TITLE:

UNDERSTANDING THE IMPACT OF FEEDING DEVELOPMENT, FOOD ACCEPTANCE AND EDUCATIONAL STRATEGIES IN PHENYLKETONURIA (PKU)

by

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ABSTRACT

Background: A phenylalanine restricted diet is the principal treatment in the inherited metabolic disorder phenylketonuria (PKU) but is practically difficult to apply in children. Feeding patterns and practices, growth and parental anxiety have not been reported in infants with PKU. There is limited information about dietary patterns, taste preferences, food acceptance and neophobia in children, impeded by inadequate tools to measure habitual food intake. Parents/caregivers have difficulty interpreting food labels, calculating protein intake and determining food suitability, compounded by conflicting advice from health professionals. Accurate, consistent, evidence based dietary information, and suitable educational tools are essential.

Methods: A series of longitudinal case-control studies report on growth, weaning age, feeding progression, the feeding environment, mealtime emotions and behaviours, dietary patterns, food acceptance and food neophobia in children with PKU compared with age/sex matched healthy children. Parental anxiety, coping strategies, and food neophobia were also examined. Further studies reported the validation of a food frequency questionnaire (FFQ) developed specifically for PKU for collecting data on habitual food intake and dietary patterns; development of national consensus statements for PKU on food labelling interpretation and protein allocation by the British Inherited Metabolic Disease Group (BIMDG) dietitians; and a randomised controlled trial to assess self-efficacy, label reading knowledge, and calculation of natural protein intake in caregivers/patients with PKU using a low protein multimedia diet app compared with using written/pictorial material.

Results: Children with PKU did not differ significantly from non-PKU children in most aspects of weaning progression, although they commenced weaning a month earlier, and experienced prolonged bottle and parental spoon feeding associated with parental anxiety. Teething and illness affected weaning protein substitute administration with difficulties peaking at age 10-18 months. Accelerated linear growth was observed in infants with PKU.

Protein, carbohydrate and fat as a percentage of energy intake were comparable with control children up to 12 months of age, when carbohydrate increased, but fat decreased compared to controls. This difference was validated by the FFQ which demonstrated energy and macronutrient reproducibility and validity compared with 24-hour dietary recalls. All children (PKU and non-PKU) demonstrated a preference for sweet foods compared with savoury, bitter and sour foods despite the early and persistent exposure to bitter tasting protein substitutes in infancy in PKU. Children with PKU were more food neophobic than their parents and non-PKU peers. The 112 food statements developed by BIMDG dietitians to guide interpretation of food labels and allocation of protein in PKU were translated into a patient-friendly app. The patients/caregivers of younger children found the app particularly informative and there were trends toward improved protein calculation and labelling knowledge with frequent app users.

Conclusion: Timing of solid food and weaning protein substitute introduction, parental approach, and consistent feeding routines were important in aiding their acceptance. Systematic introduction of weaning protein substitute facilitated normal growth and feeding development although parents required support in managing the complexity of feeding and to 'normalise' mealtime social inclusivity. Neophobia played a significant role in food refusal in PKU and may be related to a fear of eating unfamiliar high phenylalanine/protein foods. Any preference for bitter tasting foods in PKU may be modulated by the continual use of artificially sweetened protein substitutes in childhood. Measurement of energy, macronutrient and individual food intake using the validated FFQ will be useful in assessing changes in dietary patterns over time in patients with PKU. The low protein diet app will support education of parents/caregivers, extended family and nursery/school employees. Collectively, these studies enhance knowledge and provide innovative practical tools that will improve dietary management of PKU and will assist health professionals to effectively support patients with PKU and their families.

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TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION **1**

PHENYLKETONURIA **2**

1.1 Pathophysiology **3**

1.2 History **4**

1.3 Incidence **6**

1.4 Genetics **7**

1.5 Diagnosis **8**

1.6 Classification **9**

1.7 Monitoring **10**

1.8 Treatment **10**

1.8.1 Pharmacological treatment 10

1.8.2 Dietary treatment 13

1.9 Application of dietary treatment **22**

1.10 Protein substitutes, feeding development and weaning in PKU **23**

1.11 Food preferences, food acceptance and neophobia in PKU **25**

1.11.1 Assessing dietary patterns in PKU 25

1.11.2 Food acceptance and neophobia 26

1.12 Burden of care and education **28**

1.12.1 Low protein food labelling, protein calculation and interpretation of food suitability 28

1.12.2 Dietary resources and dietary instruction 30

1.13 Summary **38**

1.14 RESEARCH QUESTIONS **39**

REFERENCES **42**

CHAPTER 2: FEEDING DEVELOPMENT AND WEANING IN PHENYLKETONURIA **58**

CHAPTER 2A: FIFTEEN YEARS OF USING A SECOND STAGE PROTEIN SUBSTITUTE FOR WEANING IN PHENYLKETONURIA: A RETROSPECTIVE STUDY **59**

CHAPTER 2B: HOW DOES FEEDING DEVELOPMENT AND PROGRESSION ONTO SOLIDS IN PKU COMPARE WITH NON-PKU CHILDREN DURING WEANING? **60**

CHAPTER 2C: MEALTIME ANXIETY AND COPING BEHAVIOUR IN PARENTS AND CHILDREN DURING WEANING IN PKU: A CASE-CONTROL STUDY **61**

CHAPTER 2D: GROWTH, PROTEIN AND ENERGY INTAKE IN CHILDREN WITH PKU TAKING A WEANING PROTEIN SUBSTITUTE IN THE FIRST TWO YEARS OF LIFE: A CASE-CONTROL STUDY....62

CHAPTER 3: FOOD PREFERENCES, FOOD ACCEPTANCE AND NEOPHOBIA IN PHENYLKETONURIA....63

CHAPTER 3A: VALIDATION OF A LOW PROTEIN SEMI-QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE....64

CHAPTER 3B: FOOD ACCEPTANCE AND NEOPHOBIA IN CHILDREN WITH PHENYLKETONURIA: A PROSPECTIVE CONTROLLED STUDY 65

CHAPTER 3C: THE INFLUENCE OF PARENTAL FOOD PREFERENCE AND NEOPHOBIA ON CHILDREN WITH PHENYLKETONURIA 66

CHAPTER 4: BURDEN OF CARE AND EDUCATION....67

CHAPTER 4A: DEVELOPMENT OF NATIONAL CONSENSUS STATEMENTS ON FOOD LABELLING INTERPRETATION ANDPROTEIN ALLOCATION IN A LOW PHENYLALANINE DIET FOR PKU....68

CHAPTER 4B: UNIFORMITY OF FOOD PROTEIN INTERPRETATION AMONGST DIETITIANS FOR PATIENTS WITH PHENYLKETONURIA: 2020 NATIONAL CONSENSUS STATEMENTS....69

CHAPTER 4C: EFFICACY OF A NEW LOW PROTEIN MULTIMEDIA APP FOR PKU....70

CHAPTER 5: DISCUSSION....71

5.1 Overview of Results....72

5.2 Weaning protein substitute....72

5.3 Growth, macronutrient intake and weaning protein substitute....76

5.4 Feeding development and progression during weaning....80

5.5 Parental feeding strategies, anxiety and coping behaviour during weaning.83

5.6 Food frequency questionnaires and dietary patterns....85

5.7 Food acceptance and neophobia....87

5.8 Food labelling interpretation and protein calculation....90

5.9 A low-protein multimedia diet app for PKU....93

5.10 CONCLUSION....96

REFERENCES....98

APPENDICES....105

APPENDIX 1: SUPPLEMENTARY DATA FOR CHAPTER 2D....106

APPENDIX 2: SUPPLEMENTARY DATA FOR CHAPTER 3A....111

APPENDIX 3: SUPPLEMENTARY DATA FOR CHAPTER 4B....170

APPENDIX 4: SUPPLEMENTARY DATA FOR CHAPTER 4C....182

LIST OF FIGURES

CHAPTER 1. INTRODUCTION

Figure 1.1 Overview of the role of diet in PKU.....15
Figure 1.2 Food pyramid for PKU[114]. 15
Figure 1.3 The advantages and challenges of protein substitute composition and adherence in PKU 18
Figure 1.4 Feeding issues in Phenylketonuria (PKU).....22

CHAPTER 2A: FIFTEEN YEARS OF USING A SECOND STAGE PROTEIN SUBSTITUTE FOR WEANING IN PHENYLKETONURIA: A RETROSPECTIVE STUDY 59

Figure 1. Protein equivalent (g/kg/day) by age for weaning protein substitute, infant protein substitute and total protein

Figure 2. Number of individual incidences of weaning protein substitute and food refusal by age group

CHAPTER 2B: HOW DOES FEEDING DEVELOPMENT AND PROGRESSION ONTO SOLIDS IN PKU COMPARE WITH NON-PKU CHILDREN DURING WEANING? 60

Figure 1. Number of children displaying different negative behaviours associated with protein substitute administration at each age

Figure 2. Percentage of subjects reporting gastrointestinal symptoms at (a) 6 months, (b) 12 months, (c) 18 months, and (d) 24 months of age

CHAPTER 2C: MEALTIME ANXIETY AND COPING BEHAVIOUR IN PARENTS AND CHILDREN DURING WEANING IN PKU: A CASE-CONTROL STUDY 61

Figure 1. Mean (1) maternal and (b) child mood during children's mealtimes

Figure 2. Mean maternal feelings about giving protein substitute to their child with phenylketonuria (PKU) at different child ages in the first two years

Figure 3. Mean control maternal feelings about feeding their child solid foods at different ages in the first two years

Figure 4. Mean anxiety scores for mothers of children with PKU and control mothers

Figure 5. Total group scores for emotive symptoms experienced by mothers of PKU children and control mothers

Figure 6. Mean child blood phenylalanine levels by age

CHAPTER 2D: GROWTH, PROTEIN AND ENERGY INTAKE IN CHILDREN WITH PKU TAKING A WEANING PROTEIN SUBSTITUTE IN THE FIRST TWO YEARS OF LIFE: A CASE-CONTROL STUDY.....62

Figure 1. Mean weight, length and body mass index (BMI) z-scores for PKU and control groups by age. (a) mean weight z-scores for PKU and control groups by age; (b) mean length z-scores for PKU and control groups by age; (c) mean BMI z-scores for PKU and control groups by age

Figure 2. Mean male BMI z-scores for PKU vs control group by age

Figure 3. Mean female length z-scores for PKU vs control groups by age

Figure 4. Mean PKU BMI z-scores for male vs female by age

Figure 5. Mean protein intake g/kg/day from total protein substitute, weaning protein substitute and Phe-free infant formula

Figure 6. Mean carbohydrate intake (g/kg/day) for PKU and control groups by age

Figure 7. Mean energy intake (kcal) from both protein substitute (infant and weaning) by age

Figure 8. Mean % dietary reference values (DRV) for energy for PKU and control groups by age

CHAPTER 3A: VALIDATION OF A LOW PROTEIN SEMI-QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE.....64

Figure 1. Study design

Figure 2. Bland-Altman plots for PKU and control group macronutrient intake FFQ 1 vs. FFQ 2

Figure 3. Bland-Altman plots for PKU and control group macronutrient intake FFQ vs. dietary recalls

CHAPTER 3C: THE INFLUENCE OF PARENTAL FOOD PREFERENCE AND NEOPHOBIA ON CHILDREN WITH PHENYLKETONURIA 66

Figure 1. Median total scores for food neophobia for children with PKU and control children and their parents

Figure 2. Median total scores for general neophobia for children with PKU and control children and their parents

CHAPTER 4C: EFFICACY OF A NEW LOW PROTEIN MULTIMEDIA APP FOR PKU.....70

Figure 1. App study design and development procedures

Figure 2. “PKU Bite”® logo

LIST OF TABLES

CHAPTER 1. INTRODUCTION

Table 1.1 Types of protein substitute for PKU suitable for children 20
Table 1.2 Summary of some of the practical issues of food protein label interpretation and assessment of food suitability.....29
Table 1.3 Current apps for Inherited metabolic disorders of amino acid metabolism 33

CHAPTER 2A: FIFTEEN YEARS OF USING A SECOND STAGE PROTEIN SUBSTITUTE FOR WEANING IN PHENYLKETONURIA: A RETROSPECTIVE STUDY 59

Table 1. Median protein equivalent from weaning protein substitute, infant protein substitute, natural protein and total protein

Table 2. Issues with administration of weaning protein substitute and food/exchanges

CHAPTER 2B: HOW DOES FEEDING DEVELOPMENT AND PROGRESSION ONTO SOLIDS IN PKU COMPARE WITH NON-PKU CHILDREN DURING WEANING? 60

Table 1. Subject demographics

Table 2. First weaning foods and textures

Table 3. Average age for progression onto different textured foods

Table 4. Total fluid intake (mL) per day; PKU vs. control

Table 5. Age of subjects for attaining feeding skills (%) (n=20 PKU; n=20 control)

