

Will my taking part in the research be kept confidential?

The study is subject to ethical guidelines set out by the British Psychological Society and the NHS. All information that is collected from you during the course of the study will be treated in the strictest of confidence at all times and will only be used for the purposes of this research. Your GP will only be notified of your participation in this research with your permission.
After initially completing the consent form and recruitment information questionnaire you will be given a unique study identity code. All data will then be recorded safely using this code and not your name. The link between your name (and other personal data) and your unique study identity code will be maintained and stored securely in the School of Psychology at The University of Leeds and will only be accessible to the research team. Individuals will not be identifiable from any details in reports, presentations or scientific publications based on the results of the study. Only the research team will have access to the data, and the data will only be used for the purpose of the research. Data will only be shared with Vitaflo (International) Ltd and Arla Food Ingredients in an anonymised form.

Who is organising and funding the research?

This research is funded by the Medical Research Council, Vitaflo (International) Ltd and Arla Food Ingredients. It is a collaboration between the University of Leeds, Leeds Teaching Hospitals NHS Trust, Mark Holland Metabolic Unit (Salford Royal Foundation Trust), Vitaflo (International) Ltd and Arla Food Ingredients.

What will happen to the results of the research study?

Once all participants have completed the study, the information obtained will need to be collected and analysed before any results are published. This is likely to take at least one year to be finalised. Results of tests that will be done as part of this research will not be shared with you on an individual level. However, upon completion of the research, overall study results will be shared with all participants.

Will I receive anything for taking part?

Yes, travel expenses made to attend test session will be reimbursed within reason. Travel expenses that will be reimbursed include standard class rail fare, bus fares and petrol costs (£0.40/mile). First class rail fares or taxi fares will not be reimbursed.

If I want to take part or get more information what do I do next?

If you want to volunteer to take part in this study or if you have any questions, feel free to contact the postgraduate researcher on this project, Denise Hofman. You are also free to contact her academic supervisors or clinic staff at any time using the contact details below. Before participating you will be asked to sign a consent form to show that you have read the information above and have agreed to take part.

Contact details:
Denise Hofman
PhD Student
School of Psychology
University of Leeds
Leeds LS2 9JT
psdlh@leeds.ac.uk

Supervision:
Dr Clare Lawton
Associate Professor
c.l.lawton@leeds.ac.uk
+44 113 343 5741

Prof Louise Dye
Chair in Nutrition and Behaviour
l.dye@leeds.ac.uk
+44 113 343 5707

Other contacts:
Diane Green
Metabolic Dietitian
diane.green@srft.nhs.uk
+44 161 206 1899

Dr Reena Sharma
Consultant Adult Metabolic Medicine
reena.sharma@srft.nhs.uk
+44 161 206 1899

Appendix P. Chapter 5: Study 3, Part 1- Participant Information Sheet: Semi-adherent Early Treated Adults with PKU (ET AwPKU)

Participant Information Sheet B – Intervention

Dietary adherence, nutritional status, quality of life and cognitive functioning of Phenylketonuria (PKU) patients

This information sheet should provide you enough information about the study in order to allow you to make an informed decision about participation. Before you decide whether or not you wish to take part in the study it is important that you understand why the study is being done and what it will involve for you. Please read the following information carefully and discuss it with others if you wish. Feel free to ask questions if anything is unclear or you would like further explanation (contact details can be found at the end of this Participant Information Sheet).

What is the research about?

You are invited to take part in a study looking at a newly developed protein substitute designed specifically for adult patients suffering from PKU. The study is a collaboration between the University of Leeds, Leeds Teaching Hospitals NHS Trust, Mark Holland Metabolic Unit, Vitaflo (International) Ltd and Arla Food Ingredients.

The study will investigate differences in nutritional status, quality of life and cognitive function (e.g. memory, attention, reaction time, etc.) of adult (16+ years of age) PKU patients who are adherent to (i.e. are able to stick to) both their protein-low diet and protein substitutes and adult PKU patients who have issues adhering to their prescribed protein low diet and protein substitutes. In addition to this, the study aims to examine the effects of a 12 week intervention with a newly developed protein substitute on the same outcomes.

Why have I been asked to take part?

You have been invited to take part in this study because you have Phenylketonuria and have been identified as a potential participant by your metabolic team as you meet the study criteria (aged 16 years or over, not pregnant, difficulties adhering to your prescribed protein substitutes, (self) restricting protein intake).

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be asked to sign a consent form when you come for a screening session. Once enrolled on the study you are still free to withdraw at any time and without giving a reason.

What will I have to do?

You can express your interest to take part in the study by contacting Denise Hofman (contact details at the end of this information sheet). The study procedures will be explained to you and you will be free to ask any questions (via phone, e-mail, face-to-face) which you may have about any aspect of the study. You will have at least one week from receiving this information sheet to decide whether you want to participate.

Screening visit

If you wish to participate you will be invited to a screening visit. This visit can either take place at the Barnes Clinical trials unit (Salford Royal) or the Human Appetite Research Unit located at the School of Psychology, University of Leeds (whichever is easiest for you). During this visit you will have the opportunity to ask questions about the study. If you are still happy to participate in the research, you will then sign a consent form confirming you agree to participate in the study and complete a recruitment questionnaire which asks you various details about yourself (e.g. contact details, age, PKU, education, health). We will obtain other information from your medical records with your permission (by signing the consent form) such as previous blood phenylalanine and tyrosine levels. This data will be shared, in anonymised form, with the research team at the University of Leeds for purposes of this research. After this, you will be asked to provide us with a completed blood spot (finger prick) and you will be asked to complete a few simple tasks to assess your full scale IQ. The screening visit will last a maximum of 1 hour.

Test days

If you meet all study criteria, you will be required to attend Barnes Clinical Trials Unit (Salford Royal) or the Human Appetite Research Unit (again, whichever you prefer) for 3 separate testing sessions. Furthermore, you will be provided with the newly developed protein substitute for 12 weeks (starting at the first testing session). You will get additional information on the new protein substitutes and how to take them.

Each testing session will last about 2 hours. You are not allowed to eat or drink (apart from water) during the 2 hours leading up to your test session. During test sessions you will be asked to complete a questionnaire which assesses your quality of life. Furthermore your height, weight and body composition will be measured, you will be asked to complete a cognitive test battery and, finally, we will obtain a venous blood sample (~20mL/4 tsp). The second and third testing session should be planned no later than 6 and 12 weeks after the first testing session (see the study flowchart in Figure 1). In addition to attending the testing sessions, you will be required to send weekly bloodspots to St. James' University Hospital for assessment of your phenylalanine and tyrosine levels. You will be provided with franked envelopes for this. Furthermore, you will be required to complete a short digestive wellbeing diary daily for 7 days leading up to your first test day and will be asked to keep a study diary to assess tolerance and adherence to the new protein substitute during the 12 week intervention and complete an acceptability and palatability questionnaire during your final test day (week 12). Completion of the study diaries should only take a few minutes every day.

During these 12 weeks, the research team aims to contact you weekly via text messages/phone calls to check if you are still ok with all study procedures and are not experiencing any difficulties with the new protein substitutes or study procedures.

The results from the study will be used towards an educational qualification (PhD) by a member of the research team (Denise Hofman).

Tell me more about this new protein substitute?

The protein substitute used in this research is a flavoured, powdered, low phenylalanine supplement based on casein glycomacropeptide (CGMP). CGMP is a protein that is naturally low in phenylalanine. Research in the United States has shown that protein substitutes containing both CGMP and amino acids are more acceptable than protein substitutes based on amino acids alone: PKU patients have reported that protein substitutes containing CGMP have a better taste (less bitter and less acidic), after taste and smell. They also have a smoother texture, making them more pleasant to take than the protein substitutes currently available in the UK.

Products based on CGMP for the dietary management of PKU are widely available in other countries but not currently within the UK. Although there is data to support the use of products based on CGMP in the dietary management of PKU there is a lack of longer term data.

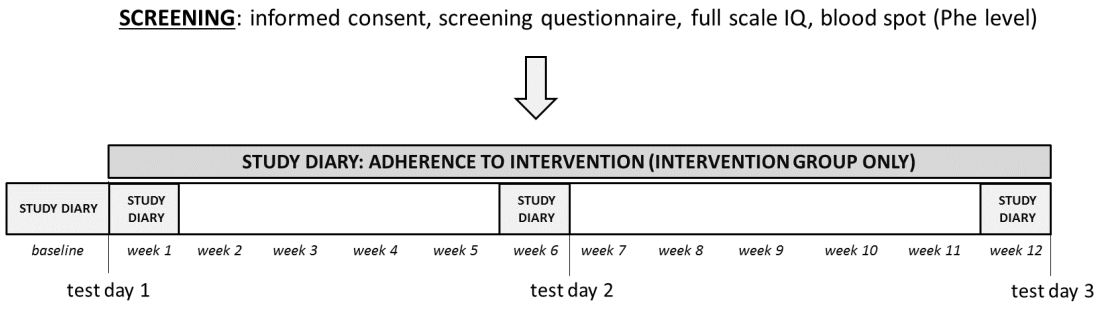
The recommended intake of the protein substitute is two sachets daily. The sachets contain a powder which can be dissolved in 100-120mL water and should be taken orally. The protein substitutes used in this research are flavoured with more adult tastes in mind. A coffee flavour that can be warmed and a blood orange flavour have therefore been developed.

How will my body composition be measured?

We use a technique called bioimpedance and this requires you to stand on a scale, dressed but in your bare feet. This machine measures the amount of fat and muscle you have in your body by passing a small electric current through your body and measuring the resistance. This is completely safe, provided that you do not have a cardiac pacemaker fitted, and you will not be able to feel anything.

What are the cognitive tests?

These are a series of computerised tests of mental performance. The tests will assess brain functions such as memory, reaction time, attention and problem solving skills. The tests are not designed to trick you. You just have to complete the tests to the best of your ability. You will have the opportunity to practise the tests and familiarise yourself with the procedures. A member of the study team will be in the room with you as part of the testing procedure so if you are unsure about anything you have to do as part of the test, you will be able to ask.



TEST DAYS: (height), weight and body composition measured, completion of a quality of life questionnaire, cognitive tests and blood sampling via venepuncture

Figure 1 – Study flow-chart

What will happen to the blood samples?

The blood spot collected during the screening visit and the blood spots you will collect at home will be analysed for phenylalanine and tyrosine levels at the metabolic lab at St. James's University Hospital, Leeds. You will be provided with franked envelopes to send your blood spots to the lab. The venous blood samples will be processed and stored in multiple micro tubes for the analysis of a full amino acid profile, protein status, vitamin and micronutrient markers of health and nutritional status. Samples will be transported to the Biochemical Genetics lab at St. James's University Hospital, where they will be stored until analysed. Analyses of the samples will take place in several specialist labs at St. James's University Hospital. All samples and data will be labelled by number only, and only the researchers collaborating on the project will have access to the data. We would like to store any

unused blood samples as we expect to do genetic mutation testing on these samples in the future, to determine your PKU genotype. We will only do this with your written consent. If you do not want us to store your bloods for this purpose, any unused plasma or serum and all the red blood cells will be destroyed. It is important to point out that you will be free to withdraw and ask for your samples to be destroyed at any time without giving a reason.

What are the benefits of taking part?

Taking part in this research will contribute to the growing research in Phenylketonuria. It will be the first research investigating the acceptability and outcomes on nutritional status, quality of life and cognitive performance of a newly developed protein substitute. Furthermore, upon completion of all 3 test days you will be provided with the newly developed protein substitute for free until it is available on prescription, if you wish. Once available on prescription, future provision of the protein substitute will be determined by your clinician or GP.

What are the disadvantages of taking part?

The research will involve you visiting either the Barnes Clinical Trials Unit (Salford Royal) or the Human Appetite Research Unit at Leeds University on **4** separate occasions in a period of approximately **4** months. Where possible, time constraints incurred by taking part in the research will be kept to a minimum. Screening and testing sessions can take place in the morning or afternoon to minimise inconvenience to you. Each session will last about 2 hours.

The risks associated with the finger prick for the bloodspot and the venepuncture include discomfort, bruising and fainting. Study staff are all fully trained in both techniques and first aid trained and will take every step to minimise any of the risks associated. Moreover, one of the trained research nurses will be present during blood sampling to further minimise any of the risks associated.

Who has reviewed this study?

All research is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by the Yorkshire and the Humber – Sheffield REC.

Will my taking part in the research be kept confidential?

The study is subject to ethical guidelines set out by the British Psychological Society and the NHS. All information that is collected from you during the course of the study will be treated in the strictest of confidence at all times and will only be used for the purposes of this research. Your GP will only be notified of your participation in this research with your permission.

After initially completing the consent form and recruitment information questionnaire you will be given a unique study identity code. All data will then be recorded safely using this code and not your name. The link between your name (and other personal data) and your unique study identity code will be maintained and stored securely in the Institute of Psychological Sciences at The University of Leeds and will only be accessible to the research team.

Individuals will not be identifiable from any details in reports, presentations or scientific publications based on the results of the study. Only the research team will have access to the data, and the data will only be used for the purpose of the research. Data will only be shared with Vitaflo (International) Ltd and Arla Food Ingredients in an anonymised form.

If you are allocated to the intervention group and only with your consent, the research team will provide Vitaflo (International) Ltd with your name, delivery address and contact telephone number. Vitaflo will keep this information safe, secure and confidential and will only use this information for supplying the study product.

Who is organising and funding the research?

This research is funded by the Medical Research Council, Vitaflo (International) Ltd and Arla Food Ingredients. It is a collaboration between the University of Leeds, Leeds Teaching Hospitals NHS Trust, the Mark Holland Metabolic Unit, Vitaflo (International) Ltd and Arla Food Ingredients.

What will happen to the results of the research study?

Once all participants have completed the study, the information obtained will need to be collected and analysed before any results are published. This is likely to take at least one year to be finalised.
Results of tests that will be done as part of this research will not be shared with you on an individual level. However, upon completion of the research, overall study results will be shared with all participants.

Will I receive anything for taking part?

You will receive these protein substitutes during the interventional phase (12 weeks) and, upon completion of all three test sessions during this period, you will keep receiving these protein substitutes if you wish.
Furthermore, travel expenses made to attend test sessions will be reimbursed within reason. Travel expenses that will be reimbursed include standard class rail fare, bus fares and petrol costs (£0.40/mile). First class rail fares or long-distance taxi fares will not be reimbursed.

If I want to take part or get more information what do I do next?

If you want to volunteer to take part in this study or if you have any questions, feel free to contact the postgraduate researcher on this project, Denise Hofman. You are also free to contact her academic supervisors or clinic staff at any time using the contact details below. Before participating you will be asked to sign a consent form to show that you have read the information above and have agreed to take part.

Contact details:
Denise Hofman
PhD Student
School of Psychology
University of Leeds
Leeds LS2 9JT
psdlh@leeds.ac.uk

Supervision:
Dr Clare Lawton
Associate Professor
c.l.lawton@leeds.ac.uk
+44 113 343 5741

Prof Louise Dye
Chair in Nutrition and Behaviour
l.dye@leeds.ac.uk
+44 113 343 5707

Other contacts:
Diane Green
Metabolic Dietitian
diane.green@srft.nhs.uk
+44 161 206 1899

Dr Reena Sharma
Consultant Adult Metabolic Medicine
reena.sharma@srft.nhs.uk
+44 161 206 1899

Appendix Q. Chapter 5: Study 3, Part 1- Informed Consent Form: Adherent Early Treated Adults with PKU (ET AwPKU)

Please read carefully and initial, date and sign for consent	***Initials***
1. I have read and understood the participant information sheet A, which outlines the study (Version 2, 18th July 2016). I have been given the opportunity to ask questions and if I have asked questions I have received satisfactory answers.	
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
3. I have been informed that my information and results will be kept anonymous.	
4. I give permission for Denise Hofman (chief investigator) to look at my medical records.	
5. I have been informed that my participation in this study will involve me having my phenylalanine level measured during screening using finger prick measurement techniques (blood spot).	
6. I have been informed that my participation in this study will involve blood sampling by means of a venepuncture to measure nutritional status (i.e.full amino acid profile, protein status (albumin, prealbumin, transferrin), c-reactive protein, and micronutrients (vitamin B12, vitamin D, vitamin E, calcium, iron, zinc, selenium, glutathione peroxidase, homocysteine and parathyroid hormone (PTH)).	
7. I have been informed that this study involves one screening and one testing session.	
8. I have been informed that this study has been approved by Integrated Research Ethics Committee (REF 16/YH/0273) and that there are no disguised procedures in this study, and that all the procedures may be taken at face value.	
9. Under the terms of the 1998 Data Protection Act, I agree to provide such personal data as may be needed, and understand that any such data will be treated confidentially and not disclosed to others without my expressed permission.	
10. I would like my GP (Dr.....) to be notified about my participation in the study and I give my permission for you to contact them.	
11. I give consent for my anonymised data from this research to be used for future projects and/or shared with other researchers in the future.	
12. I am willing to take part in this study, and whilst doing so, will not take part in any other clinical study.	

Name of Participant Signature Date

Name of Person Taking Consent Signature Date

Appendix R. Chapter 5: Study 3, Part 1- Informed Consent Form: Semi-adherent Early Treated Adults with PKU (ET AwPKU)

Please read carefully and initial, date and sign for consent	***Initials***
1. I have read and understood the participant information sheet B, which outlines the study (Version 4, 24th March 2017). I have been given the opportunity to ask questions and if I have asked questions I have received satisfactory answers.	
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
3. I have been informed that my information and results will be kept anonymous.	
4. I give permission for Denise Hofman (chief investigator) to look at my medical records.	
5. I give consent for my name, address and contact telephone number to be provided to Vitaflo (International) Ltd, so that supplies of study product (CGMP-AA Protein substitute) may be sent directly to my home for the purposes of this research study and beyond.	
6. I have been informed that my participation in this study will involve me having my phenylalanine and tyrosine levels measured regularly using finger prick measurement techniques (blood spots).	
7. I have been informed that my participation in this study will involve blood sampling by means of four venepunctures to measure nutritional status (i.e. full amino acid profile, protein status (albumin, pre-albumin, transferrin), c-reactive protein, and micronutrients (vitamin B12, vitamin D, vitamin E, calcium, iron, zinc, selenium, glutathione peroxidase, homocysteine and parathyroid hormone (PTH)).	
8. I have been informed that this study involves one screening and three testing sessions.	
9. I have been informed that this study has been approved by Integrated Research Ethics Committee (REF 16/YH/0273) and that there are no disguised procedures in this study, and that all the procedures may be taken at face value.	
10. Under the terms of the 1998 Data Protection Act, I agree to provide such personal data as may be needed, and understand that any such data will be treated confidentially and not disclosed to others without my expressed permission.	
11. I would like my GP (Dr.....) to be notified about my participation in the study and I give my permission for you to contact them.	
12. I give consent for my anonymised data from this research to be used for future projects and/or shared with other researchers in the future.	
13. I am willing to take part in this study, and whilst doing so, will not take part in any other clinical study.	