Table 6. Mean age (months) of feeding skills development

Table 7. Percentage of children fed in different environments at different ages

Table 8. Median number of meals or snacks per day

CHAPTER 2C: MEALTIME ANXIETY AND COPING BEHAVIOUR IN PARENTS AND CHILDREN DURING WEANING IN PKU: A CASE-CONTROL STUDY 61

Table 1. Means and standard deviations for phenylketonuria (PKU) coping health inventory for parents (CHIP) subsets compared with normative data

Table 2. Means and standard deviations for PKU CHIP subsets compared with control mothers

CHAPTER 2D: GROWTH, PROTEIN AND ENERGY INTAKE IN CHILDREN WITH PKU TAKING A WEANING PROTEIN SUBSTITUTE IN THE FIRST TWO YEARS OF LIFE: A CASE-CONTROL STUDY.....62

Table 1. Total energy intake (kcal/day) and percentage of total energy intake attributed to protein substitutes (infant and weaning) and food (mean (range))

CHAPTER 3A: VALIDATION OF A LOW PROTEIN SEMI-QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE.....64

Table 1. Meal patterns – percentage of subjects consuming meals and snacks, or missing meals for FFQ 1, FFQ 2 and dietary recalls (PKU and control groups)

Table 2. Percentage of subjects consuming natural protein exchanges (1g protein = 50mg phenylalanine) and protein substitute at meals and mid meals (PKU group only)

Table 3. Nutrients – median (IQR) intakes FFQ 1 vs. FFQ 2 and FFQ vs. dietary recalls for PKU and control groups; and FFQ PKU vs. control

CHAPTER 3B: FOOD ACCEPTANCE AND NEOPHOBIA IN CHILDREN WITH PHENYLKETONURIA: A PROSPECTIVE CONTROLLED STUDY 65

Table 1. Mean values for categorisation of foods by age group

Table 2. Mean values for ranking of foods by age group

Table 3. Mean (range) number of times/week certain foods/food groups consumed

Table 4. Comparison of mean response for general and food neophobia questions

CHAPTER 3C: THE INFLUENCE OF PARENTAL FOOD PREFERENCE AND NEOPHOBIA ON CHILDREN WITH PHENYLKETONURIA 66

Table 1. Subject demographics – parental age, ethnicity and highest educational level achieved

Table 2. Median frequency (per week) of consumption of certain food/food groups consumed by parents and children from both PKU and control groups

Table 3. Comparison of food neophobia and food variety with mother's educational level

CHAPTER 4A: DEVELOPMENT OF NATIONAL CONSENSUS STATEMENTS ON FOOD LABELLING INTERPRETATION ANDPROTEIN ALLOCATION IN A LOW PHENYLALANINE DIET FOR PKU……68

Table 1. Variation in dietary advice given to patients by BIMDG dietitians

Table 2. Summary of consensus statements

Table 3. Calculating food protein exchanges from protein analysis on the food label

Table 4. New exchanges (previously exchange-free)

Table 5. Summary of final consensus statements for PKU

CHAPTER 4B: UNIFORMITY OF FOOD PROTEIN INTERPRETATION AMONGST DIETITIANS FOR PATIENTS WITH PHENYLKETONURIA: 2020 NATIONAL CONSENSUS STATEMENTS.....69

Table 1. Category 1: Foods high in protein or phenylalanine

Table 2. Category 2: Foods allocated without restriction

Table 3. Category 3: Manufactured foods allocated as part of an exchange system based on protein or phenylalanine content

Table 4. Category 4: Exchange fruit and vegetables (Phenylalanine >75mg/100g)

CHAPTER 4C: EFFICACY OF A NEW LOW PROTEIN MULTIMEDIA APP FOR PKU.....70

Table 1. Characteristics of subjects

Table 2. Baseline use of apps and dietary information

Table 3. Frequency of using apps

Table 4. Percentage of respondents accessing different sources of dietary information at least weekly at baseline, 12 weeks and 6 months

Table 5. Self-efficacy and patient activation measure mean scores for app and control groups at baseline, 12 weeks and 6 months

Table 6. Mean percentage of correct answers on protein-labelling knowledge/ interpretation questionnaire by all respondents

Table 7. Accuracy of calculating protein intake from diet diaries

Table 8. Median blood Phe levels at baseline, 12 weeks and 6 months

Table 9. Most useful features about the app

Table 10. Preferred method of obtaining diet information

Table 11. Feedback about “PKU Bite®” app

CHAPTER 5. DISCUSSION

Table 5.1 Energy and macronutrient composition of infant, weaning and third stage protein substitutes for PKU.....74

LIST OF SUPPLEMENTARY DATA/APPENDICES

APPENDIX 1: SUPPLEMENTARY DATA FOR CHAPTER 2D.....106
Table S1. Longitudinal modelling results for weight z-scores
Table S2. Longitudinal modelling results for head circumference z-scores
Table S3. Longitudinal modelling results for BMI z-scores
Table S4. Longitudinal modelling results for length z-scores
Table S5. Longitudinal modelling results for length-for-age difference
Table S6. Longitudinal modelling results for total protein equivalent g/kg/day
Table S7. Longitudinal modelling results for protein intake g/100kcal
Table S8. Longitudinal modelling results for carbohydrates g/kg/day
Table S9. Longitudinal modelling results for total energy kcal/day
Table S10. Total energy intake as a percentage of DRV for PKU and control groups males vs females (mean (range))

APPENDIX 2: SUPPLEMENTARY DATA FOR CHAPTER 3A.....111
Table S1. Mean z-scores for BMI, weight and height for PKU and control children
Table S2. FFQ food items mean g/week FFQ 1 vs. FFQ 2 (PKU and control)
Table S3. FFQ food items mean g/week FFQ vs. dietary recalls (PKU and control)
Table S4. Kappa coefficient statistics
Supplementary S1. Script for FFQ (PKU)
Supplementary S2. Script for FFQ (control)
Supplementary S3. Script for 24-h dietary recall (PKU)
Supplementary S4. Script for 24-h dietary recall (PKU)
Appendix A. Low protein food frequency questionnaire
Appendix B. Low protein picture portion size guide for food frequency questionnaire
Appendix C. Control food frequency questionnaire
Appendix D. Control picture portion size guide for food frequency questionnaire

APPENDIX 3: SUPPLEMENTARY DATA FOR CHAPTER 4B.....170
Appendix A. Low protein labelling consensus statements for people with PKU on ≤10g/day of natural protein

APPENDIX 4: SUPPLEMENTARY DATA FOR CHAPTER 4C.....182
Supplementary data S1. Low protein labelling knowledge questionnaire

LIST OF ABBREVIATIONS

AA	Amino Acids
ACBS	Advisory Board for Borderline Substances
ANOVA	Analysis of Variance
BAI	Beck Anxiety Inventory
BBB	Blood Brain Barrier
BCAA	Branch Chain Amino Acids
BCH	Birmingham Children's Hospital NHS Foundation Trust
BDA	British Dietetic Association
BH4	Tetrahydrobiopterin
BIMDG	British Inherited Metabolic Diseases Group
BIMDG-DG	British Inherited Metabolic Diseases Group – Dietitians Group
BMI	Body Mass Index
BWC	Birmingham Women's and Children's NHS Foundation Trust
GF	Gluten Free
GMP	Glycomacropeptide
CGMP Casein	Glycomacropeptide
CHIP	Child Health Inventory for Parents
CI	Confidence Interval
CHO	Carbohydrate
CNS	Central Nervous System
DMIMD	Dietary Management of Inherited Metabolic Disorders
DRV	Dietary Reference Value
DTAC	Digital Technology Assessment Criteria
EAR	Estimated Average Requirements
ESPKU	European Society for Phenylketonuria
EU	European Union
FAO	Food and Agriculture Organisation (of the United Nations)
FFQ	Food Frequency Questionnaire
GP	General Practitioner
IMD	Inherited Metabolic Disease
iOS	iPhone Operating System
IQ	Intelligence Quotient
IQR	Interquartile Range
IRAS	Integrated Research Application System

LAT1	L-Amino Acid Transporter 1
LCP	Long Chain Polyunsaturated Fatty Acid
LEU	Leucine
LNAA	Large Neutral Amino Acids
LP	Low Protein
NBS	Newborn Screening
NHS	National Health Service (UK)
NRES	National Research Ethics Committee
NSPKU	National Society for PKU (UK)
PAH	Phenylalanine Hydroxylase
PE	Protein Equivalent
PEG PAL	Pegylated Phenylalanine Ammonia-Lyase
Phe	Phenylalanine
PKU	Phenylketonuria
PPA	Phenyl Pyruvic Acid
PS	Protein Substitute
RCT	Randomised Controlled Trial
RDI	Reference Daily Intake
REC	Research Ethics Committee
SD	Standard Deviation
SLPF	Special Low Protein Food
SSIEM	Society for the Study of Inborn Errors of Metabolism
UK	United Kingdom
UNU	United Nations University
USA	United States of America
USDA	United States Department of Agriculture
WHO	World Health Organisation
WPS	Weaning Protein Substitute

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Dr Schar Medical Nutrition Magazine for health care professionals in inborn errors of metabolism (distributed internationally) (2015): Taste acceptance in PKU: do bitter tasting L-amino acid supplements influence this?

NSPKU News & Views 2017: New PKU low protein labelling guidelines

NSPKU News & Views 2018: Low protein APP for PKU

PKU Bite® App on Google Play January 2022 and App Store February 2022.

LIST OF ABSTRACTS, POSTERS AND PRESENTATIONS RELATED TO THIS THESIS

ABBREVIATIONS:

BIMDG: British Inherited Metabolic Disease Group

DMIMD: Dietary Management of Inherited Metabolic Disorders

ESPKU: European Society for Phenylketonuria

ICIEM: International Congress of Inborn Errors of Metabolism

NSPKU: National Society for PKU (UK)

SSIEM: Society for the Study of Inborn Errors of Metabolism

PRESENTATIONS RELATED TO THIS THESIS:

SSIEM Innsbruck, Austria 2-5 September 2014

Taste preferences of young children with PKU.

BIMDG Dietitian's Group Meeting, Liverpool 26 November 2015

Evans S. Results of PKU Low Protein Labelling Questionnaire

Vitaflo Metabolic Dietitian's Meeting, Liverpool 27 November 2015

Practicalities of weaning: second stage protein substitutes for weaning: 15 years of experience.

DMIMD, London 22 April 2016

Weaning patterns and practices in PKU: a longitudinal case-control study.

BIMDG conference, Birmingham 30 June 2016

Comparing food preferences and neophobia in parents of children with PKU and age-matched control children.

BIMDG conference, London 30 June 2017

UK BIMDG Dietetic Consensus Guidelines on Low Protein Labelling Interpretation and Food Allocation for Patients and Caregivers With PKU.

Feeding infants & young children with PKU, Birmingham 18 July 2017

Taste preferences & neophobia in children with PKU.

BIMDG Conference, Nottingham 21 June 2018

Low Protein App.

Update on labelling guidelines.

NSPKU Family Low Protein Conference, Birmingham 11 November 2018

Low protein App.

BCH Teenage PKU Event, Birmingham 24 November 2018

Low protein App.

NSPKU study day, Liverpool 29 November 2018

Low protein labelling guidelines and App.

Nutricia promotional filming for Anamix First Spoon publications, Birmingham 6 December 2018

Weaning protein substitute.

DMIMD remote conference 22 April 2021

Dietetic management in IMD: the App.

ESPKU remote conference 15 October 2021

A mobile app to guide food suitability of food choice in the PKU diet.

Vitaflo remote conference 3 December 2021

A mobile media dietary application for PKU.

SSIEM Dietitians Group remote Annual Symposium 3 March 2022

A new dietary app for PKU to guide protein label interpretation.

CONFERENCE ABSTRACTS/POSTERS RELATED TO THIS THESIS:

SSIEM Innsbruck, Austria 2-5 September 2014

Evans S, Wildgoose J, Cochrane B, Daly A, Chahal S, MacDonald A. Feeding patterns and practices in young children with PKU during the first two years of life: acceptability and efficacy of a new protein substitute. J Inherit Metab Dis, 2014. Vol 37 Issue S1:27-185.

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Evans S, Daly A, Ashmore C, Chahal S, MacDonald A. Weaning patterns and practices in PKU: a longitudinal case-control study.

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Evans S, Yilmaz O, Pinto A, Daly A, MacDonald A. Low Protein Mobile and Web-Based Diet Applications: What's Out There?

Evans S, BIMDG dietitians Group. UK BIMDG Dietetic Consensus Guidelines on Low Protein Labelling Interpretation and Food Allocation for Patients and Caregivers With PKU.

Evans S, Daly A, Wildgoose J, Cochrane B, Chahal S, Ashmore C, MacDonald A. Growth of Children With Phenylketonuria Taking a Semi-Solid Weaning Protein Substitute is Comparable to Control Children in the First 2 Years of Life.