Name of Participant Signature Date

Name of Person Taking Consent Signature Date

Appendix S. Chapter 5: Study 3, Part 1- Screening Questionnaire

Screening questionnaire

(First page to be removed after screening to anonymise questionnaire)

CONTACT DETAILS

FIRST NAME(S): _____

SURNAME: _____

ADDRESS:

_____

_____

DAYTIME TEL: _____

EVENING TEL: _____

MOBILE TEL: _____

E-MAIL: _____

GENERAL INFORMATION

1. Please specify your age, date of birth (D.O.B.) and gender:

AGE: _____ D.O.B. _____/_____/_____

GENDER: Male □ Female □

2. What is your occupation?

□ Employed (please complete questions 2a and 2b)

□ Unemployed (please continue with question 3)

□ Student (please complete question 2c)

□ Housewife/homemaker (please continue with question 3)

a. If your answer to question 2 was "employed", please specify whether you are working full-time or part-time:

Full-time □ Part-time □

b. If your answer to question 2 was "employed", please specify your occupational group (please tick the box of the occupational groups that best fits your current job):

- o Higher managerial, administrative or professional
  e.g. established doctor, solicitor, director in large organization (200+ employees), top level civil servant/public service employee
- o Intermediate managerial, administrative or professional
  e.g. newly qualified (under 3 years) doctor, solicitor, director in small organization, middle manager in large organization, principal officer in civil service/local government
- o Supervisory or clerical; junior managerial, administrative or professional
  e.g. office worker, foreman with 25+ employees, salesperson etc.
- o Skilled manual worker
  e.g. nurse, midwife, skilled carpenter, plumber, painter, bricklayer, bus/ambulance driver, HGV driver, mechanic/AA patrolman etc.
- o Semi-skilled or unskilled manual worker
  e.g. manual workers, all apprentices to skilled trades, hairdresser, caretaker, shop assistant etc.
- o Full time carer of other household member

c. If your answer to question 2 was "student", please specify whether you are studying full-time or part-time:

Full-time □ Part-time □

3. What is you highest completed level of education?

- Level 1

  e.g. GCSE grades D-G, NVQ Level 1, Skills For Life level 1, BTEC award certificate and diploma level 1, OCR National

- Level 2

  e.g. GCSE grades A-C, NVQ Level 2, BTEC award certificate and diploma level 2*

- Level 3

  e.g. AS and A level, NVQ Level 3, Advanced Extension award, International Baccalaureate, OCR National

- Level 4

  e.g. NVQ Level 4, BTEC Professional award, Certificate of higher education

- Level 5

  e.g. NVQ Level 4, Higher Diploma, HND, HNC, Foundation degree

- Level 6

  e.g. BTEC Award advanced professional, Bachelors degree, Graduate Diploma

- Level 7

  e.g. Masters degree, Postgraduate diploma, NVQ Level 5, BTEC advanced professional award certificate and diploma level 7

- Level 8

  Doctorate

4. Did you ever drop out of school or repeat a year?

YES ☐ NO ☐

If YES, please specify: _____

5. What is your legal marital or same-sex civil partnership status?*

- o Single
- o In a relationship
- o Married
- o Divorced/Separated
- o Widowed
- o In a registered same-sex civil partnership
- o Formerly in a same-sex civil partnership which is now legally dissolved
- o Surviving partner from a same-sex civil partnership
- o Prefer not to say
- o What is your living situation?
- o Live with friend(s)
- o Live with partner
- o Live with partner + child(ren)
- o Live with child(ren)
- o Live at home (with parent(s)/legal guardian(s))
- o Live alone
- o Prefer not to say

HEALTH AND WELLBEING

6. Do you suffer from any other conditions apart from PKU? *

- ☐ Yes (please continue with question 7)
- ☐ No (please continue with question 8)

7. Which condition(s)?

- o Anorexia
- o Anxiety
- o Asthma
- o Depression

- o Heart disease
- o Type I diabetes
- o Other, please specify: _____

8. Are you currently taking any medication (prescribed or over-the-counter)?

   YES □ NO □

   If YES, please specify:

YOUR PKU DIET

9. What is your dietary allowance? _____ exchanges / day

10. How often do you attend the metabolic clinic for a check-up?

    _____ times / year

11. Do you take blood spots to monitor your phenylalanine levels?

Yes □ No □

12. What protein substitutes do you currently use (if any)?

---

13. How many protein substitutes do you take every day?

---

14. How many times a day do you generally take your protein substitutes?

---

15. When do you generally take your protein substitutes (i.e. which times of day)?

---

16. Which of the following characteristics of (your) protein substitutes are most important to you (multiple answers possible)?

- o Appearance (packaging)
- o Convenience
- o Taste
- o Smell
- o After taste
- o Size
- o Variety
- o Texture (e.g. viscosity, lumps etc.)

o Other, please specify _____

17. How often do you skip your protein substitutes?

18. What is the reason for skipping your protein substitutes?

19. How often do you eat the following foods?

	2 or more times a day	Once a day	3-5 times a week	1-2 times a week	1-3 times a month	Rarely	Never
Beans and peas *e.g. broad beans, baked beans (not green beans)*	○	○	○	○	○	○	○
Bread and cereals *e.g. white/brown/wholemeal bread, cake, cereals*	○	○	○	○	○	○	○
Dairy products *e.g. milk, yoghurt, cheese*	○	○	○	○	○	○	○
Eggs	○	○	○	○	○	○	○
Fish and seafood *e.g. tuna, salmon, shrimps*	○	○	○	○	○	○	○
Fruit *e.g. apples, pears, oranges, bananas*	○	○	○	○	○	○	○
Meats *e.g. beef, lamb, pork, chicken, bacon, sausages*	○	○	○	○	○	○	○
Meat substitutes *e.g. soya, quorn, tofu*	○	○	○	○	○	○	○
Nuts and seeds	○	○	○	○	○	○	○
Low-protein foods *e.g. pasta, rice, bread, milk, chocolate*	○	○	○	○	○	○	○
Potatoes *Jacket, boiled, mashed but NOT sweet potatoes*	○	○	○	○	○	○	○
Vegetables *e.g. carrots, tomatoes, courgette, cucumber*	○	○	○	○	○	○	○
Rice and pasta	○	○	○	○	○	○	○

ADDITIONAL INFORMATION

20. Can we keep this information on file and contact you about future studies?

Yes □ No □

21. Do you have any holidays planned during the next 9 months?

Yes □ No □

If YES, please specify:

From ___/___/_____ until ___/___/_____

From ___/___/_____ until ___/___/_____

From ___/___/_____ until ___/___/_____

22. If you have any additional comments, please note them below:

Thank you very much for filling out this questionnaire!!

Appendix T. Chapter 5: Study 3, Part 1- Protein intake of adherent and semi-adherent Early Treated Adults with PKU (ET AwPKU) at baseline

	Adherent ET AwPKU						Semi-adherent ET AwPKU				
	From diet *g*	From protein substitute *g*	Total protein *g*	Body weight *kg*	Protein intake *g/kg*		From diet *g*	From protein substitute *g*	Total protein *g*	Body weight *kg*	Protein intake *g/kg*
1	10	80	90	50.4	1.79	1	30	20	50	85.1	0.59

c

2	15	60	75	93.2	0.80	2	46	0	46	68.2	0.67
3	15	65	80	84.1	0.95	3	42	0	42	70.9	0.59
4	22	55	77	66.9	1.15	4	52	0	52	83.2	0.63
5	15	60	75	55.9	1.34	5	28	40	68	98.5	0.69
6	25	60	85	55.5	1.53	6	40	30	70	94.4	0.74
7	15	80	95	118.7	0.80	7	15	40	55	93.6	0.59
8	22	80	102	112.4	0.91	8	15	30	45	68	0.66
9	11	45	56	60	0.93	9	30	20	50	67.2	0.74
10	12	60	72	81.1	0.89	10	20	30	50	72	0.69

Appendix U. Chapter 5: Study 3, Part 1- Repeated measures cognitive performance ANT tasks

Table 1: SPSS Repeated measures results for performance of adherent and semi-adherent ET AwPKU on the Flanker (FL) task

	Speed of correct responses (msec)	Number of errors (n)
Main effect terms		
Group	F(1,18)=.007, p=.94, η_p^2<.001	F(1,18)=14.40, p=.13, η_p^2=.12
Type of stimulus [1]	**F(1,18)=23.10, p<.001, η_p^2=.56**	**F(1,18)=10.32, p=.005, η_p^2=.36**
Interaction term		
Group*Type of stimulus	F(1,18)=.34, p=.57, η_p^2=.02	F(1,18)=2.26, p=.13, η_p^2=.13

[1] Type of stimulus: compatible vs. incompatible trials

Table 2: SPSS Repeated measures results for performance for performance of adherent and semi-adherent ET AwPKU on the Memory Search 2D Objects (MS2D) task

	Speed of correct responses (msec)	Number of errors (n)
Main effect terms		
Group	F(1,18)=.12, p=.73, η_p^2<.01	F(1,18)=.28, p=.61, η_p^2=.02
Type of stimulus [1]	**F(1,18)=242.86, p<.001, η_p^2=.93**	**F(1,18)=24.47, p<.001, η_p^2=.58**
Interaction term		
Group* Type of stimulus	F(1,18)=.38, p=.55, η_p^2=.02	F(1,18)=.25, p=.63, η_p^2=.01

[1] Type of stimulus: low (1 target) vs. high (3 targets) working memory load

Table 3: Repeated measures results for performance for performance of adherent and semi-adherent ET AwPKU on the Sustained Attention Dots (SAD) task

	Mean series time (MST; msec)[1]	Number of errors (n)
Main effect terms		
Group	F(1,18)=.30, p=.59, η_p^2=.02	F(1,18)=.69, p=.42, η_p^2=.04
Time[2]	**F(1,18)=8.23, p=.01, η_p^2=.31**	F(1,18)=3.37, p=.08, η_p^2=.16
Interaction term		
Group*Time	F(1,18)=1.85, p=.19, η_p^2=.09	F(1,18)=.004, p=.95, η_p^2<.001

[1] MST: average RT over a number series (each series contains 12 trials)

[2] Time: first 10 series (120 trials) vs. last 10 series (120 trials)

Table 4: Repeated measures results for performance for performance of adherent and semi-adherent ET AwPKU on the Set Shifting Visual (SSV) task

	Speed of correct responses (msec)	Number of errors (n)
Main effect terms		
Group	F(1,17)=.004, p=.95, η_p^2<.001	F(1,17)=1.35, p=.26, η_p^2=.07
Type of stimulus [1]	**F(1,17)=10.77, p=.004, η_p^2=.39**	**F(1,17)=4.56, p=.048, η_p^2=.21**
Interaction term		
Group* Type of stimulus	F(1,17)=.34, p=1.16, η_p^2=.06	F(1,18)=.37, p=.55, η_p^2=.02

[1] Type of stimulus: compatible vs. incompatible trials

Table 5: SPSS Repeated measures results for performance for performance of adherent and semi-adherent ET AwPKU on the Tracking (TR) and Pursuit (PU) tasks

	Accuracy of movement[1]	Stability of movement[2]
Main effect terms		
Group	F(1,18)=.25, p=.63, η_p^2=.01	F(1,18)=.84, p=.37, η_p^2=.04
Task[3]	**F(1,18)=89.57, p<.001, η_p^2=.83**	**F(1,18)=13.84, p=.002, η_p^2=.44**
Interaction term		
Group*Task	F(1,18)=.20, p=.66, η_p^2=.01	F(1,18)=.12, p=.73, η_p^2<.01

[1]Accuracy of movement: mean deviation of the moving target/trajectory
[2] Stability of movement: standard deviation (SD) of the trajectory
[3] Task: tracking (TR) vs. pursuit (PU)

Appendix V. Chapter 6: Study 3, Part 2- Individual participant (ppt) ratings of attributes of CGMP-AA Protein Substitute

Ppt	Aftertaste	Appearance	Packaging/ presentation	Smell	Taste	Texture/ mouthfeel
1	4	2	4	2	1	1
2	3	4	5	5	4	3
3	4	3	5	3	4	4
4	3	2	4	5	3	4
5	5	3	5	5	5	3
6	3	2	2	2	3	4
7	3	3	4	3	4	3
8	3	3	4	2	2	4
9	4	4	4	4	4	3
10	3	4	4	1	4	3

Key: ppt: participant; 1 = I really did not like it!!; 2 = I did not like it!; 3 = Neither liked or disliked it; 4= I liked it!; 5 = I loved it!!)