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Evans S, Ashmore C, Daly A, Dhadwar P, Syed A, Jackson R, Pinto A, MacDonald A. A new dietary app for PKU to guide protein label interpretation.

GRANT APPLICATIONS

BWCH Charities Research Fund (2021) Dietary App for phenylketonuria: can it improve knowledge, self-efficacy and dietary management? £18,750

Nutricia Metabolics Research Fund (2020) Dietetic management in IMD: the App €20,000

BCH Research Foundation (2017) £60,000 A low protein food application (APP) for inherited metabolic disorders (IMD) of amino acid metabolism

BCH Research Foundation (2015) £10,000 Dietary patterns analysis in PKU

BCH Research Foundation (2014) £10,000 Taste preferences and food neophobia in children with PKU

CHAPTER 1: INTRODUCTION

PHENYLKETONURIA

Phenylketonuria (PKU) is a rare inherited metabolic disorder (IMD) due to a deficiency or absence of the hepatic enzyme, phenylalanine hydroxylase (PAH), resulting in partial or complete inability to catalyse the hydroxylation of the essential amino acid phenylalanine to tyrosine [1-4]. If untreated, this leads to deficiency of tyrosine and accumulation of phenylalanine in the blood and brain tissue. It causes intellectual disability, microcephaly, seizures, hyperactivity, aberrant behaviour, psychiatric symptoms, neuromotor disturbances, a musty odour and eczema [1, 2, 4-6]. Traditional treatment is a phenylalanine restricted diet supplemented with phenylalanine-free amino acids or low phenylalanine casein glycomacropeptide (CGMP). In a subset of milder cases, an oral pharmacological adjunct treatment, sapropterin, is used in combination with dietary phenylalanine restriction. In patients over 16 years of age, a subcutaneously administered phenylalanine ammonium lyase enzyme treatment, pegvaliase (Palynziq), may be used in the USA and some European countries. Early and continuous lifelong treatment prevents major neurocognitive deficits and enables individuals to develop normally, achieve expected educational outcomes, obtain employment, and live independently as adults [1, 2, 4, 6-14]

However, even with early and continuous treatment, individuals with PKU on average, have a lower intelligence quotient (IQ) than non-PKU peers and they experience impairment in executive functioning (e.g. planning, problem solving, information processing, and attention), psychiatric, behavioural and social problems, cerebellar ataxia, tremor and visual abnormalities [1, 2, 4, 6-16]. These deficits are frequently associated with higher mean lifetime, blood phenylalanine concentrations or increased

variability. Some deficits may still occur irrespective of degree of metabolic control [4, 8, 9, 16-22]

1.1 Pathophysiology

Normally, the essential amino acid phenylalanine is hydrolysed to the amino acid tyrosine by the hepatic enzyme PAH. This requires the presence of cofactors: reduced pterin tetrahydrobiopterin (BH4), ferrous iron, and molecular oxygen [1, 4, 7, 23]. BH4 is oxidised in the reaction and enzymatically recycled back into BH4 for further hydroxylation of phenylalanine [7]. In PKU, absence or deficiency of PAH leads to hyperphenylalaninaemia (HPA) and hypotyrosinaemia [7]. Deamination of phenylalanine leads to the formation of phenylketones such as phenylpyruvate which are subsequently excreted in the urine [7].

The main pathological manifestations of PAH deficiency affect the brain, associated with excessive blood and brain phenylalanine concentrations, although HPA alone does not fully explain the mechanism of brain dysfunction in PKU, which remains unclear [7, 24-26]. There is evidence of hypomyelination/demyelination and changes in brain white matter in PKU that are implicated in the brain damage seen in untreated or poorly treated patients. Reasons for white matter change are multifactorial and may include alterations in lipid metabolism; impaired glucose metabolism, myelin synthesis, and protein synthesis; abnormal epigenetic alterations; and oxidative stress associated with HPA [7, 27-32]. There is also evidence of grey matter abnormalities and other central nervous system (CNS) dysfunctions associated with PKU [27, 33].

There is a relationship between plasma phenylalanine concentrations and cerebral protein synthesis, with decreased synthesis at concentrations above 600 μmol/L [26]. Large neutral amino acids (LNAA) enter the brain across the blood brain barrier (BBB) via the carrier, L-amino acid transporter 1 (LAT1), which has a higher affinity for phenylalanine than for the other LNAA (histidine, isoleucine, leucine, methionine, threonine, tryptophan, valine, tyrosine). High blood phenylalanine levels competitively inhibit the transport of other LNAA, such as tryptophan (a precursor of the neurotransmitter serotonin) and tyrosine (a precursor of the neurotransmitters dopamine and norepinephrine) at the BBB causing reductions in protein and neurotransmitter synthesis and demyelination [25, 30]. The same mechanisms and white matter changes appear to operate even in early and continuously treated individuals with blood phenylalanine levels within the range expected [25].

There is also evidence of reduced antioxidant defenses and oxidative stress associated with high blood phenylalanine concentrations, indicated by increased lipid peroxidation and microglial activation in mouse models [7, 30]. HPA is also implicated in epigenetic alterations of gene expression patterns in the brain [7].

1.2 History

Imbecillitas phenylpyruvica (the original name for PKU), was first described by a Norwegian chemist and physician, Asbjorn Folling, in 1934 [34, 35] when he identified the phenylketone, phenyl pyruvic acid (PPA) in the urine of two siblings with developmental delay [36]. Folling suggested that the condition was due to an excess of the amino acid phenylalanine which was converted to PPA and excreted in the urine [23].

It was subsequently identified as a recessively inherited disorder and named phenylketonuria (PKU) [37]. It was unclear what caused this disorder, although a deficiency of tyrosine and an excess of phenylalanine were considered and several attempts to identify a dietary treatment ensued [5, 23, 38, 39]. Reducing dietary protein intake by more than half was discovered to initially reduce urinary PPA but also led to weight loss and a subsequent increase in PPA (due to endogenous breakdown of tissue protein) [38]. It was not until 1953 that the enzyme deficiency was identified [40].

In 1951, Bickel, Gerrard, and Hickman at Birmingham Children's Hospital, UK, in consultation with chemist Dr Louis Woolf from London, developed the first phenylalanine restricted diet to treat PKU in a 2 year old girl [5, 39, 41]. Using a casein hydrolysate formula, phenylalanine was selectively removed by adsorption chromatography with charcoal and administered as a drink along with a low phenylalanine diet [5, 39, 41]. As the separation process also removed tyrosine and tryptophan these were supplemented in the final formula [41]. On a daily intake of 17g of protein and 0.3g of phenylalanine, growth was satisfactory, blood phenylalanine levels normalised, no PPA was excreted in the urine, the musty odour and eczema disappeared, and psychosocial behaviour, motor function and concentration improved but it could not reverse the brain damage already sustained [5]. Two phenylalanine challenges rapidly produced behavioural and motor skill deterioration, thereby substantiating the efficacy of the diet [5]. In 1954 their phenylalanine-free casein hydrolysate became commercially available in a powdered form [41]. It was established that a low phenylalanine diet supplemented with a low phenylalanine protein hydrolysate formula and sufficient carbohydrate and fat to prevent catabolism were essential components of dietary treatment for PKU [42].

Bickel suggested that to achieve the best outcome, dietary treatment needed to commence in infancy [5, 39]. In 1961 the first sibling of a patient with PKU, tested and diagnosed soon after birth, and treated with a low phenylalanine diet, verified that both normal development and IQ were possible [36]. The first large-scale screening program commenced in 1958 in Cardiff, testing the urine of 6-week old infants [41, 43]. Then in 1963 universal newborn screening (NBS) launched in the USA using dried blood spots (Guthrie test) [35, 44].

Protein substitute based on phenylalanine-free amino acids were developed in the 1960s but during these early years, nutritional deficiencies, malnutrition and even death were associated with dietary treatment [42]. In 1980, the first UK nutritionally complete amino acid formula was produced and in 1988 the first age-specific protein substitutes were developed [42, 45]. There have been significant improvements in the composition and presentation of protein substitutes in the last 20 years [42, 46-51] involving alterations in the quantity and type of fat and carbohydrate [52], including addition of long chain polyunsaturated fatty acids [53-56], prebiotic oligosaccharides [57], use of casein glycomacropeptide [58-71], and prolonged release protein substitutes [72-76].

1.3 Incidence

PKU, whilst rare, is the most frequently occurring inherited metabolic disorder (IMD) with an estimated global incidence of approximately 6 in 100,000 (range: 0.3 - 38.1 per 100,000) [77] or 1 in 24,000 live births [78]. Prevalence of PKU shows considerable geographic variability, with an estimated incidence in Europe of 1 in 10,000 newborn babies, but higher rates in countries such as Italy (1 in 4000), Ireland (1 in 4500)

and Turkey (1 in 6000) and much lower rates in Finland (1 in 100,000), Japan (1 in 125,000) and Thailand (1 in 225,000) [1, 2, 77-79]. In the USA incidence is estimated to be approximately 1 in 25,000 [78]. Globally, the highest regional prevalence is seen in the Eastern Mediterranean followed by Europe and the lowest incidence in Southeast Asia [77, 78]. Incidence tends to be higher in countries with a high prevalence of consanguinity within the population [4, 77]. The phenotype also varies from country to country and region to region with some countries in the Mediterranean such as Spain experiencing milder forms, and Eastern Europe experiencing the more severe classic PKU [4, 80].

The UK reports an incidence of approximately 1 in 10,000 [78] and the West Midlands 1 in 12,400 [81]. It is estimated that there are approximately 2400 patients (40% children) with PKU monitored by IMD centres in England with 77% of these on active dietary treatment, 48% of whom are children [82]. In 2017-2018, there were 113 positive diagnoses from 755,704 newborn screenings (1.22 in 10,000) across the UK [83].

1.4 Genetics

PKU is an autosomal recessive condition [5, 6, 77] caused by mutations in the gene coding for phenylalanine hydroxylase (PAH), located on chromosome 12q23.1, consisting of 13 exons and covering 100kb of genetic data in total. There are over 1550 different mutations in the gene coding for PAH that are known to be associated with PAH deficiency [84] resulting in a range of genotypes and phenotypes. Approximately 75% of patients have 2 different mutations and one may influence the expression of the other [80, 85].

The position and nature of the mutation determines the phenotype, varying from very mild to severe HPA [4]. Patient genotyping is not required for diagnosis but can provide information on metabolic phenotype [2, 8, 80]. Based on gene variants, the level of BH4 responsiveness can be predicted in around 71% of patients, and PKU severity (based on level of residual PAH activity) in 78%, but the nature of the relationships is complex and not fully understood for all mutation combinations [80].

1.5 Diagnosis

At birth, infants have normal blood phenylalanine and are initially asymptomatic, however, if untreated, neurological damage is progressive, gradually becoming apparent over several months as a normal protein intake is established leading to elevated blood phenylalanine levels [3, 77]. In patients with PKU, every 4 week delay in starting treatment, causes a reduction in IQ of 4 points [86]. Therefore, diagnosis and treatment with a phenylalanine restricted diet within the first 10 days of birth is advocated to prevent neurological damage and ensure normal development [1, 2, 4, 77, 86].

PKU is now screened by national newborn screening (NBS) programs in many countries [2, 8]. In the UK, due to routine neonatal screening at 5 days of age, children are diagnosed early, and treatment is usually commenced within 12 days after birth [87]. At diagnosis, infants may present with blood phenylalanine concentrations >1000μmol/L and all patients with untreated levels >360μmol/L should be treated [2, 8].

Despite NBS, late diagnoses still occur due to absence or partial coverage of NBS in the country of origin (e.g., Africa and the Middle East); birth before the implementation of NBS (e.g., Saudi Arabia and Egypt), or technical failure of NBS (e.g., false negative

results or failure to follow up positive results) [88]. In the USA and Europe, most missed cases are due to immigrants and refugees from countries with no NBS [88].

1.6 Classification

In non PKU individuals, blood phenylalanine concentrations are between 50-110 µmol/L [4]. Traditionally, categories for classification of PKU were defined as *mild*, *moderate* or *severe/classic* based on the highest untreated blood phenylalanine concentration following diagnosis [1]. In *classic or severe PKU* there is total or almost total deficiency of PAH, leading to high pretreatment blood phenylalanine concentrations typically >1200 µmol/l, *moderate PKU* with levels of 900–1200 µmol/l, *mild PKU* with levels of 600–900 µmol/l and *mild HPA* with levels 120-600 µmol/l [3, 4, 6, 9]. It is estimated that approximately 55% of cases have classical PKU [80]. However, because of early screening, infants may not have reached the peak blood phenylalanine concentration without treatment at the time of sampling, so this method of classification is potentially erroneous [1, 4, 9].

The European PKU guidelines proposed a new classification based on treatment needs (dietary, BH4 or both) to maintain blood phenylalanine in the recommended target range [1, 2, 89]. Using this new classification, no intervention is required if untreated blood phenylalanine concentrations are <360µmol/L; up to the age of 12 years, treatment is recommended if untreated blood phenylalanine is between 360-600µmol/L, and lifelong treatment is recommended if untreated blood phenylalanine is >600µmol/L at any age [1, 2].

1.7 Monitoring

All patients with PKU require long-term monitoring of blood phenylalanine to assess dietary control and adherence as well as nutritional, clinical, anthropometric, biochemical, psychosocial and neurocognitive follow up. Minimum requirements for management and follow up are based on age, adherence and clinical status [1, 2, 90]. Treatment and follow-up should be conducted by specialised metabolic centres ideally with access to physicians, dietitians, biochemistry, neuropsychology/ psychology and a social worker [1, 2]. Blood phenylalanine samples should be taken at the same time of day and preferably after fasting (i.e., in the morning after an overnight fast) as this is when phenylalanine levels are at their highest [2, 91, 92].

1.8 Treatment

The core treatment for PKU is a phenylalanine restricted diet accompanied by a low or phenylalanine-free amino acid or casein glycomacropeptide (CGMP) protein substitute, special low protein foods, and calculated/measured amounts of some protein containing foods to meet phenylalanine requirements within tolerance limits. Pharmacological treatments such as sapropterin, LNAA and pegvaliase that may reduce the need for dietary restriction and protein substitutes are suitable for use in subgroups of patients.

1.8.1 Pharmacological treatment

Tetrahydrobiopterin (BH4)

Around 25-30% of patients with mild or moderate PKU, benefit from a pharmacological treatment, sapropterin dihydrochloride, that acts as a pharmacological

chaperone [8, 80, 88, 93-103]. This is usually given as an adjunct treatment with a phenylalanine restricted diet, but occasionally can fully replace dietary treatment. In responsive patients, it decreases blood phenylalanine and/or an increases dietary phenylalanine tolerance. There is also less variability in blood phenylalanine concentrations [1, 2, 6, 8, 9, 104].

Patients can be tested for BH4 responsiveness firstly by genotyping, followed by a sapropterin loading test which analyses blood phenylalanine before and after a single daily dose of 20mg/kg over varying periods of time (24 hours, 48 hours or 28 days) depending on national practices [1, 2, 8]. Initial BH4 responsiveness is identified by a >30% reduction in blood phenylalanine with a BH4 loading test. Long term effectiveness is demonstrated by improved metabolic control with >75% of blood phenylalanine levels within target range without any decrease in natural protein intake, or an increase in natural protein tolerance of ≥100% with blood phenylalanine concentrations remaining consistently within the target range [1, 2, 104].

Large Neutral Amino Acids (LNAA)

Administration of LNAAs, alone or in combination with a phenylalanine restricted diet, has the potential to improve health outcomes for adolescents or adults with PKU with poor dietary adherence by reducing brain phenylalanine concentrations [2, 6, 9, 90, 105]. It is not recommended for children under the age of 10 years due to insufficient data on safety and efficacy [2, 6, 9].

LNAAs typically provide 20–30% of the total protein requirement with 70–80% of protein supplied by natural sources [6, 9, 90, 106]. In some countries LNAA are used for

treatment in adults only but evidence of their efficacy is controversial [1]. Currently there is insufficient data to make international recommendations about LNAA use.

Pegvaliase (Palynziq)

PEGylated recombinant phenylalanine ammonia-lyase (PEG-PAL) or pegvaliase is an enzyme substitute approved in the USA and the European Union for lowering blood phenylalanine [107-110]. This enzyme, administered as a daily subcutaneous injection, catalyses the conversion of L-phenylalanine to transcinnamic acid and ammonia that are subsequently metabolized in the liver and excreted in the urine, without the need for PAH or the cofactor BH4 [107-110]. It is effective in lowering blood phenylalanine concentrations in patients on unrestricted diets and unlike sapropterin, is suitable for all phenotypes of PKU [8, 9]. However, as there is no catalytic conversion of phenylalanine to tyrosine by PAH, tyrosine deficiency remains a risk [110]. In addition, phase 2 and 3 studies identified that an early decrease in blood phenylalanine was not sustained, and increasing frequency or dosage was associated with an increase in adverse events including anaphylaxis and hypophenylalaninaemia; although these effects improve over time (6-12 months) [108-112]. Consequently, some patients may be reluctant to accept the treatment [112]. Monitoring of dietary intake is essential to ensure an adequate intake particularly with the removal of protein substitutes [107]. It has not been tested for safety and efficacy in children so is currently only recommended in patients ≥16 years of age with blood phenylalanine levels >600µmol/L and for whom other available treatment options (sapropterin and/or diet) have not been successful in maintaining metabolic control [107, 108, 110].

Gene therapy

With advancing medical technology, additional treatments utilising gene therapy to deliver a working copy of the PAH gene into the patient's liver cells, or correcting the defective gene by gene editing, are undergoing clinical trials [16, 113].

1.8.2 Dietary treatment

For most patients with PKU, a phenylalanine restricted diet is the mainstay of treatment. The aim of dietary treatment is to safeguard neurological function, achieve optimal growth and development by meeting recommended nutritional requirements, and maintain normal feeding patterns.

The principles of dietary treatment are (Figure 1.1):

1. Substrate reduction: To limit the substrate load on the blocked metabolic pathway, dietary phenylalanine is restricted to around 20-25% of normal intake by avoidance of foods naturally high in phenylalanine/protein such as meat, fish, eggs, cheese, nuts, seeds, soya, and aspartame, an artificial sweetener [2, 6, 114]. Considering phenylalanine is an essential amino acid, it cannot be totally excluded, and so is allocated in daily measured amounts (according to individual tolerance) using a phenylalanine/protein food exchange system [3, 114]. In the UK, one exchange is equivalent to that amount of food that provides 1g protein or 50 mg phenylalanine.
2. Supplementation: Natural protein is replaced with a low or phenylalanine-free synthetic protein substitute that provides all essential amino acids except for phenylalanine to meet requirements for growth and development. It is based on

amino acids or low phenylalanine CGMP. Additional macro- and micro-nutrients are usually added to these supplements [3, 105, 114, 115]. The nutrient content of protein substitutes for infants are designed to replicate nutritionally complete standard infant formula whilst those for older children and adults commonly contain nutrients to meet reference nutrient intakes (RNI) in a prescribed amount.

3. Maintenance of anabolic state: Provision of a normal energy intake is essential to prevent catabolism and subsequent endogenous production of toxic metabolites. This is often achieved with the use of special low protein staple foods such as bread, pasta and cereals [3, 114, 115]. In addition, some regular foods that are naturally low in protein can be consumed without measurement including: fats (butter, margarine, oils), sugar, jams, honey, syrups, aspartame free sweet drinks (soft drinks, squash, juices), and foods with a protein content ≤0.5/100g (Phe ≤25mg/100g) such as vegan cheeses, sweets and starches (e.g., arrowroot, cornstarch) [114]. However, as in any healthy diet, foods high in sugars and fats need to be moderated and a balanced diet incorporating a wide variety of foods is advocated within the boundaries of dietary restrictions (Figure 1.2).

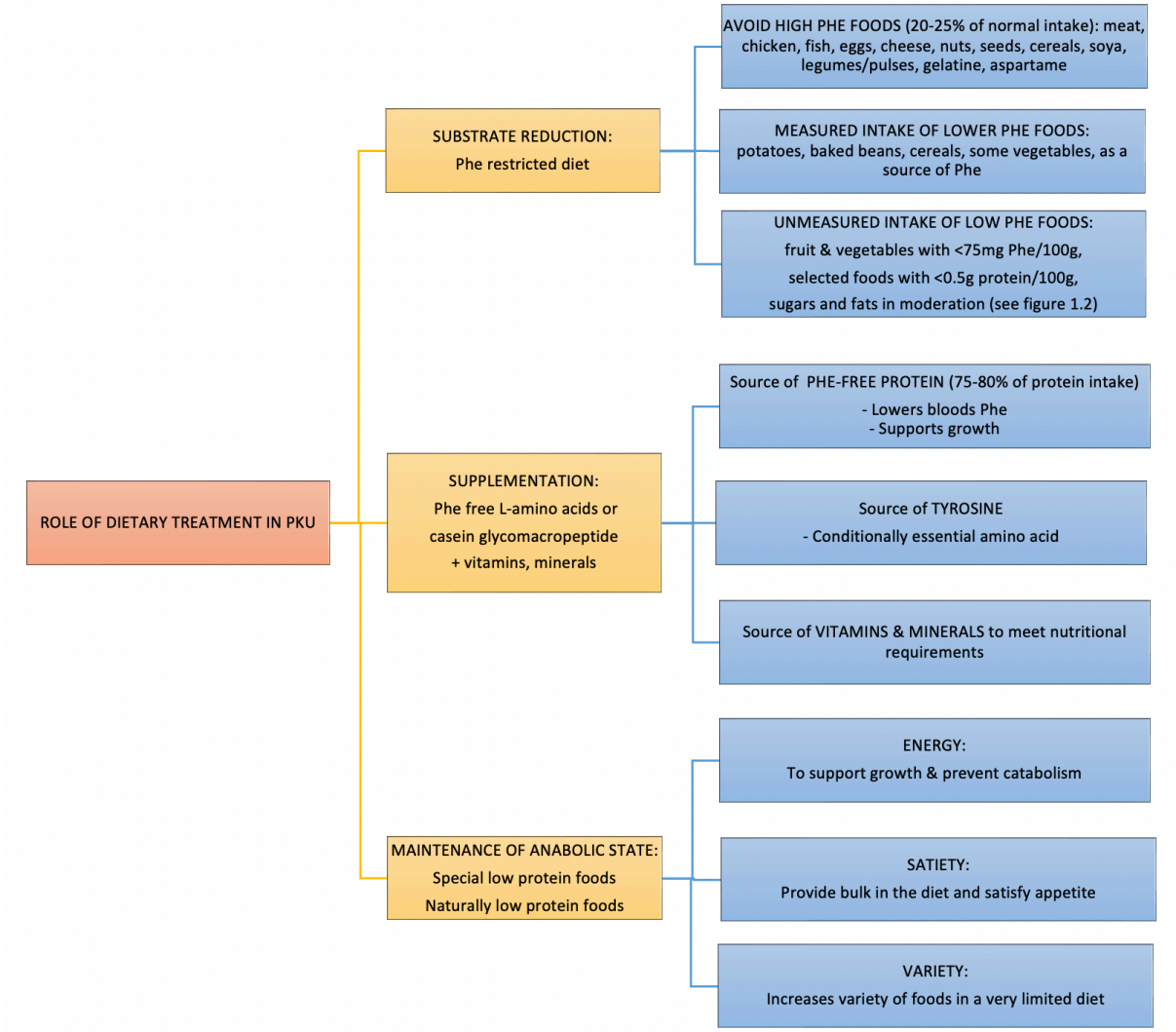

Figure 1.1 Overview of the role of diet in PKU

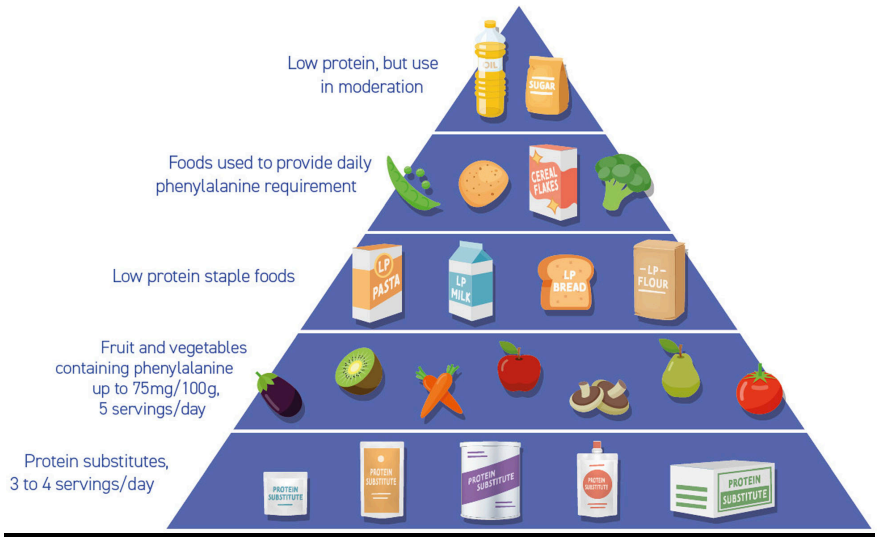

Figure 1.2 Food pyramid for PKU[114].

Phenylalanine tolerance is defined as the amount of dietary phenylalanine (in mg/day or mg/kg/day) or natural protein (g/day, assuming a phenylalanine content of 50mg / gram of protein) that an individual with PKU can tolerate to maintain blood phenylalanine consistently within therapeutic target range [2] [114]. The amount of dietary phenylalanine tolerated varies between individuals based on severity of PKU (genotype and residual PAH activity), dosage, frequency and adherence with protein substitute administration, BH4 responsiveness, age and growth [2, 8, 47, 90, 91, 114, 116]. Some patients with classical PKU may only tolerate 150 mg/day of phenylalanine (3g/day natural protein).