Appendix W. Chapter 6: Study 3, Part 2- Individual participant (ppt) feedback about CGMP-AA Protein Substitute

What did you like about CGMP-AA Protein Substitute?

1. The taste is much nicer than lophlex. I am able to enjoy and slowly sip it.
2. Not acidic; no aftertaste lingering all day in mouth; no foul smell when preparing and taking it; does not upset stomach.
3. The taste, as all other supplements have a horrible bitter aftertaste, this is much better! I also like how I only have to take 2 a day, this has made it a lot easier to adhere to.
4. Flavour that was aimed at a more mature pallet. Goes down very easily and does not make me feel sick or bloated.
5. Everything about it, it is very nice.
6. Easy to drink and less to take than normal.
7. Felt like I had more energy.
8. It doesn't give me belly ache after drinking it.
9. I like the taste as it tastes like coffee.
10. Ease of use; mainly improvement in taste.

What did you dislike about CGMP-AA Protein Substitute?

1. The powder sachets were not ideal, not mobile enough so I missed out on taking a lot because it wasn't practical with my busy schedule. Also, it needed to either be piping hot or have loads of ice in it to taste nice → again, not practical.
2. Takes a lot of shaking to get rid of all powder – sometimes there are small lumps; the 'fishy' smell if you leave it lying around! Need to clean container straight away – do not put in dishwasher.
3. Can be 'bitty' in texture at times and does leave slight smell on breath – however much better than any previous supplements tried.
4. N/A
5. Nothing.
6. Flavour- not a fan of coffee!
7. More convenient way to drink e.g. already mixed 'ready to go'.
8. The little bits (hard to blend) and the after taste.
9. Made your urine smell of the drink.
10. Aftertaste; the smell it leaves in your flask after use.

Additional feedback:

1. No matter how hard you shake it, there is still lots of bits in which makes it very difficult to drink. Also, if you leave it for a while it turns black and stinks!
2. Feel mood has improved a lot; it has lower my blood pressure – a lot! Not really noticed any side effects – think I go for a wee more; if I take on empty stomach I get runs so I always take after food and then it's fine.
3. None.
4. Overall a very easy, convenient and tasty product. Enjoyed using this substitute and it helped improved my control of PKU.
5. It is very good. If we could have a variety of flavours that would be really good. Overall very happy with it.
6. Odour to urine – coffee smell after taking!
7. More variety in flavours.
8. None.
9. Nice to have a couple of flavours
10. None.

Appendix X. Chapter 6: Study 3, Part 2- Mean (SD, 95% CI) measures of nutritional status of semi-adherent early treated adults with PKU (ET AwPKU) at each test session subsample (n=6)

	Reference range (µmol/L)	T1			T2			T3			
		mean	SD	95% CI	mean	SD	95% CI	mean	SD	95% CI	*F*, *p* and η_p^2 (session)
Albumin† (g/L)	35-50	43.83	6.31	37.22-50.45	40.50	3.67	36.64-44.36	39.00	4.38	34.40-43.60	*F*(2,14)=.69, *p*=.52, η_p^2=.09
Pre-albumin† (g/L)	0.20-0.50	0.26	0.05	0.21-0.32	0.29	0.05	0.24-0.34	0.30	0.03	0.27-0.33	*F*(2,15)=1.22, *p*=.32, η_p^2=.14
Transferrin† (g/L)	2.00-3.20	2.44	0.40	2.02-2.85	2.49	0.74	1.71-3.26	2.08	0.28	1.73-2.42	*F*(2,14)=.97, *p*=.40, η_p^2=.12
Alkaline Phosphatase (U/L)	30-130	73.67	11.78	61.31-86.02	65.67	11.67	53.42-77.92	60.50	14.35	45.44-75.56	*F*(2,14)=2.97, *p*=.08, η_p^2=.30
Calcium† (mmol/L)	2.20-2.60	2.37	0.15	2.21-2.52	2.23	0.21	2.00-2.45	2.22	0.19	2.02-2.41	*F*(2,14)=1.19, *p*=.33, η_p^2=.15
Copper (µmol/L)	11-22	18.48	6.95	11.19-25.77	20.00	9.67	9.86-30.14	18.07	6.95	10.77-25.36	*F*(2,15)=.10, *p*=.91, η_p^2=.01
Homocysteine (µmol/L)	<18	10.20	4.44	4.69-15.71	10.60	3.78	5.90-15.30	10.20	3.27	6.14-14.26	*F*(2,12)=.02, *p*=.98, η_p^2<.01
Iron† (µmol/L)	14-31 (♂)	21.50	12.59	-91.59-134.58	14.40	2.97	-12.28-41.08	15.00	1.70	-0.25-30.25	*F*(2,9)=.74, *p*=.51, η_p^2=.14
	11-29 (♀)	16.13	5.02	8.14-24.11	13.85	3.96	7.54-20.15	17.40	3.144	11.93-22.87	*F*(2,3)=.55, *p*=.63, η_p^2=.27
Phosphate† (mmol/L)	0.80-1.50	1.10	0.15	0.94-1.26	1.06	0.12	0.93-1.19	1.01	0.19	0.80-1.21	*F*(2,14)=.67, *p*=.53, η_p^2=.09
Parathyroid hormone (PTH) (pmol/L)	1.50-7.60	3.74	1.07	2.41-5.07	5.13	2.12	2.91-7.36	5.15	2.82	2.19-8.11	*F*(2,14)=.73, *p*=.50, η_p^2=.09
Selenium‡ (µmol/L)	0.80-2.00	0.95	0.15	0.79-1.11	1.04	0.16	0.87-1.20	1.02	0.18	0.83-1.20	*F*(2,14)=.91, *p*=.43, η_p^2=.12
Vitamin A (µmol/L)	1.05-3.39	2.29	0.18	2.00-2.57	1.90	0.58	0.98-2.82	2.74	1.04	1.08-4.40	*F*(2,15)=1.44, *p*=.27, η_p^2=.16
Vitamin B12† (ng/L)	211-911	451.20	149.62	265.43-636.97	520.60	197.36	275.54-765.66	472.80	221.07	198.31-747.29	*F*(2,12)=.17, *p*=.84, η_p^2=.03
Vitamin D (nmol/L)	>75	**74.80**	43.15	21.22-128.38	78.58	48.36	18.53-138.63	76.02	43.68	21.78-130.26	*F*(2,12)=.009, *p*=.99, η_p^2<.01
Vitamin E (µmol/L)	12-42	25.38	3.16	20.34-30.41	21.23	4.75	13.66-28.79	26.85	6.69	16.20-37.50	*F*(2,9)=1.32, *p*=.32, η_p^2=.23
Zinc‡ (µmol/L)	9.80-17.90	8.93	3.00	5.79-12.08	9.13	2.41	6.60-11.67	9.07	0.90	8.12-10.01	*F*(2,14)=.77, *p*=.48, η_p^2=.10

*Notes: †serum; ‡plasma; *p<.05; **p<.01; ***p<.001 (Tukey post hoc; compared to T1)*

Appendix Y. Chapter 6: Study 3, Part 2- Mean (SD, 95% CI) amino acid concentrations of semi-adherent early treated adults with PKU (ET AwPKU) at each test session – subsample (n=6)