The total protein intake (from natural protein and protein substitutes) should supply more than Dietary Reference Intakes (RDI) or approximately 40% above the FAO/WHO/UNU 2007 age-related safe levels of protein to compensate for the reduced availability of amino acids in protein substitutes, the ineffective absorption of natural protein which is largely plant based, and to optimise the effect of amino acids on blood phenylalanine control [1, 2, 6, 90, 114]. Across Europe recommendations for protein equivalent intake are usually 2-3g/kg/day for 0-1 years of age, 1.5-2g/kg/day for 1-10 years and 1g/kg/day for >10 years [117]).

Special low protein foods

Special low protein foods (SLPFs) generally contain ≤50mg phenylalanine or ≤1g protein per 100g [1]. They provide a source of energy to meet requirements, bulk to satisfy appetite, and increase variety to help normalise a restrictive diet [2, 3, 6, 8, 118, 119]. They may provide as much as 50% of estimated daily energy requirements [2, 6,

114], and the range and quantity of products consumed is highly variable due to individual tastes and appetite. Patients with milder phenotypes, with a higher natural protein tolerance are less dependent on their use [6, 119]. SLPFs include milk replacements, bread, pasta, cereals, flour, pizza bases, burger/sausage mix, biscuits, crackers, cakes, desserts, and chocolate; many are based on food starches (wheat, potato and maize) that are low in protein and high in carbohydrate [118].

Protein substitutes in PKU

Age targeted low or phenylalanine-free protein substitutes are the main source of protein, particularly in classical PKU providing at least 75% of daily nitrogen requirements [114]. If inadequate amounts of protein substitutes are given, amino acids become rate limiting for protein synthesis and this leads to catabolism and a subsequent rise in blood phenylalanine [1, 114]. They are usually supplemented with, tyrosine (due to inefficient in vivo production), essential amino acids, vitamins, minerals, trace elements and long chain fatty acids, to make up the deficit in micronutrients caused by a severely restricted diet [6, 114] (Figure 1.3). Protein substitutes contain branch chain amino acids (BCAA) (isoleucine, leucine and valine) and LNAAs, including histidine, methionine, threonine, and tryptophan.

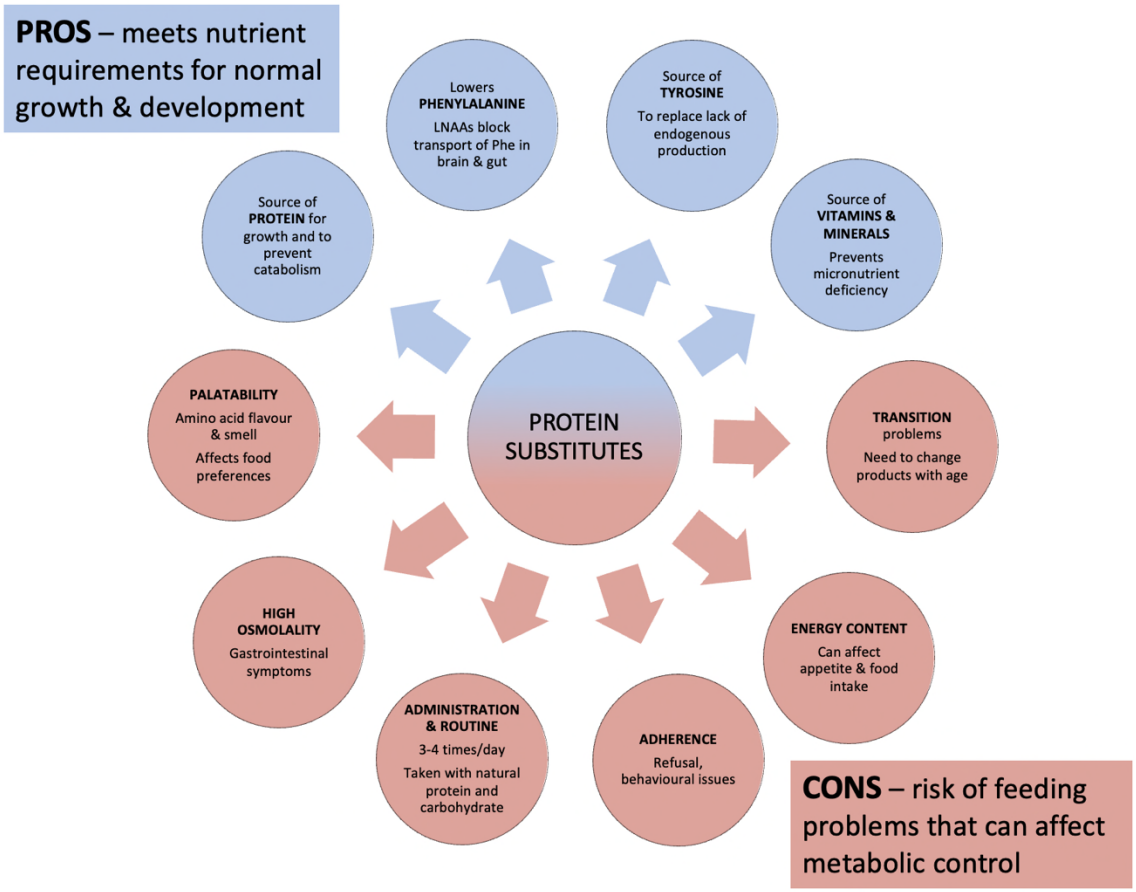

Figure 1.3 The advantages and challenges of protein substitute composition and adherence in PKU

The quantity, composition, diurnal distribution, acceptability and administration of protein substitutes can influence blood phenylalanine control [6, 47-50, 52, 91, 92, 116, 120]. When protein substitutes containing free amino acids are administered in bolus amounts during the day, they are rapidly absorbed causing a rapid increase in blood amino acids that may exceed the capacity of anabolic processes to incorporate them into nascent proteins and subsequently, it increases urinary nitrogen excretion [48, 75, 116, 121-123]). Protein retention is greater with more slowly absorbed protein sources (e.g., casein), inhibiting protein catabolism and oxidation of amino acids. Smaller and repeated dosages of amino acids lead to less phenylalanine fluctuation [47, 48, 75, 91, 116, 121-123]. In the presence of amino acids, insulin stimulates protein synthesis, thus adequate energy in

the form of glucose, along with BCAA (especially leucine) are important [121]. Protein substitutes are therefore rich in leucine and LNAA to promote protein synthesis by competitively reducing the uptake of phenylalanine via LAT1 and balancing the transport of amino acids across the BBB.

Amino acids are bitter tasting and have an unpleasant odour, that influences protein substitute adherence [2, 10, 124-128] (Figure 1.3). Very early exposure to bitter flavours (commencing in the first weeks of life) may impact on the taste preferences of children and adults with PKU [129]. Amino acid-based protein substitutes have a high osmolality (concentration of solution expressed as total number of solute particles per kg) which may delay gastric emptying and subsequently lead to gastrointestinal symptoms such as diarrhoea, abdominal cramps, distention, nausea, vomiting and constipation [1, 6, 114].

There is also a need to transition from one age-appropriate protein substitute to the next, initially at 6 months (second stage weaning protein substitute), then again at 3 to 5 years (third stage protein substitute) as nutritional requirements change. A choice of products for different ages is therefore necessary [8, 114]. Consistency and routine are important aspects of protein substitute administration, and numerous strategies are employed to encourage adherence. Table 1.1 provides a summary of types of protein substitute available in the UK.

Table 1.1 Types of protein substitute for PKU suitable for children

Stage	Type	Composition	Features	Issues
1	Infant protein substitutes (powder/ liquid)	L-amino acids	• phenylalanine-free • given following neonatal diagnosis • similar nutritional composition to regular infant formula • early infant exposure to amino acids accustoms their taste to amino acids	• may need reconstitution with water • poor taste
2	Semi-solid weaning protein substitutes (powder/ ready to use puree)	L-amino acids	• phenylalanine-free • semi-solid consistency given from a spoon • consistency can be adjusted to suit developmental age • higher in protein equivalent than infant protein substitutes • introduce from 6 months • low volume/low energy so infant has appetite for solid foods • added vitamins, minerals, long chain fatty acids, carbohydrate and fat	• may need reconstitution with water • poor taste but most infants adapt if introduced at 6 months • difficult to administer during teething/ illness • thickens on standing • high osmolality
2/3	Powders suitable from 1y+	L-amino acids	• phenylalanine-free • concentrated in amino acids so low volume • +/- vitamins, minerals, long chain fatty acids, carbohydrate and fat • flexible as the amount of water added can be adjusted to give as a semi-solid or drink • available in different flavours	• need reconstitution with water • poor taste • less convenient • high osmolality
3	Ready to use liquid protein substitutes	L-amino acids	• phenylalanine-free • low volume • convenient • usually with added vitamins, minerals, long chain fatty acids, carbohydrate and fat • usually low in carbohydrate/fat/ energy • suitable for a range of ages • available in different flavours	• poor taste • high osmolality
3	Casein glycomacro-peptide with amino acids (CGMP-AA) (powder/ liquid)	peptide based substitute with added amino acids	• low phenylalanine • powdered, liquid and bars • improved taste and palatability • lower osmolality • may be more slowly digested & absorbed (unproven) • may promote satiety (unproven) • improves nitrogen retention	• contains residual phenylalanine (1.8mg/1g protein equivalent) • may increase blood phenylalanine in well-treated children if given as a sole source of protein substitute

			• less blood phenylalanine variability • prebiotic, antimicrobial and immunomodulatory effects • with added vitamins, minerals, long chain fatty acids, carbohydrate and fat	• human studies on long-term effects are limited • may require reconstitution with water
3	Slow-release protein substitutes suitable from 3y+	Amino acids coated with ethyl cellulose and alginate	• phenylalanine-free • granules • mixed with food or fruit juice • prolonged release and physiological absorption of amino acids • improved taste, smell and palatability	• only short term studies reported
3	Tablets or capsules	L-amino acids	• phenylalanine-free • +/- vitamins and minerals • masks taste and smell of amino acids	• high numbers required to meet full protein requirement • often used in combination with other protein substitutes to aid variety
3	Snack bars	L-amino acids	• phenylalanine-free • +/- vitamins and minerals • masks taste and smell of amino acids	• large amounts required to meet full protein requirement • often used in combination with other protein substitutes to aid variety

Adapted from Yilmaz et al 2022 [130] and MacDonald et al 2020 [114]

CGMP, a low phenylalanine protein derived from cheese whey, has an improved taste compared to amino acid supplements. Pure CGMP is naturally low or deficient in phenylalanine and the essential LNAAs, histidine, leucine, tryptophan and tyrosine, and is two to three times higher in the LNAAs, threonine and isoleucine than amino acid protein substitutes [2, 9, 58, 60, 65]. Consequently, CGMP protein substitutes adapted for PKU are supplemented with deficient LNAAs, vitamins, minerals and trace elements to meet age-specific nutritional requirements. CGMP may have several benefits compared to amino acids but is unsuitable for children under 4 years of age (Table 1.1).

1.9 Application of dietary treatment

Overall, there are numerous difficulties with the application of dietary treatment in PKU that may lead to disordered feeding patterns, aberrant eating behaviour and delayed feeding progression (Figure 1.4). The social stigma associated with consuming a restrictive diet and the difficulty in obtaining suitable foods means that eating outside the home is largely avoided [131]. Patients with PKU are considered at risk of food poverty i.e., the inability to obtain an adequate and nutritious diet in socially acceptable ways [132].