	Reference range (µmol/L)	T1			T2			T3			
		mean	SD	95% CI	mean	SD	95% CI	mean	SD	95% CI	*F*, *p* and η_p^2 (session)
Alanine (Ala)	248-778	233.50	67.41	162.76-304.24	257.17	68.61	185.17-329.17	266.67	36.18	228.70-304.64	*F*(2,15)=.50, *p*=.62, η_p^2=.06
Arginine (Arg)	30-198	37.33	10.93	25.86-48.80	38.50	10.58	27.40-49.60	43.83	13.70	29.45-58.21	*F*(2,15)=.52, *p*=.61, η_p^2=.06
Asparagine (Asn)	35-128	38.17	13.99	23.48-52.85	46.17	13.21	32.30-60.03	45.33	7.15	37.83-52.83	*F*(2,15)=.83, *p*=.46 η_p^2=.10
Aspartic acid (Asp)	45-125	9.00	1.22	7.48-10.52	9.75	0.96	8.23-11.27	12.60	3.65	8.07-17.13	*F*(2,11)=3.16, *p*=.08, η_p^2=.37
Citrulline (Cit)	13-52	31.00	9.17	21.38-40.62	34.17	8.98	24.75-43.59	35.50	7.97	27.14-43.86	*F*(2,15)=.42, *p*=.66, η_p^2=.05
Cysteine (Cys)	17-124	41.33	7.31	33.66-49.01	39.67	9.99	29.18-50.15	44.17	8.75	34.98-53.35	*F*(2,15)=.41, *p*=.67, η_p^2=.05
Glutamate (Glu)	46-428	36.33	20.88	14.42-58.24	41.83	28.21	12.23-71.44	41.17	12.89	27.64-54.69	*F*(2,15)=.13, *p*=.89, η_p^2=.02
Glutamine (Gln)	270-1159	420.67	110.07	305.15-536.18	459.67	111.97	342.17-577.17	479.17	91.11	383.55-574.78	*F*(2,15)=.49, *p*=.63, η_p^2=.06
Glycine (Gly)	185-552	233.83	121.79	106.03-361.64	256.50	140.08	109.50-403.50	269.50	118.02	145.65-393.35	*F*(2,15)=.12, *p*=.89, η_p^2=.02
Histidine (His)†	81-193	64.83	15.55	48.52-81.15	66.33	13.02	52.67-79.99	72.83	12.45	59.77-85.90	*F*(2,15)=.58, *p*=.58, η_p^2=.07
Isoleucine (Iso)†	35-127	47.67	12.16	34.91-60.43	48.00	23.77	23.05-72.95	53.50	8.60	44.48-62.52	*F*(2,15)=.25, *p*=.79, η_p^2=.03
Leucine (Leu)†	80-229	101.67	19.58	81.12-122.22	100.50	36.91	61.76-139.24	112.17	18.96	92.27-132.06	*F*(2,15)=.35, *p*=.71, η_p^2=.05
Lysine (Lys)†	165-378	132.17	32.29	98.28-166.06	133.17	30.21	101.46-164.87	153.00	32.58	118.81-187.19	*F*(2,15)=.82, *p*=.46, η_p^2=.10
Methionine (Met)†	9-52	12.67	4.80	7.63-17.71	14.00	4.65	9.12-18.88	14.67	3.01	11.51-17.83	*F*(2,15)=.35, *p*=.71, η_p^2=.04
Ornithine (Orn)	117-279	38.50	15.95	21.76-55.24	36.67	19.12	16.60-56.73	41.50	10.45	30.54-52.46	*F*(2,15)=.15, *p*=.86, η_p^2=.02
Phenylalanine (Phe)†	120-700[1]	1073.67	217.22	845.71-1301.62	1102.83	189.29	904.18-1301.48	1255.17	284.54	956.56-1553.77	*F*(2,15)=1.04, *p*=.38, η_p^2=.12
Proline (Pro)	123-451	103.17	23.37	78.64-127.69	104.33	51.41	50.39-158.28	114.50	22.69	90.69-138.31	*F*(2,15)=.19, *p*=.83, η_p^2=.03
Serine (Ser)	68-256	79.50	21.01	57.45-101.55	88.33	37.47	49.01-127.66	85.67	27.10	57.22-114.11	*F*(2,15)=.14, *p*=.89, η_p^2=.02
Taurine (Tau)	80-344	76.17	62.52	10.55-141.78	83.33	37.32	44.17-122.50	85.17	21.47	62.64-107.70	*F*(2,15)=.07, *p*=.93, η_p^2<.01
Threonine (Thr)†	60-231	88.50	24.95	62.31-114.69	136.83	45.21	89.39-184.28	121.17	17.98	102.30-140.04	***F*(2,15)=3.66, *p*=.05, η_p^2=.33**
Tyrosine (Tyr)‡	57-110	43.17	11.11	31.51-54.82	53.17	35.27	16.16-90.18	53.17	19.23	32.99-73.35	*F*(2,15)=.35, *p*=.71, η_p^2=.04
Valine (Val)†	117-359	205.33	34.05	169.60-241.07	195.00	69.36	122.21-267.79	205.33	12.64	192.06-218.60	*F*(2,15)=.11, *p*=.90, η_p^2=.01

Notes: †Essential Amino Acid; ‡Conditionally Essential Amino Acid in PKU

Appendix Z. Chapter 6: Study 3, Part 2- Mean (SD, 95% CI) self-reported health and symptom PKU-QoLQ scores for each test session – subsample (n=6)

	T1			T2			T3			T3 vs. T1[1]			
	Mean	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	**+**	**-**	**=**	***F*, *p* and η_p^2 (session)**
Self-rated health	58.33	25.82	31.24-85.43	41.67	20.41	20.25-63.09	45.83	18.82	26.08-65.58	**3**	1	2	$F(2,15)=.94$, $p=.41$, $\eta_p^2=.11$
Aggressiveness	8.33	12.91	-5.21-21.88	0.00	0.00	n/a	4.17	10.21	-5.54-14.88	2	0	**4**	$F(2,15)=1.15$, $p=.34$, $\eta_p^2=.13$
Anxiety	66.67	20.41	45.25-88.09	**25.00****	15.81	8.41-41.59	**12.50*****	13.69	-1.87-26.87	**6**	0	0	**$F(2,15)=16.95$, $p<.001$, $\eta_p^2=.69$**
Headaches	50.00	27.39	21.26-78.74	**12.50***	13.69	-1.87-26.87	16.67	12.91	3.12-30.21	**5**	0	1	**$F(2,15)=6.89$, $p=.008$, $\eta_p^2=.48$**
Irritability	8.33	12.91	-5.21-21.88	0.00	0.00	n/a	4.17	10.21	-5.54-14.88	**4**	0	2	$F(2,15)=1.15$, $p=.34$, $\eta_p^2=.13$
Lack of concentration	54.17	29.23	23.50-84.84	**16.67***	12.91	3.12-30.21	25.00	22.36	1.53-48.47	**4**	1	1	**$F(2,15)=4.59$, $p=.03$, $\eta_p^2=.38$**
Moodiness	62.50	13.69	48.13-76.87	**20.83*****	10.21	10.12-31.54	**25.00****	15.81	8.41-41.59	**5**	0	1	**$F(2,15)=17.50$, $p<.001$, $\eta_p^2=.70$**
Sadness	45.83	10.21	35.12-56.54	**12.50****	13.69	-1.87-26.87	25.00	22.36	1.53-48.47	**4**	0	2	**$F(2,15)=6.45$, $p=.01$, $\eta_p^2=.46$**
Slow thinking	45.83	29.23	15.16-76.50	20.83	10.21	10.12-31.54	25.00	15.81	8.41-41.59	**3**	0	**3**	$F(2,15)=2.67$, $p=.10$, $\eta_p^2=.26$
Stomach aches	8.33	20.41	-13.09-29.75	8.33	20.41	-13.09-29.75	8.33	12.91	-5.21-21.88	2	1	**3**	$F(2,15)<.001$, $p=1.00$, $\eta_p^2<.001$
Tiredness	83.33	20.41	61.91-104.75	**45.83***	18.82	26.08-65.58	50.00	31.62	16.81-83.19	**3**	0	**3**	**$F(2,15)=4.29$, $p=.03$, $\eta_p^2=.36$**
Trembling hands	50.00	27.39	21.26-78.74	**12.50***	13.69	-1.87-26.87	25.00	22.36	1.53-48.47	2	1	**3**	**$F(2,15)=4.57$, $p=.03$, $\eta_p^2=.38$**

*[1] + improved; - worsened; = unchanged; ? unknown; *p<.05; **p<.01; ***p<.001 (Tukey post hoc; compared to T1)*

Appendix AA. Chapter 6: Study 3, Part 2- Mean (SD, 95% CI) self-reported PKU-QoLQ impact scores for each test session – subsample (n=6)

	T1			T2			T3			T3 vs. T1[1]			
	Mean	SD	95% CI	Mean	SD	95% CI	Mean	SD	95% CI	+	-	=	*F*, *p* and η_p^2 (session)
Emotional impact	45.83	18.28	26.65-65.02	37.50	28.06	8.05-66.95	47.50	22.53	23.86-71.14	**3**	**3**	0	$F(2,15)=.32$, $p=.73$, $\eta_p^2=.04$
Social impact	22.92	18.45	3.56-42.27	6.25	7.91	-2.05-14.55	22.57	22.80	-1.36-46.50	2	2	2	$F(2,15)=1.77$, $p=.20$, $\eta_p^2=.19$

[1] *+ improved; - worsened; = unchanged; ? unknown; *p<.05; **p<.01; ***p<.001 (Tukey post hoc; compared to T1)*

Appendix BB. Chapter 6: Study 3, Part 2- Repeated measures cognitive performance ANT tasks (All participants)

Table 1: SPSS Repeated measures results for performance of semi-adherent ET AwPKU on the Flanker (FL) task – all participants (n=10)

	Speed of correct responses (msec)	Number of errors (n)
Main effect terms		
Session	F(2,25)=.03, p=.97, η_p^2<.01	F(2,25)=.34, p=.71, η_p^2=.03
Type of stimulus[1]	**F(1,25)=21.95, p<.001, η_p^2=.47**	**F(1,25)=8.15, p=.009, η_p^2=.25**
Interaction term		
Session*Type of stimulus	**F(2,25)=4.71, p=.02, η_p^2=.27**	F(2,25)=1.26, p=.30, η_p^2=.09

[1] Condition: compatible vs. incompatible trials

Table 2: SPSS Repeated measures results for performance of semi-adherent ET AwPKU on the Memory Search 2-Dimensional Objects (MS2D) task – all participants (n=10)

	Speed of correct responses (msec)	Number of errors (n)
Main effect terms		
Session	F(2,25)=.99, p=.39, η_p^2=.07	F(2,25)=.99, p=.38, η_p^2=.07
Type of stimulus[1]	**F(1,25)=352.53, p<.001, η_p^2=.93**	**F(1,25)=23.13, p<.001, η_p^2=.48**
Interaction term		
Session*Type of stimulus	F(2,25)=1.63, p=.22, η_p^2=.12	F(2,25)=1.12, p=.34, η_p^2=.08

[1] Condition: low (1 target) vs. high (3 targets) working memory load

Table 3: SPSS Repeated measures results for performance of semi-adherent ET AwPKU on the Sustained Attention Dots (SAD) task – all participants (n=10)

	Mean series time (MST; msec)[1]	Number of errors (n)
Main effect terms		
Session	F(2,25)=.28, p=.76, η_p^2=.02	F(2,25)=.06, p=.94, η_p^2<.01
Time[2]	**F(1,25)=14.63, p=.001, η_p^2=.37**	**F(1,25)=5.08, p=.03, η_p^2=.17**
Interaction term		
Session*Time	F(2,25)=.24, p=.79, η_p^2=.02	F(2,25)=.44, p=.96, η_p^2<.01

[1] MST: average RT over a number series (each series contains 12 trials)

[2] Time: first 10 series (120 trials) vs. last 10 series (120 trials)

Table 4: SPSS Repeated measures results for performance of semi-adherent ET AwPKU on the Set Shifting Visual (SSV) task – all participants (n=10)

	Speed of correct responses (msec)	**Number of errors (n)**
Main effect terms		
Session	F(2,25)=.86, p=.44, η_p^2=.06	F(2,25)=.41, p=.67, η_p^2=.03
Type of stimulus[1]	**F(1,25)=12.13, p=.002, η_p^2=.33**	F(1,25)=2.39, p=.14, η_p^2=.09
Interaction term		
Session*Type of stimulus	F(2,25)=.46, p=.64, η_p^2=.04	F(2,25)=.34, p=.71, η_p^2=.03

[1] Condition: compatible vs. incompatible trials

Table 5: Repeated measures results for performance of semi-adherent ET AwPKU on the Tracking (TR) and Pursuit (PU) tasks – all participants (n=10)