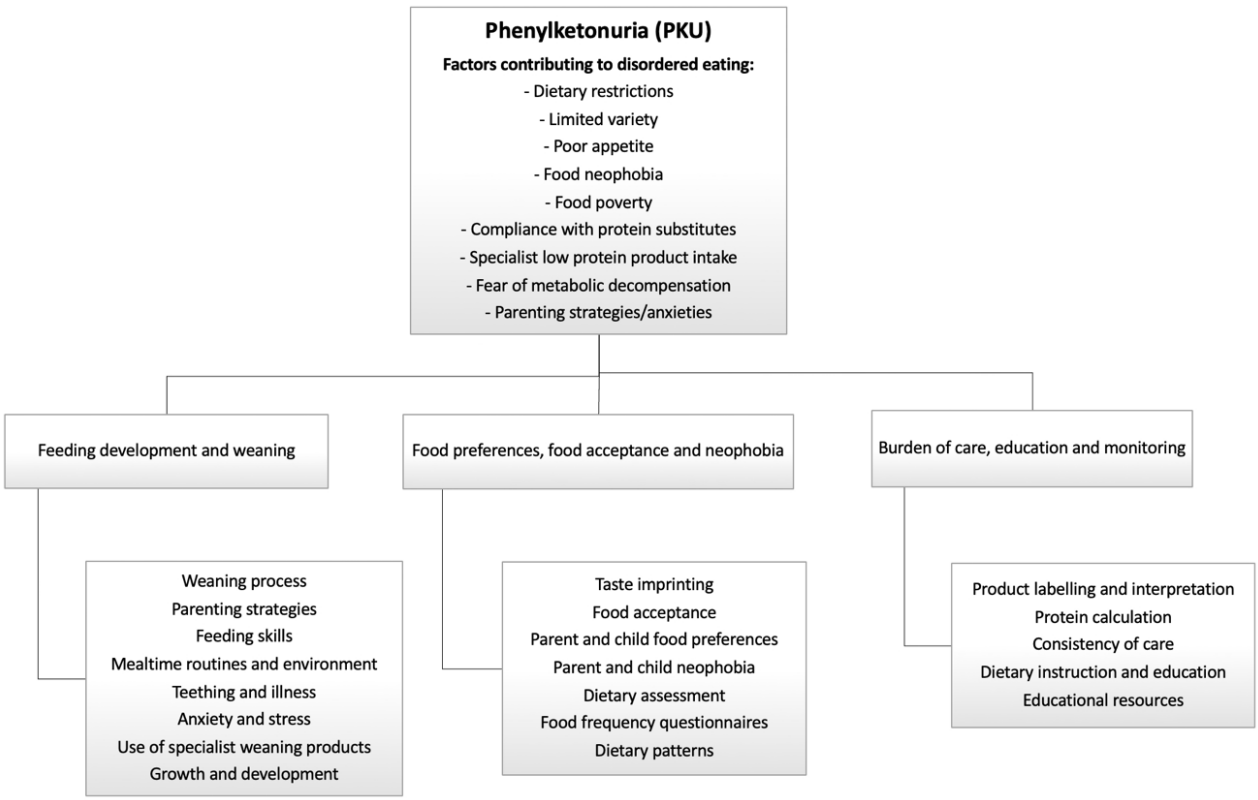

Figure 1.4 Feeding issues in Phenylketonuria (PKU)

There are issues surrounding the weaning process, assessing dietary patterns, development of food preferences, food acceptance and food neophobia. Furthermore, there is a lack of consensus on how to apply the diet and educate patients in terms of

interpreting food labelling, calculating phenylalanine/protein intake and determining suitability of foods for inclusion in the diet.

1.10 Protein substitutes, feeding development and weaning in PKU

Weaning is the time when solid foods are introduced to complement breastfeeding or infant formula feeding and to meet developmental and nutritional needs. Some of the food preferences and feeding patterns seen in PKU are likely to have their origin in the early years and during the early weaning process [133, 134]. As well as understanding the normal process of weaning, parents of children with PKU have the additional anxiety of dealing with administration of a second stage protein substitute, introducing SLPFs and learning how to calculate phenylalanine exchanges [133]. The knowledge that dietary indiscretions and inability to persuade children to eat all their phenylalanine allocation or take protein substitutes, may result in inadequate blood phenylalanine control and irreversible neurological disability, causes a significant amount of anxiety for parents particularly during mealtimes [133]. Any abnormal focus on food and eating that has a negative impact on food behaviour [135], can overshadow the important social aspects of eating [132].

Across Europe, weaning in PKU generally commences between the age of 17 to 26 weeks of age [134, 136] but there is wide variation in the age at which second stage protein substitutes and phenylalanine containing foods are given [136] and this is not based on scientific evidence [133, 136, 137]. It is thought that introducing solid food before the 6 months of age recommended by the World Health Organisation [138], can be an advantage in minimising neophobic responses and improving food acceptance in

PKU [133]. Balancing the need for a prescribed intake of protein substitute with the introduction of foods and the demands of new tastes and textures is challenging. As with any child there is a need to gradually reduce the intake of liquid diet whilst increasing solid food intake and maintaining protein and energy requirements to aid normal growth and development.

In PKU, although there are protein substitutes specifically designed for the weaning age (from 6 months), no studies have formally investigated the best approach to introducing these second stage protein substitutes. Studies have also not examined the meal patterns, self-feeding skills, feeding environment and mealtime behaviour of weaning children with PKU compared with non-PKU children. It might be expected that parents of infants with PKU feel more in control of their child's intake if they continue to spoon feed, to ensure that adequate amounts of prescribed natural protein and protein substitute are consumed, but this has not been investigated. Studies have not examined levels of anxiety and coping behaviours of parents of infants with PKU during the weaning years.

There has also been concern that early growth may be suboptimal in PKU [11, 139-144]. Studies of extensively hydrolysed protein formula used for cow's milk protein intolerance, showed slower growth than infants fed standard cow's milk based infant formula [145-147]. It is hypothesised that this may be due to compositional differences in protein source, availability, amino acid composition, fat type and quantity, and absorption and bioavailability of individual nutrients [146]. Second stage weaning protein substitutes for PKU are amino acid based and tend to be high in carbohydrate and low in fat and this may affect growth, although this has not been systematically studied.

Therefore, studies examining growth, energy and macronutrient intake in PKU infants prescribed a second stage protein substitute are warranted.

1.11 Food preferences, food acceptance and neophobia in PKU

1.11.1 Assessing dietary patterns in PKU

Whilst much is known about what patients with PKU should eat, less is known about what they consume including meal choices and food patterns. There is also increasing concern about the development of other co-morbidities such as obesity [148-152]. High sugar and carbohydrate (particularly high glycaemic index) intakes and the type of protein substitute used could contribute to excessive weight gain in PKU. In addition, a proportion of children with mild or moderate PKU are treated with sapropterin, [93-103, 153] but little is known about how this will change food choices, particularly the type and variety of food eaten and impact on body mass index (BMI).

Measuring food and nutrient intake to identify feeding patterns in PKU relies on the selection of a suitable research tool for dietary assessment. This is challenging as no single method measures dietary intake without error [154]. In addition, those used for the general population are generally unsuitable due to the restrictive nature of a low protein diet. A food frequency questionnaire (FFQ) provides a list of foods and respondents are required to say how often they consume each food in broad terms such as the number of times per day/per week/per month. FFQ are useful due to minimal demands on health professional time, low participant commitment and lower cost [154]. The results obtained represent usual intakes over time and can be used to rank subjects into low, medium or high intake groups. Dietary pattern analysis is used to study total food intake and the

potentially synergistic effect of food and nutrients [155, 156] and is based on habitual food consumption [157]. FFQs are particularly well suited to dietary pattern analysis by providing a broad picture of food and nutrient consumption. This may be more predictive of disease risk than individual foods or nutrients.

It is important to validate a FFQ to ensure that it measures what it claims to measure, yields consistent reliable/reproducible results, can differentiate between different subject groups and compares well with an existing standard [158]. This improves the quality of the data collected and enables comparisons to be made between studies using the same tool. There is no validated UK FFQ for a phenylalanine restricted diet. An American FFQ has been validated for use in adolescents and adults with PKU but this is based on foods and portion sizes consumed in the USA, it does not cater for young children with PKU and focusses on validity in measuring protein intake but not energy or other macronutrients [159]. It is important to develop a UK validated FFQ tool to examine dietary patterns in PKU.

1.11.2 Food acceptance and neophobia

Unlike standard infant formula, which is composed of whole proteins, low protein substitutes are composed of bitter tasting amino acids, similar to protein hydrolysate formula used in cow's milk protein intolerance [129, 160]. The administration and acceptance of protein substitutes in PKU is critical for metabolic control. Studies in infants taking hydrolysate formula and subjective evidence in patients with PKU prescribed amino acids have suggested that there is a narrow age window for successful introduction in terms of acceptance [161]. Furthermore, infants fed bitter tasting protein

hydrolysate formulas from birth have been shown to eat more savoury, bitter, and sour-tasting weaning foods than those given sweeter tasting milk-based formula or breast milk [162]. Whilst anecdotal evidence suggests that savoury and bitter foods may be preferred in PKU, little is known about the impact of this on the dietary patterns of children with PKU.

The development and maintenance of food acceptance in PKU is essential. Food preferences are normally controlled by a multitude of emotional, personal, age, cultural, and situational factors and are widely agreed to be important determinants of eating behaviour in young children. Neophobia and food fussiness have previously been reported in PKU [135]. Parents may play a significant role in the development of these traits in terms of social and environmental factors around mealtimes, attitudes toward food, and role modelling with respect to their own food preferences [124]. A higher degree of food neophobia has been associated with unpleasant mealtime emotions, a mother's own degree of neophobia, and with the maternal practice of coaxing children to eat in response to food refusal [163, 164]. Children with PKU often eat in isolation, due to preparation of separate and differing meals than the rest of the family, and to enable parents to focus more attention on ensuring meals are consumed; children also require more prompting to eat as they have less interest in food [135, 165, 166]. To what extent this influences longer term food choice in children with PKU is unknown. Use of a validated PKU FFQ may help to establish differences in taste preferences and food choice in children.

1.12 Burden of care and education

Children with PKU are dependent on complex life-long dietary treatment [1, 4, 6, 167]. There is much intricate information for parents, and later patients, to learn. Implementation of this special diet is challenging. Difficulties with treatment adherence are common, but some of this could be due to dietary misunderstandings and so repeated education and continuing support are vital.

1.12.1 Low protein food labelling, protein calculation and interpretation of food suitability

Understanding which foods can be included in the diet and in the amounts permitted is complex. Natural protein prescriptions vary between individuals and even within the same individual over time, so no one rule fits all. There are no definitive dietary guidelines on what constitutes one protein/phenylalanine exchange and how to interpret protein values on food labels.

Difficulty in interpreting the protein content on food labels may occur for numerous reasons (Table 1.2), including unclear, inconsistent, misleading or changes in labelling [168-170]. A recent review of regular and 'free-from' supermarket foods identified 55% had uncertain protein labelling, with misleading, omitted, or erroneous information in 43% of 'free-from' foods and 62% of regular foods [169]. Accidental consumption of the phenylalanine containing artificial sweetener aspartame by adults and children with PKU is commonly attributed to manufacturers changing ingredients on food labels [168]. Similarly, in a recent survey of 246 adult patients and caregivers of children with PKU, 31% found it difficult to calculate food protein exchanges from food labels [170].

Table 1.2 Summary of some of the practical issues of food protein label interpretation and assessment of food suitability

Issues	Problems for patient/caregivers in PKU
The amount of phenylalanine per gram of protein is inconsistent in some foods e.g., fruits and vegetables contain less than foods such as milk and cereals.	Patients/caregivers cannot assess suitability of fruits and vegetables from protein content on food labels.
EU regulations are not strict about accurate protein analysis on food labels e.g., *EU regulation No. 1169/2011:* protein content of manufactured food ≤0.5g/100g is not legally required to be declared on a food label. Therefore, food labels may list 0g protein/100g despite including protein containing ingredients (e.g., gelatine). Similarly, in the USA, a protein content of <1g per serve size does not need to be declared on the food label.	Some patients with PKU are only allowed 4 or 5g/day natural protein and need to account for small amounts of protein from manufactured foods. Accumulation of 'extra' dietary phenylalanine from lower protein containing foods over the day can lead to poor dietary control.
Phenylalanine is declared on all SLPFs e.g., bread, even though the amounts may be negligible.	This causes confusion for parents/caregivers and inappropriate phenylalanine calculation.
The phenylalanine content of some foods (e.g., herbs and spices) may be high even though they are used in minute amounts.	Patients/caregivers are confused on how to interpret protein values.
When calculated, if a food contains protein <0.5g per portion, some individuals may count this as a protein exchange, others consider it exchange-free.	Patients/caregivers are confused about how to calculate and interpret protein values in daily diets.
Food labels even for the same type of food vary in how nutrient information is presented (e.g., cooked versus uncooked weight) and for powdered products (e.g., custard powder, milkshake powder) the nutrient content may be reported as reconstituted with ingredients that are not used in the PKU diet (e.g., with milk)	Patents/caregivers are confused as to the protein content of the food: should they be measuring it in the cooked or uncooked form; and how do they know how much protein is in a powdered product without the added milk which they cannot have?
New manufactured products are constantly appearing on the market.	Patients/caregivers need information about their suitability for inclusion in the diet.
Manufacturers can change ingredient content without notice and new ingredients may contain phenylalanine. e.g., with the recent UK sugar tax, many drink manufacturers reduced the sugar content and added artificial sweeteners such as aspartame (containing a source of phenylalanine). Sugar-free and sugar containing drink labels of the same brand may look similar, so it is easy to select the wrong one.	Patients/caregivers can easily purchase incorrect products.

Even amongst dietitians there is conflicting advice and not surprisingly this causes confusion for patients and families who commonly use social media to discuss issues and seek guidance. There is also disparity between health professionals and patients regarding interpretation of food labels [171]. This presents a huge challenge for dietitians. A means of simplifying the messages, improving long-term retention of knowledge and providing readily available and easily accessible dietary information is required.