	Accuracy of movement[1]	**Stability of movement[2]**
Main effect terms		
Session	F(2,24)=.49, p=.62, η_p^2=.04	F(2,24)=1.95, p=.16, η_p^2=.14
Task[3]	**F(1,24)=100.28, p<.001, η_p^2=.81**	**F(1,24)=12.94, p=.001, η_p^2=.35**
Interaction term		
Session*Task	F(2,24)=.16, p=.85, η_p^2=.01	F(2,24)=.06, p=.94, η_p^2<.01

[1]Accuracy of movement: mean deviation of the moving target/trajectory

[2] Stability of movement: standard deviation (SD) of the trajectory

[3] Task: tracking (TR) vs. pursuit (PU)

Appendix CC. Chapter 6: Study 3, Part 2- Mean (SD, 95% CI) speed of correct responses (msec) and number of errors (n) on the Baseline Speed (BS) and Flanker (FL) tasks for all test sessions

	T1			T2			T3			
	mean	SD	95% CI	mean	SD	95% CI	mean	SD	95% CI	*F*, *p* and η_p^2 (session)
Baseline Speed (BS)										
Speed (msec)	274.67	50.59	221.57-271.50	271.50	46.48	222.73-320.28	268.60	40.86	217.87-319.33	$F(2,14)=.02$, $p=.98$, $\eta_p^2<.01$
Flanker (FL)										
Part 1 – Compatible										
Speed (msec)	459.17	101.33	352.83-565.51	419.33	95.38	319.24-519.43	430.25	132.61	219.24-641.26	$F(2,13)=.22$, $p=.81$, $\eta_p^2=.03$
Errors (n)				0.17	0.41	-0.26-0.60	0.25	0.50	-0.55-1.05	$F(2,13)=.68$, $p=.52$, $\eta_p^2=.10$
Part 1 – Neutral										
Speed (msec)	476.67	112.71	358.39-594.95	444.00	111.63	326.85-561.15	454.00	82.93	322.05-585.95	$F(2,13)=.15$, $p=.86$, $\eta_p^2=.02$
Errors (n)	1.17	0.98	0.13-2.20	0.33	0.52	-0.21-0.88	0.75	0.96	-0.77-2.27	$F(2,13)=1.52$, $p=.26$, $\eta_p^2=.19$
Part 2 – Compatible										
Speed (msec)	487.83	117.68	364.34-611.33	512.50	122.66	383.78-641.22	523.00	151.13	335.35-710.65	$F(2,13)=.06$, $p=.90$, $\eta_p^2=.02$
Errors (n)*	1.67	1.37	0.23-3.10	1.00	1.26	-0.33-2.33	0.80	1.30	-0.82-2.42	$F(2,13)=.38$, $p=.52$, $\eta_p^2=.09$
Part 2 – Incompatible										
Speed (msec)	558.83	178.50	371.51-746.16	510.50	111.08	393.93-627.07	586.20	146.18	404.69-767.71	$F(2,13)=.30$, $p=.70$, $\eta_p^2=.05$
Errors (n)	2.17	1.94	0.13-4.20	2.17	2.40	-0.35-4.69	1.40	2.19	-1.32-4.12	$F(2,13)=.52$, $p=.81$, $\eta_p^2=.03$

Appendix DD. Chapter 6: Study 3, Part 2- Mean (SD, 95% CI) outcome measures on both parts of the Memory Search 2-Dimensional Objects (MS2D) task – subsample (n=6)

	T1			T2			T3			
	mean	SD	95% CI	mean	SD	95% CI	mean	SD	95% CI	*F*, *p* and η_p^2 (session)
Part 1										
Hits (n)	23.33	1.21	22.06-24.60	22.67	1.75	20.83-24.50	23.83	0.41	23.40-24.26	$F(2,15)=1.31$, $p=.30$, $\eta_p^2=.15$
Speed (msec)	580.33	128.37	445.61-715.05	571.67	183.53	379.06-764.27	638.67	195.41	433.60-843.74	$F(2,15)=.27$, $p=.77$, $\eta_p^2=.04$
Correct (n)	23.50	0.84	22.62-24.38	23.67	0.52	23.12-24.21	23.33	0.52	22.79-23.88	$F(2,15)=.41$, $p=.67$, $\eta_p^2=.05$
Speed (msec)	737.67	271.57	452.67-1022.66	687.50	316.98	354.85-1020.15	769.83	314.21	440.09-1099.58	$F(2,15)=.11$, $p=.89$, $\eta_p^2=.02$
Misses (n)	0.67	1.21	-0.60-1.94	1.33	1.75	-0.50-3.17	0.17	0.41	-0.26-0.60	$F(2,15)=1.31$, $p=.30$, $\eta_p^2=.15$
Speed (msec)	530.00	94.75	-321.32-1381.32	709.67	481.08	-485.41-1904.74	709.67	481.08	-485.41-1904.74	$F(2,3)=.19$, $p=.84$, $\eta_p^2=.11$
False alarms (n)	0.50	0.84	-0.38-1.38	0.33	0.52	-0.21-0.88	0.67	0.52	0.12-1.21	$F(2,15)=.41$, $p=.67$, $\eta_p^2=.05$
Speed (msec)	558.00	18.38	392.82-723.18	864.00	446.89	-3151.16-4879.16	580.75	126.38	379.66-781.84	$F(2,5)=1.29$, $p=.36$, $\eta_p^2=.34$
Part 2										
Hits (n)	17.83	4.54	13.07-22.59	19.50	3.56	15.76-23.24	21.00	3.46	17.36-24.64	$F(2,15)=1.00$, $p=.39$, $\eta_p^2=.12$
Speed (msec)	2571.00	533.40	2011.23-3130.77	1899.67	397.12	1482.92-2316.42	2040.33	578.20	1433.55-2647.12	$F(2,15)=2.91$, $p=.09$, $\eta_p^2=.28$
Correct (n)	20.00	6.03	13.67-26.33	21.67	1.63	19.95-23.38	23.67	0.52	23.12-24.21	$F(2,15)=1.54$, $p=.25$, $\eta_p^2=.17$
Speed (msec)	3424.50	361.68	3044.94-3804.06	2712.67	423.32	2268.42-3156.91	2926.83	874.88	2008.70-3844.97	$F(2,15)=2.23$, $p=.14$, $\eta_p^2=.23$
Misses (n)	6.17	4.54	1.41-10.93	5.33	3.72	1.43-9.24	3.00	3.46	-0.64-6.64	$F(2,15)=1.05$, $p=.38$, $\eta_p^2=.12$
Speed (msec)	3130.00	714.14	2380.56-3879.44	2553.33	522.92	2004.57-3102.10	2867.50	1306.40	788.72-4946.28	$F(2,13)=.72$, $p=.51$, $\eta_p^2=.10$
False alarms (n)	4.00	6.03	-2.33-10.33	1.50	1.76	-0.35-3.35	0.33	0.52	-0.21-0.88	$F(2,15)=1.59$, $p=.24$, $\eta_p^2=.18$
Speed (msec)	3491.00	985.90	1922.21-5059.79	3159.67	987.91	705.57-5613.77	2409.50	1501.19	-11078.14-15897.14	$F(2,6)=.66$, $p=.55$, $\eta_p^2=.18$

Appendix EE. Chapter 6: Study 3, Part 2- Mean (SD, 95% CI) Mean Series Time (MST; msec) and number of errors (n) on the Sustained Attention Dots (SAD) task for all test sessions

	T1			T2			T3			
	Mean	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	***F*, *p* and η_p^2 (session)**
Inhibition										
MST (msec)	10.79	2.57	8.09-13.49	9.95	2.93	6.87-13.03	9.66	2.62	6.90-12.41	$F(2,15)=.28$, $p=.76$, $\eta_p^2=.04$
Errors (n)	23.17	11.67	10.92-35.41	16.00	6.90	8.76-23.24	14.33	6.74	7.26-21.41	$F(2,15)=1.73$, $p=.21$, $\eta_p^2=.19$
Sustained attention										
MST (msec) – first 10 series	10.26	2.06	8.10-12.42	9.72	2.39	7.21-12.23	9.59	2.36	7.12-12.07	$F(2,15)=.15$, $p=.87$, $\eta_p^2=.02$
Errors (n) – first 10 series	4.83	4.36	0.26-9.40	3.50	2.59	0.78-6.22	2.83	3.37	-0.70-6.37	$F(2,15)=.50$, $p=.61$, $\eta_p^2=.06$
MST (msec) – last 10 series	12.39	3.63	8.57-16.20	11.14	3.54	7.42-14.85	10.77	2.97	7.65-13.89	$F(2,15)=.37$, $p=.70$, $\eta_p^2=.05$
Errors (n) – last 10 series	4.33	1.75	2.50-6.17	4.00	3.22	0.62-7.38	4.33	1.97	2.27-6.40	$F(2,15)=.04$, $p=.96$, $\eta_p^2<.01$

Appendix FF. Chapter 6: Study 3, Part 2- Mean (SD, 95% CI) speed of correct responses (msec) and number of errors (n) on the Set Shifting Visual (SSV) task for all test sessions

	T1			T2			T3			
	mean	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	***F*, *p* and η_p^2 (session)**
Part 1 – Compatible										
Speed (msec)	457.00	127.47	323.23-590.77	480.67	125.16	349.32-612.01	474.50	118.53	350.11-598.89	$F(2,15)=.06$, $p=.94$, $\eta_p^2<.01$
Errors (n)	0.17	0.41	-0.26-0.60	0.50	0.55	-0.07-1.07	0.50	0.55	-0.07-1.07	$F(2,15)=.87$, $p=.44$, $\eta_p^2=.10$
Part 2 – Incompatible										
Speed (msec)	1036.17	416.08	599.52-1472.82	957.33	303.21	639.13-1275.54	919.33	322.65	580.73-1257.93	$F(2,15)=.17$, $p=.84$, $\eta_p^2=.02$
Errors (n)	1.83	1.94	-0.20-3.87	1.33	0.52	0.79-1.88	1.33	1.21	0.06-2.60	$F(2,15)=.27$, $p=0.77$, $\eta_p^2=.04$
Part 3 – Compatible										
Speed (msec)	1207.17	230.79	964.97-1449.36	910.67	177.46	724.43-1096.90	945.50	199.09	736.57-1154.43	**$F(2,15)=3.80$, $p=.046$, $\eta_p^2=.34$**
Errors (n)	1.33	1.37	-0.10-2.77	0.83	1.17	-0.39-2.06	2.50	2.59	-0.22-5.22	$F(2,15)=1.33$, $p=.30$, $\eta_p^2=.15$
Part 3 – Incompatible										
Speed (msec)	1382.83	353.98	1011.35-1754.32	1192.33	362.25	812.18-1572.49	1198.17	255.08	930.48-1465.86	$F(2,15)=.66$, $p=.53$, $\eta_p^2=.08$
Errors (n)	2.17	1.94	0.13-4.20	1.50	1.38	0.05-2.95	2.67	1.51	1.09-4.25	$F(2,15)=.78$, $p=.48$, $\eta_p^2=.09$