1.12.2 Dietary resources and dietary instruction

There is a need for consensus and development of shared resources to support families with PKU [133]. Caregivers/patients with PKU require accurate, reliable and readily available information about the protein content of foods, to aid self-care skills in interpretation of food protein suitability.

People have different learning styles, so no one educational tool will suit all patients. A combination of teaching methods (visual, auditory, and tactile) is necessary [172]. A range of resources and methods are currently available including written/pictorial booklets, social media (e.g., Twitter), individual clinic verbal face-to-face sessions, group teaching sessions (e.g., cooking, shopping), peer support groups and web-based teaching modules [114]. Dietitians spend a considerable amount of clinical time responding to patient/caregiver telephone calls, and email queries about low protein labelling interpretation and product suitability.

Involving patients and carers in dietary plans, decision making, and development of educational materials are important in obtaining commitment to the treatment, particularly with adolescents. There is discrepancy between patients and clinicians' views

on the perceived effectiveness of nutrition education tools and this has significant implications for long-term treatment adherence [173]. Patients, particularly those under the age of 10 years, report that their families are the best source of education and written material the least effective, whilst parents prefer one-on-one counselling [173]. Motivational interviewing improves self-efficacy and self-management behaviour in adolescents and adults with PKU [174].

The challenge is how best to disseminate consistent and accurate educational material to users and how to make it readily accessible to all UK patients with PKU. Mobile apps are an important educational resource and many of the problems in acquiring instant access to the protein content and suitability of foods could be overcome by access to a mobile low protein diet app available on mobile phones and tablets. Apps may be more motivational particularly for teens and preadolescent children.

Studies examining technology education in PKU are limited. An online search of apps suitable for protein metabolic disorders, conducted in 2016 [175], identified 22 apps (18 PKU) from 11 countries (Table 1.3). Only 4 were developed by health professionals/organisations, the remainder by commercial dietary companies, parents/patients with the condition or independent/unknown individuals. Not all were available free of charge, only 5 were available on both iOS and Android, whilst 3 were web-based requiring internet access to function. The majority focused on protein calculators, low protein recipes and monitoring intake/blood results rather than the suitability of foods for inclusion in the diet. Few had been validated by a healthcare organisation or certified by a recognised body. Food databases on apps reflect local diets so are not suitable for use internationally due to differing guidelines for allocation of dietary protein. No current apps for PKU are inclusively: developed by health

professionals, available on both iOS an Android, free to download, based on UK foods, and able to answer user questions on food suitability.

Table 1.3 Current apps for Inherited metabolic disorders of amino acid metabolism

App	Developer	Compatibility	Price	Language	Features	Cons
AccuGo for PKU	Jeremy Cross © 2015 (parent) (USA)	Requires iOS 6.0 or later Compatible with iPhone, iPad, and iPod touch	£0.79	English	+ track phenylalanine or protein + track 10,15,20,25 or 50mg exchanges + includes pre-loaded and searchable hand-picked USDA-based food items + add your own foods and values to database or share with community + enter food amounts using a variety of units + works offline regardless of Wi-Fi or cellular availability + online synchronisation on >1 device + overview of day's running total phenylalanine, exchanges and calories + email daily records + historical records + built in calendar	+ not free + only on iOS + USA based food items + developed by parent rather than health professional so focus may differ + no guidance on how to determine appropriateness of foods
DietWell™ for PKU	Cambrooke Foods, LLC © 2009 Cambrooke (USA)	Requires iOS 4.3 or later Compatible with iPhone, iPad, and iPod touch	$4.99	English	+ add new items, save frequently used ones + share custom items, recipes and meals with friends + monitor daily goals with the graphing feature (intake and blood phenylalanine) + phenylalanine, protein, protein equivalent, calories for >7500 USDA foods including LP foods and formula. + enter food amounts using a variety of units + email daily records + no Wi-Fi needed except to email logs + free updates	+ not free + only on iOS + USA based food items + commercial bias + no guidance on how to determine appropriateness of foods
My PKU USA	Nutricia (USA)	Requires iOS 7.0 or later Compatible with iPhone, iPad, and iPod touch	Free	English, Dutch	+ set and track daily PKU protein substitute and protein intake and set reminders so you don't miss a serving + track and monitor your phenylalanine levels over time and set blood test reminder notifications + enter food amounts using a variety of units + scan food items + create a PDF so you can print or email the report	+ only on iOS + commercial bias + USA based food/formula options + no guidance on how to determine appropriateness of foods

App	Developer	Compatibility	Price	Language	Features	Cons
Phenylketonuria (PKU)	Vitaflo International Limited (UK)	Requires iOS 6.0 or later Compatible with iPhone, iPad, and iPod touch	Free	English	+ Vitaflo's PKU Guide helps to explain the diagnosis and dietary management of the genetic condition PKU	+ only on iOS + commercial bias + information provision only
Metabolic Diet App	BC Children's Hospital Foundation and the Rare Diseases Foundation (Canadian)	Compatible with iPhone, iPad, android, laptop, computer	Free	English	+ functions include nutrient intake counts, own foods and recipes, and daily food diary + on the desktop version, there is an export food log which can be shared with healthcare providers + track and plan meals + suitable for 15 different IMDs + energy, CHO, fat and protein content of foods and amino acid content specific to condition + login to access and personalise data + export food log on desktop version e.g. email dietitian	+ USA based food items + no guidance on how to determine appropriateness of foods
My PKU Diet	T J Lee (USA)	Android 3 and up	Free	English	+ tool to manage daily diet records for PKU and personal LP foods	+ only on Android + source of information uncertain i.e. validated by a health professional? + no guidance on how to determine appropriateness of foods
Cambrooke foods for PKU	T J Lee (USA)	Android 3 and up	Free	English	+ nutritional and product info for all the PKU foods that are manufactured by Cambrooke Foods only + barcode scanner	+ commercial bias + only on Android
De PKU Kenner	van McWood Solutions (Initiative of the Dutch PKU association)	Requires iOS 7.1 or later Compatible with iPhone, iPad, and iPod touch	€ 0,99	Dutch	+ has protein content of foods on your phone + keep track of how much protein and blood values + get reminders + large number of products, the amount of protein (grams) per volume (provide unit) + unit in which the quantity is given varies by product + values in the standard table are from the NEVO table and checked by metabolic dieticians + a direct initiative of the Dutch PKU association in collaboration with the Foundation for Research PKU	+ not free + only on iOS + Dutch based food items + no guidance on how to determine appropriateness of foods

App	Developer	Compatibility	Price	Language	Features	Cons
Phenylalanine Rechner	Andre Reus	Requires Android, Windows Phone & Google Chrome Other platforms (such as iOS) can use the app on website	Free	English, German	+ combines calculator, pencil, paper and nutrition table + calculated per 100g phe levels for a given weight + search with English and German terms + daily schedule and list + bookmark foods (favourites) + add own foods + warning when exceed daily protein/phe allowance	+ limited use on iOS + based on USA dietary system so fruits and vegetables counted as exchanges + source of information uncertain
PKU Daily Food Diary Light	Maria Pia Izzo © 2013	Requires iOS 5.0 or later Compatible with iPhone, iPad, and iPod touch	Free	Italian, English, German, Spanish, Northern Sami	+ an application that helps people with PKU in the daily calculation of the amount of protein they can take	+ only on iOS + source of information uncertain i.e. validated by a health professional? + no guidance on how to determine appropriateness of foods
PKU Maik Barz	Maik Barz	Requires iOS 8.0 or later Compatible with iPhone, iPad, and iPod touch	Free	English Deutsch Dutch	+ in the daily overview you can see how much phe you still need in order to complete your daily amount + create recipes, add foods, supplement alerts, monitor phenylalanine intake	+ only on iOS + source of information uncertain i.e. validated by a health professional? + no guidance on how to determine appropriateness of foods
Aphetit	Merck Sante © Merck Serono SAS	Requires iOS 7.0 or later Compatible with iPhone, iPad, and iPod touch	Free	French	+ approximately 30 recipes available and easy to make + convert levels of phenylalanine in the units used by different labs around regions in France + monitor phe intake and blood phe levels	+only on iOS + commercial bias + no guidance on how to determine appropriateness of foods
PKyoU	Bio Marin Pharmaceutical's	Requires iOS 6.0 or later Compatible with iPhone, iPad, and iPod touch	Free	Italian	+ functions include nutrient intake counts, own foods and recipes, and daily food diary + monitor adherence	+ only on iOS + commercial bias + no guidance on how to determine appropriateness of foods

App	Developer	Compatibility	Price	Language	Features	Cons
PheFeed	© Mark Brenneman (USA) (patient)	Requires iOS 8.1 or later Compatible with iPhone, iPad, and iPod touch	Free	English	+ like people's PKU dishes to save them to your profile + comment on people's PKU dishes to ask them about the recipe or other tips	+ only on iOS + developed by patient rather than health professional so focus may differ + no guidance on how to determine appropriateness of foods
How much phenylalanine	Virginia Schuett (Dietitian) (USA) initiated by a parent who was a web developer	Any mobile device with internet access	Subscrip-tion (National PKU News Service)	English	+ all foods in LP food list + get phe, protein, calories, exchanges for foods + calculator to convert your quantities into exchanges + record your intake and blood phe so can track + 7000+ food items	+ requires internet connection + not free although funds go into PKU organisation + USA based food items
CalculAA	Jorge Acosta Alvarado Latin American Congress of IEM, Institute of Food Technoology Uni Chile	Requires iOS 7.1 or later Compatible with iPhone, iPad, and iPod touch	Free	Spanish, ??Chilean	+ set and track your daily intake for PKU and maple syrup urine disease (MSUD) counting mg of phenylalanine and leucine	+ only on iOS + no guidance on how to determine appropriateness of foods
eDietetik	IJS, Jožef Stefan Institute, Computer Systems	Requires iOS 4.3 or later Compatible with iPhone, iPad, and iPod touch and Android	Free	Slovenian	+ for celiac disease, diabetes, PKU, high blood pressure + people can scan the barcode of the food product by using smartphone camera and it will inform you about the ingredients, which may be in excessive quantities for you or your child	+ source of information uncertain i.e. validated by a health professional? + no guidance on how to determine appropriateness of food
PKU Tarifleri	Multimedia	Compatible with Android 4.2 and up	Free	Turkish	+ LP recipes for patients with PKU + food categories: salads, pasta and rice, yogurt and ice cream, breakfast, pastry, veggie meatballs, soups and desserts	+ only on Android + ? Turkish based food items + source of information uncertain i.e. validated by a health professional? + no guidance on how to determine appropriateness of foods

App	Developer	Compatibility	Price	Language	Features	Cons
EWG's Food Source	Environmental Working Group	Requires iOS 8.0 or later Compatible with iPhone, iPad, and iPod touch	Free	English	+ an easy-to-navigate database created to help consumers make healthier, greener food choices + it is not a PKU APP but it can be adapted for PKU + counts calories, fat, sugar, protein, sodium, fibre, fruit and vegetables + looks at ingredients and potential contaminants, and level of processing + database of >80,000 products, 5000 ingredients and 1500 brands + compare similar products + comparison with daily requirements based on age and gender	+ only on iOS + USA based food items + not specific to IMDs + no guidance on how to determine appropriateness of foods
PKU Diet Management	(USA)	Android 2.3.3 and up (Google play)	Free	English	+ track phe, protein, calories, CHO and 18 vitamins and minerals – compares with RDI + barcode scanner	+ only on Android + source of information uncertain i.e. validated by a health professional? + no guidance on how to determine appropriateness of foods
PKU Diary	ReFlex Wireless (USA)	Android 3 and up	Free	English	+ helps people to manage their phenylalanine level by monitoring protein intake	+ only on Android + source of information uncertain i.e. validated by a health professional? + no guidance on how to determine appropriateness of foods
PKU Manager	Andre Reus	Android 4 and up	Free	Spanish, English	+ search for foods, add, edit foods	+ only on Android + source of information uncertain i.e. validated by a health professional? + no guidance on how to determine appropriateness of foods

LP = low protein; PE = protein equivalent; USA = United States of America; USDA = United States Department of Agriculture; iOS = iPhone operating system; PDF = portable document format; IMD = inherited metabolic disease; UK = United Kingdom; NEVO = Dutch food composition database; Phe = phenylalanine; RDI = Reference Daily Intake [175]

Patients with PKU require strict control of the protein content of their diet. Patients/caregivers need information to assess individual food products (not just protein content, but specific information regarding suitability) and be able to make decisions without having to consult a dietitian. No existing apps for PKU have been validated for efficacy via a randomised controlled trial (RCT) with a user group. An app that will provide concordance in label interpretation and standardisation of dietary instruction, has the potential to improve knowledge, adherence, long-term metabolic control and empower individuals to take greater responsibility in managing dietary treatment. It would also be useful for schools, nurseries, and other family members.