Appendix GG. Chapter 6: Study 3, Part 2- Mean (SD, 95% CI) accuracy (mm) and stability (mm) on the Tracking (TR) and Pursuit (PU) tasks for all test sessions

	T1			T2			T3			
	mean	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	**mean**	**SD**	**95% CI**	***F*, *p* and η_p^2 (session)**
Tracking (TR)										
Accuracy[1] (mm)	1.07	1.21	-0.20-2.34	0.76	0.75	-0.03-1.55	1.48	1.50	-0.09-3.04	$F(2,15)=.55$, $p=.59$, $\eta_p^2=.07$
Stability[2] (mm)	2.00	0.90	1.06-2.95	1.40	0.45	0.92-1.87	2.10	1.06	0.99-3.21	$F(2,15)=1.22$, $p=.32$, $\eta_p^2=.14$
Pursuit (PU)										
Accuracy[1] (mm)	4.12	0.51	3.57-4.66	4.10	0.68	3.39-4.82	3.96	0.52	3.42-4.50	$F(2,15)=.13$, $p=.88$, $\eta_p^2=.02$
Stability[2] (mm)	2.89	0.82	2.03-3.75	2.36	0.37	1.97-2.74	2.81	0.93	1.84-3.78	$F(2,15)=.90$, $p=.43$, $\eta_p^2=.11$

[1] Accuracy (mm): mean deviation of the trajectory that was followed
[2] Stability (mm): standard deviation of the trajectory that was followed

Appendix HH. Chapter 6: Study 3, Part 2- Repeated measures cognitive performance ANT tasks (subsample)

Table 1: SPSS Repeated measures results for performance of semi-adherent ET AwPKU on the Flanker (FL) task – subsample (n=6)

	Speed of correct responses (msec)	Number of errors (n)
Main effect terms		
Session	F(2,14)=.14, p=.87, η_p^2=.02	F(2,14)=.35, p=.71, η_p^2=.05
Condition[1]	**F(1,14)=12.35, p=.003, η_p^2=.47**	F(1,14)=3.59, p=.08, η_p^2=.20
Interaction term		
Session*Condition	F(2,14)=3.56, p=.06, η_p^2=.02	F(2,14)=.29, p=.76, η_p^2=.04

[1] Condition: compatible vs. incompatible trials

Table 2: SPSS Repeated measures results for performance of semi-adherent ET AwPKU on the Memory Search 2-Dimensional Objects (MS2D) task – subsample (n=6)

	Speed of correct responses (msec)	Number of errors (n)
Main effect terms		
Session	F(2,15)=1.39, p=.28, η_p^2=.16	F(2,15)=1.42, p=.27, η_p^2=.16
Condition[1]	**F(1,15)=361.59, p<.001, η_p^2=.96**	**F(1,15)=12.93, p=.003, η_p^2=.17**
Interaction term		
Session*Condition	F(2,15)=2.82, p=.09, η_p^2=.27	F(2,15)=1.49, p=.26, η_p^2=.17

[1] Condition: low (1 target) vs. high (3 targets) working memory load

Table 3: SPSS Repeated measures results for performance of semi-adherent ET AwPKU on the Sustained Attention Dots (SAD) task – subsample (n=6)

	Mean series time (MST; msec)[1]	Number of errors (n)
Main effect terms		
Session	F(2,15)=.30, p=.75, η_p^2=.04	F(2,15)=.35, p=.71, η_p^2=.04
Time[2]	**F(1,15)=10.54, p=.005, η_p^2=.41**	F(1,15)=.37, p=.61, η_p^2=.02
Interaction term		
Session*Time	F(2,15)=.34, p=.71, η_p^2=.04	F(2,15)=.35, p=.71, η_p^2=.04

[1] MST: average RT over a number series (each series contains 12 trials)
[2] Time: first 10 series (120 trials) vs. last 10 series (120 trials)

Table 4: SPSS Repeated measures results for performance of semi-adherent ET AwPKU on the Set Shifting Visual (SSV) task – subsample (n=6)

	Speed of correct responses (msec)	**Number of errors (n)**
Main effect terms		
Session	F(2,15)=1.91, p=.18, η_p^2=.20	F(2,15)=2.04, p=.17, η_p^2=.21
Condition[1]	**F(1,15)=14.83, p=.002, η_p^2=.50**	F(1,15)=.94, p=.35, η_p^2=.06
Interaction term		
Group*Condition	F(2,15)=.27, p=.77, η_p^2=.03	F(2,15)=.12, p=.89, η_p^2=.02

[1] Condition: compatible vs. incompatible trials

Table 5: SPSS Repeated measures results for performance of semi-adherent ET AwPKU on the Tracking (TR) and Pursuit (PU) tasks – subsample (n=6)

	Accuracy of movement[1]	**Stability of movement[2]**
Main effect terms		
Session	F(2,15)=.39, p=.68, η_p^2=.05	F(2,15)=2.11, p=.16, η_p^2=.22
Task[3]	**F(1,15)=170.96, p<.001, η_p^2=.83**	**F(1,15)=10.22, p=.006, η_p^2=.41**
Interaction term		
Session*Task	F(2,15)=.52, p=.61, η_p^2=.06	F(2,15)=.08, p=.93, η_p^2=.01

[1]Accuracy of movement: mean deviation of the moving target/trajectory
[2] Stability of movement: standard deviation (SD) of the trajectory
[3] Task: tracking (TR) vs. pursuit (PU)

Appendix II. Chapter 6: Study 3, Part 2- Participant 7 individual results

Gender: Male

Age at screening: 39 years

Phe level at screening: 1042 µmol/L

Weight at screening: 93.6 kg

Protein intake at screening:

- from diet: ~15 grams
- from protein substitute: ~40 grams
- total protein intake: ~55 grams or 0.59 g/kg bodyweight

Weight at T3: 89.6 kg

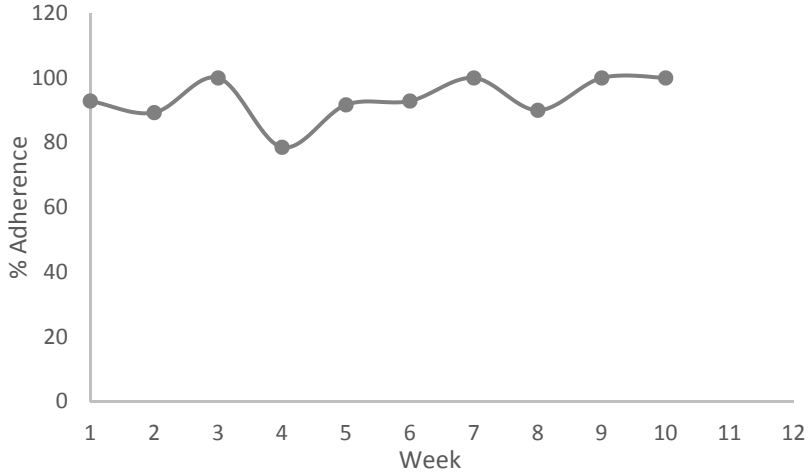
Protein intake after 12 weeks:

- from diet: ~33 grams
- from protein substitute: ~70 grams
- total protein intake: ~103 grams or 1.15 g/kg bodyweight

Notes: Did not complete final 2 weeks of the study diary, but did return 3-day food diary.

Self-reported adherence

Average self-reported adherence (%) to CGMP-AA Protein substitute throughout the trial: 94.19%.



Self-reported average weekly adherence to CGMP-AA Protein Substitute – participant 7

Nutritional status on all test days

Measures of nutritional status on all test days – participant 7

		Session		
	Reference range	**1**	**2**	**3**
Albumin† (g/L)	*35-50*	52	42	**34**
Pre-albumin† (g/L)	*0.20-0.50*	0.30	0.30	0.28
Transferrin† (g/L)	*2.00-3.20*	2.66	**1.86**	**1.79**
Alkaline Phosphatase (U/L)	*30-130*	54	47	37
Calcium† (mmol/L)	*2.20-2.60*	2.31	**2.06**	**1.85**
Copper (µmol/L)	*11-22*	12.3	11.8	11.5
CRP (mg/L)	*<5*	<5	<5	<5
Homocysteine (µmol/L)	*<18*	**18**	17	16
Iron† (µmol/L)	*14-31*	30.4	16.5	16.2
Phosphate† (mmol/L)	*0.80-1.50*	1	0.97	**0.7**
PTH (pmol/L)	*1.50-7.60*	HAEM	4	5.5
Selenium‡ (µmol/L)	*0.80-2.00*	1.12	0.97	0.88
Vitamin A (µmol/L)	*1.05-3.39*	2.11	1.99	1.59
Vitamin B12† (ng/L)	*211-911*	273	275	218
Vitamin D (nmol/L)	*>75*	**34**	**45.3**	**43.1**
Vitamin E (µmol/L)	*12-42*	21.1	22.9	18.5
Zinc‡ (µmol/L)	*9.80-17.90*	14.1	**7.9**	**9.4**

Notes: † serum; ‡ plasma; reference ranges and deficiencies highlighted in grey; elevated levels highlighted red

Full Amino Acid profile on all test days – participant 7

	Reference range (µmol/L)	Session 1	2	3
Alanine (Ala)	*248-778*	310	390	259
Arginine (Arg)	*30-198*	35	56	49
Asparagine (Asn)	*35-128*	**33**	41	45
Aspartic acid (Asp)	*45-125*	?	?	?
Citrulline (Cit)	*13-52*	37	47	41
Cysteine (Cys)	*17-124*	43	30	54
Glutamate (Glu)	*46-428*	**43**	**40**	46
Glutamine (Gln)	*270-1159*	584	652	622
Glycine (Gly)	*185-552*	348	429	414
Histidine (His)†	*81-193*	**71**	84	**80**
Isoleucine (Iso)†	*35-127*	58	94	62
Leucine (Leu)†	*80-229*	122	174	137
Lysine (Lys)†	*165-378*	**128**	175	**145**
Methionine (Met)†	*9-52*	17	23	19
Ornithine (Orn)	*117-279*	**53**	**72**	**56**
Phenylalanine (Phe)†	*120-700*[1]	**933**	**1174**	**1001**
Proline (Pro)	*123-451*	123	196	139
Serine (Ser)	*68-256*	97	131	110
Taurine (Tau)	*80-344*	201	110	90
Threonine (Thr)†	*60-231*	100	218	141
Tyrosine (Tyr)‡	*57-110*	**44**	122	**55**
Valine (Val)†	*117-359*	227	334	228

Notes: [1] recommended reference range for AwPKU in the UK prior to implementation of new European guidelines (van Wegberg et al., 2017); †Essential Amino Acid; ‡Conditionally Essential Amino Acid (in PKU); reference ranges and deficiencies highlighted in grey; elevated levels highlighted red

Appendix JJ. Chapter 6: Study 3, Part 2- Instructions for taking Dried Blood Spots (DBS) at Home

INSTRUCTIONS FOR TAKING DRIED BLOOD SPOTS (DBS) AT HOME

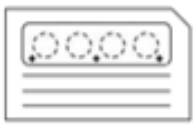
(Version 1, 10th June 2016)

You are enrolled in a study looking at the effects of a newly developed dietary protein substitute for patients suffering from PKU. To assess its effects on phenylalanine and tyrosine blood levels, we would like you to take **weekly dried blood spots** (DBS) and post them to the Biochemical Genetics lab at St. James's University hospital in Leeds, using the filter papers and pre-labelled envelopes provided by the research team. Even if you are not receiving the dietary supplement, we would like you to complete these measures as this will allow us to better investigate the effects of the new supplement.

Please try to take a blood spot on the same day and time of day every week you are enrolled in the study using the instructions on the next two pages. If you are having trouble filling all circles, please have a look at the tips on the last page. If you have any concerns or questions about the finger pricks, do not hesitate to contact the research team, contact details on the last page of this booklet.

SUPPLIES

Spring-loaded lancets / lancet device & needles



Filter papers

Pre-labelled envelopes

OPTIONAL ITEMS

Alchol wipes

Plasters

Instructions:

1. Wash your hands

Wash your hands thoroughly with soap and **warm** water. Dry with a clean towel.

Note: you may use an alcohol wipe to clean the puncture site, but if you do, you will need to discard the first drop of blood before applying to filter paper.

2. Select a puncture site

Try to use the sides of the fingertips as the tops are very sensitive. Choose a different puncture site each time you test. Repeated punctures in the same spot may cause soreness and calluses.

3. Use lancet to prick finger

Press the opening of the lancet firmly against the puncture site and push down on the trigger. Carefully lift the lancet away from the skin without smearing the blood sample.

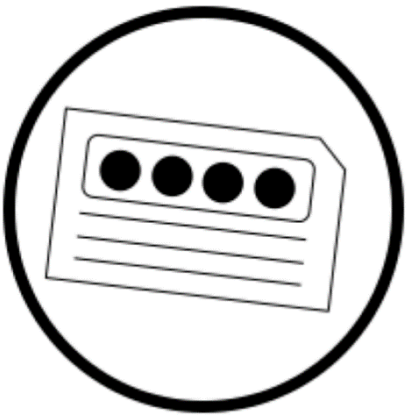
4. Start blood collection

Turn your hand over and let a drop of blood form on your fingertip.

5. Fill the filter paper

Fill filter paper circles completely with blood by applying gentle pressure to the puncture site so that the blood drops onto the filter paper. Do not actually touch the finger on the paper. In fact, the finger and the paper should not touch anything at all to avoid contamination of the sample. Do not squeeze the finger too hard as this can dilute the blood. The drop should become so large that it false freely from the finger and should nearly fill the circle on the filter paper.

Fill from one side only, but make sure that there is enough blood to soak through to the other side. Blood should be visible from both sides of all the circles. Two spots are adequate but they must fill, or very nearly fill, the circles.



6. Let filter paper dry

Let filter paper specimen air dry completely (2-3 hours). Choose a safe place where the filter paper will not be contaminated. Be careful that the paper is not placed on a surface where the blood will soak through.

7. Mail the filter paper to the lab

Complete the required information on the filter paper. Using the pre-labelled envelopes you received from the research team, mail the completed filter paper and form to the testing laboratory (the Willinck institute, St. James's University Hospital, Leeds) **within 24 hours** of collection.

TIPS

1. It is easier to get blood from **warm hands**. Try rinsing your hands under / hold your hands in warm water or holding a warm object (e.g. cup of tea, heating pad, etc.).
2. **Use gravity**: shake your hands downward / hold them below waist level.
3. **Massage your fingers** to get blood flowing to your fingertips.
4. Try **different puncture** sites. Some people like to use the sides of their fingertips because there are less nerve endings there. Others like to use the fingertips because, even though it may hurt more, they get all the blood they need from only one finger prick.
5. You may have to **adjust the lancet** to a deeper setting to get a large enough drop of blood.
6. **Do not over-saturate** the circles (i.e. do not layer drops of blood on top of each other) – this could lead to an invalid blood sample.

THANK YOU FOR YOUR PARTICIPATION IN THIS STUDY

YOUR HELP IS HIGHLY APPRECIATED!

If you have any concerns or questions regarding finger pricks or other aspects of the study, please do not hesitate to contact Denise Hofman, postgraduate researcher on the project.

E-mail: psdlh@leeds.ac.uk

Appendix KK. Chapter 6: Study 3, Part 2- Acceptability and Palatability Questionnaire (Version 1; 10th June 2016)

Participant ID _____ **Date: _____**

We would like to ask you to answer a few questions about the CGMP Protein substitute.

1. In which places did you take the CGMP Protein substitute?

Please tick all that apply

○ At home ○ Out and about

○ At work ○ Other

If other, please specify: _____

2. How much did you like the following attributes of the CGMP Protein substitute?

Please indicate your answer by ticking only one circle per attribute

	I loved it!!	I liked it	Neither like or dislike	I did not like it	I really did not like it!!
Appearance	○	○	○	○	○
Smell	○	○	○	○	○
Taste	○	○	○	○	○
Aftertaste	○	○	○	○	○
Texture/mouth feel	○	○	○	○	○
Packaging/presentation	○	○	○	○	○

3. How did you find taking/drinking the CGMP Protein substitute during the past 12 weeks?

Please tick one only

Very easy	Quite easy	OK	Difficult	Very difficult
○	○	○	○	○

4. How would you rate the convenience/ease of use of the CGMP Protein substitute?
Please tick one only

Very convenient	Convenient	OK	Inconvenient	Very inconvenient
○	○	○	○	○

5. Was the CGMP Protein substitute easier to use than your normal supplement?
Please tick one

Yes ○ No ○ Not applicable ○

6. What do you like about the CGMP Protein substitute?

7. What do you dislike about the CGMP Protein substitute?

8. If you have any additional comments, please provide them below

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE!!

Appendix LL. Chapter 6: Study 3, Part 2- Adverse Event Form (Version 1, 10-06-2016)

Participant number: ***Date of enrolment in study:*** ___/___/___

Study title	Introducing a Casein Glycomacropeptide (CGMP)-based Food for Special Medical Purposes (FSMP) in the dietary management of semi-adherent Phenylketonuria (PKU) patients.
Chief Investigator	Denise Hofman, Postgraduate Research Student, School of Psychology, University of Leeds

Has the participant had any Adverse Events during this study? ☐ **Yes** ☐ **No** ***(If yes, please list all Adverse Events below)***

Severity	Study Intervention Relationship	Action Taken Regarding Study Intervention	Outcome of AE	Expected	Serious
1 = Mild 2 = Moderate 3 = Severe	1 = Definitely related 2 = Possibly related 3 = Not related	1 = None 2 = Discontinued permanently	1 = Resolved, No Sequel 2 = AE still present- no treatment 3 = AE still present-being treated 4 = Residual effects present-not treated 5 = Residual effects present- treated 6 = Death 7 = Unknown	1 = Yes 2 = No	1 = Yes 2 = No (If yes, complete SAE form)

Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken	Outcome of AE	Expected?	Serious Adverse Event?	Initials
1.									
2.									
3.									

Appendix MM. Chapter 6: Study 3, Part 2- Study Diary

Bristol stool chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges (passed easily)
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces, **Entirely liquid**

As part of the study diary you will be asked to complete a table to provide information on the timing and nature of your daily bowel movements. When completing this table, please record the time of day of each bowel movement and, by referring to the Bristol stool chart (above), the type number (1-7) that best describes your stool/s that day. If you wish to record any additional comments about each bowel movement there is space to add this.

Day: Mon Tue Wed Thu Fri Sat Sun

Participant ID: _____ **Date: _____/_____/_____**

Time of completion: ____:____(am/pm)

Bowel Function

Please refer to the Bristol stool chart for stool type

Time	Type (tick)							Comments
	1	2	3	4	5	6	7	

Wellbeing

Please indicate the extent to which you have experienced the following feelings/symptoms today by ticking the circle that best describes your experience.

	None	Minimal	Moderate	A lot/Very	Extreme
Wind	○	○	○	○	○
Constipation	○	○	○	○	○
Diarrhoea	○	○	○	○	○
Bloating	○	○	○	○	○
Feeling sluggish	○	○	○	○	○
Bowel pain/cramp	○	○	○	○	○
Stomach pain/cramp	○	○	○	○	○
Heartburn	○	○	○	○	○
Burps	○	○	○	○	○
Nausea	○	○	○	○	○
Vomiting	○	○	○	○	○

Other information

Please use this space to make a note of anything else you have felt/noticed today

Prescribed dose *(e.g. grams or number of pouches or pots/day)*: _____ **Participant ID:** _____ **Start Date:** ____/____/_____

Time of completion: ____:____**(am/pm)**

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Actual volume taken *e.g. ½ pouch, pot, a mouthful*							
Time taken *e.g. 9am, 3pm*							
Any reason(s) as to why the prescribed dose was not taken? *e.g. feeling unwell, nausea, vomiting*							

Any additional comments?