1.13 Summary

There are many barriers that impede the attainment of dietary treatment goals in PKU [10, 176]. Difficulties of administrating bitter tasting protein substitutes may influence development of food neophobia, taste preferences and alter weaning experiences [129, 133]. A phenylalanine restricted diet has implications for growth, nutritional status and quality of life [3]. It also affects patient/parental anxiety, food choice, mealtime strategies and the feeding environment. The dietary restriction is complex and difficult to understand. This is compounded by inconsistencies in dietary advice due to a lack of health professional consensus on dietary issues such as food labelling, its interpretation and protein calculation. New and novel approaches to dietary education are essential to motivate and encourage patients with PKU to take responsibility for their dietary management with confidence. This thesis, through a series of questions, aims to address these issues to further improve dietary support in PKU.

1.14 RESEARCH QUESTIONS

QUESTION 1 (CHAPTER 2a). Is a specially designed weaning protein substitute for PKU more appropriate during the weaning years and is it well accepted?

In PKU, the aim of this retrospective study was to examine the introduction and transition from a liquid infant formula protein substitute to a semi-solid weaning protein substitute during weaning in terms of child developmental progression, difficulties with administration, and strategies used. This included a review of weaning age, growth, metabolic control, dosage of liquid and semi-solid weaning protein substitutes at each stage in the stepwise transition process, introduction of natural protein foods, and issues with administration of food and protein substitutes.

QUESTION 2 (CHAPTER 2b). How do the weaning patterns and practices of children with PKU differ to normal healthy children?

The aim of this 20-month prospective case-controlled study was to observe longitudinal feeding development and progression in infants with PKU compared with a group of age, ethnicity, and gender matched control children. This included progression with textured foods, self-feeding motor skills development, the feeding environment, gastrointestinal symptoms and behaviour at mealtimes.

QUESTION 3 (CHAPTER 2c). How do maternal and child anxiety and coping mechanisms during the transition from a liquid to a solid diet compare between children with PKU and healthy control children?

The aim of this 2-year prospective case-control study was to compare mealtime emotions, anxieties and coping strategies between a group of mothers with infants with PKU and a group of age, ethnicity and gender matched control infants during the

transition from a liquid to a solid diet; and to determine the effect of the additional burdens of protein substitute administration and natural protein restriction. This included an examination of mealtime emotions for parent and child, parental anxieties, and coping behaviours around feeding and protein substitute administration.

QUESTION 4 (CHAPTER 2d). Does the growth and macronutrient intake of infants with PKU differ from that of control children during the first 2 years of life?

This prospective, longitudinal case-control study aimed to compare the growth, energy and macronutrient (protein, carbohydrate, fat) intakes of infants with PKU compared to age, ethnicity and gender matched control children during the transition from liquid to solid diet and the transition from a liquid to semi-solid protein substitute.

QUESTION 5 (CHAPTER 3a). Can a food frequency questionnaire be validated for use in determining food preferences and dietary patterns of children with PKU compared with normal healthy children?

The aim of this study was to develop a validated UK food frequency questionnaire (FFQ) for use in children with PKU based on food items commonly eaten in PKU and the 'general' population. Once validated, the questionnaire can be used to compare food intakes and preferences of PKU and control children and can be used to examine dietary patterns in PKU.

QUESTION 6 (CHAPTER 3b). Do children with PKU given amino acid protein substitutes from infancy prefer bitter tasting foods compared to normal healthy children?

The aim of this randomised controlled study was to investigate the flavour preference of young children with PKU compared with normal healthy control children and to establish if 'flavour imprinting' occurs in PKU as a result of early exposure to bitter

tasting amino acids. In addition, it explores differences in food neophobia and food preferences (measured by food frequency questionnaire) between PKU and non-PKU children.

QUESTION 7 (CHAPTER 3c). Do parents of children with PKU differ from control parents with respect to food preferences and neophobia; and how does this influence their children?

The aim of this prospective observational study was to determine if food preferences (measured by food frequency questionnaire) and neophobia seen in children with PKU is influenced by parental food preferences and neophobia; and if this differs to control children compared with their parents.

QUESTION 8 (CHAPTER 4a). How can dietitians simplify and minimise the disparity and inconsistencies in food labelling interpretation and protein calculation for individuals with PKU and their caregivers?

The aim of this prospective study was to identify areas of disparity between dietitians in the UK in terms of interpretation of how different foods should be allocated in low protein diets for PKU and to describe the process used to develop national consensus guidelines utilising Delphi methodology.

QUESTION 9 (CHAPTER 4b). What are the UK National Consensus Statements on Food Protein Interpretation for PKU?

This paper follows on from chapter 4a, describing the final collaborative statements agreed by UK IMD dietitians for food labelling interpretation and protein allocation in a low protein diet for PKU, how they have been categorised and justification for their

inclusion. This will provide consistency in the dietary education provided for patients with PKU across the UK.

QUESTION 10 (CHAPTER 4c). In PKU, does the use of a mobile multi-media low protein diet app improve the knowledge and accuracy of patient/caregiver protein calculations/food choices when selecting fresh, manufactured and specialised dietary products in a low protein diet? And does regular use of a mobile app increase both self-efficacy in dietary management and reduce dependence on dietitians?

The aim of this randomised controlled trial was to develop a mobile multi-media app as a UK national resource for patients/caregivers prescribed a low protein diet for PKU. Its efficacy was then tested in a 12-week randomised controlled parallel intervention study and compared with use of 'traditional' written dietary resources.

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CHAPTER 2: FEEDING DEVELOPMENT AND WEANING IN PHENYLKETONURIA

CHAPTER 2A: FIFTEEN YEARS OF USING A SECOND STAGE PROTEIN SUBSTITUTE FOR WEANING IN PHENYLKETONURIA: A RETROSPECTIVE STUDY

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CLINICAL NUTRITION

Fifteen years of using a second stage protein substitute for weaning in phenylketonuria: a retrospective study

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Keywords
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Abstract

Background: In phenylketonuria (PKU), during weaning, it is necessary to introduce a second stage phenylalanine (Phe)-free protein substitute (PS) to help meet non-Phe protein requirements. Semi-solid weaning Phe-free PS have been available for >15 years, although no long-term studies have reported their efficacy.

Methods: Retrospective data from 31 children with PKU who commenced a weaning PS were collected from clinical records from age of weaning to 2 years, on: gender; birth order; weaning age; anthropometry; blood Phe levels; age commenced and dosage of weaning PS and Phe-free infant L-amino acid formula; natural protein intake; and issues with administration of PS or food.

Results: Median commencement age for weaning was 17 weeks (range 12–25 weeks) and, for weaning PS, 20 weeks (range 13–37 weeks). Median natural protein was 4 g day^{-1} (range 3–11 g day^{-1}) and total protein intake was >2 g kg^{-1} day^{-1} from weaning to 2 years of age. Children started on 2–4 g day^{-1} protein equivalent (5–10 g day^{-1} of powder) from weaning PS, increasing by 0.2 g kg^{-1} day^{-1} (2 g day^{-1}) monthly to 12 months of age. Teething and illness adversely affected the administration of weaning PS and the acceptance of solid foods. Altogether, 32% of children had delayed introduction of more textured foods, associated with birth order (firstborn 80% versus 38%; $P = 0.05$) and food refusal when teething (80% versus 29%; $P = 0.02$).

Conclusions: Timing of introduction of solid foods and weaning PS, progression onto more textured foods and consistent feeding routines were important in aiding their acceptance. Any negative behaviour with weaning PS was mainly associated with food refusal, teething and illness. Parental approach influenced the acceptance of weaning PS.

Introduction

The principle treatment of phenylketonuria (PKU) is a low phenylalanine (Phe) diet. This is limited in natural protein (commonly less than 10 g day^{-1}) and is supplemented with Phe-free L-amino acids. In early infancy, feeding is relatively straightforward. Infants obtain their Phe source from breast milk or standard infant formula, with the amount titrated according to blood Phe concentrations [1]. This is supplemented with an L-amino acid Phe-free infant protein substitute formula to meet the remaining non-Phe protein and nutrient requirements. When solids are introduced, feeding becomes more complex for both caregivers and health professionals. Many dietary changes are instituted so that feeding meets the developmental needs of an infant, although attaining satisfactory growth and blood Phe control is essential. If this stage is managed poorly, it may negatively impact on future feeding and long term metabolic control [2].

In all infants, the primary aim of weaning is to progressively replace an exclusively liquid diet with a mixed diet incorporating solid foods. In PKU, breast milk or

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infant formula milk is replaced by solids containing the equivalent quantity of Phe. Ensuring an adequate intake of non Phe L amino acids and energy intake is essential. As infants increase in weight, the infant protein substitute should increase accordingly in line with non Phe protein requirements. However, when an infant is over 6 months of age, more than 600 mL day^{-1} of infant protein substitute is not in concordance with good feeding practice. For all infants, a high volume of fluid intake limits appetite for solid foods, thereby deterring exposure to different tastes and textures and contributing to bottle feeding dependence. At the age of weaning, when the aim is to progress onto more solid food, children with PKU should ideally replace their infant protein substitute with a supplement that has a higher concentration of Phe free L amino acids and is given in a semi solid consistency [3]. During the weaning stage, dietitians have tried many different techniques to introduce protein substitutes that are more concentrated in protein equivalent. This has included adding a second source of non infant Phe free L amino acid to bottles of infant protein substitute formula or adding a small quantity of water to a non infant Phe free L amino acid supplement and administering this as a paste [4]. Although this had the advantage of lowering the volume of protein substitute, the design and composition resulted in an inconsistent and often undesirable texture [2]. However, the administration of a paste is generally less problematic and is associated with less parental and child stress [3], although the success and impact of these techniques remains unreported.

In the UK in 2001, a weaning Phe free L amino acid supplement designed to be given as a gel (PKU gel; Vitaflo International Ltd, Liverpool, UK) was introduced. We have retrospectively evaluated the medical/dietetic records of infants prescribed this product, examining the introduction and administration of the weaning protein substitute during the first 2 years of life.

Materials and methods

Subjects

Retrospective data were collected from patient records for 31 consecutive children (16 male) with PKU diagnosed by newborn screening who were prescribed a second stage weaning protein substitute (PKU gel; Vitaflo International Ltd) administered as a gel or paste during weaning from one specialist PKU centre. They all took a Phe free infant L amino acid formula (PKU Anamix Infant: Nutricia, Trowbridge, UK) in combination with breast or infant formula milk to provide a source of Phe. Fourteen subjects were breast fed for at least 1 month (median 3 months; range 1 12 months). The method, timing and sequence of introduction of solids was the same irrespective of whether infants were breastfed or formula fed. The decision to commence introduction of solid foods was initiated by parents/carers. Infants with biopterin defects were excluded.

In the UK, the aim of dietary management in children from 0 3 years is to establish dietary Phe tolerance by titrating the amount of Phe intake until blood Phe concentrations were within target range (120 360 μmol L^{-1}), prescribe a total of 3 g kg^{-1} day^{-1} protein equivalent (from natural protein and Phe free protein substitute) and encourage low protein foods that are given without measurement (e.g. low protein fruits and vegetables, containing less than 75 mg Phe per 100 g of product, and low protein special foods).

The present study was conducted as a service evaluation based on guidelines from the National Research Ethics Service and did not require ethical review by a NHS Research Ethics Committee. However, it was conducted in accordance with basic ethical principles with respect for the confidentiality of participants and in line with the Data Protection Act 2000 and was registered as an audit within the study centre. Written consent was obtained from parents/caregivers.

Study product

PKU gel (Vitaflo International Ltd) is a powdered Phe free protein substitute containing essential and non essential amino acids, carbohydrate, vitamins, minerals and trace elements for the dietary management of children with PKU from 6 months of age [in line with World Health Organization (WHO) recommendations for weaning age; however, in PKU weaning is recommended to commence earlier at 17 26 weeks [2]]. It provided 10 g of protein equivalent per 24 g of powder and was prepared with the addition of water to produce a gel/paste that was administered by spoon and is of similar texture and consistency to early weaning foods.

For an equivalent protein content, the weaning protein substitute has a vitamin and mineral composition similar to the infant protein substitute but is lower in energy [67 kJ (16 kcal) per 2 g protein versus 280 kJ (67 kcal) per 2 g protein], with the aim being to reduce the impact on appetite and thereby encourage appetite for solid foods (both natural protein sources and low protein foods).

Study design

This was a single centre retrospective study. Patient records from 2001 for children with PKU taking the same second stage weaning protein substitute were accessed and the data collected were: gender; birth order; family

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members with PKU; weaning age; age commenced weaning protein substitute; anthropometry [weight and weight for age *z* score (WHO)] (at the closest point to age 6, 12 and 24 months); blood Phe levels; weaning protein substitute and infant protein substitute formula prescription by age; protein equivalent from infant protein substitute formula, weaning protein substitute and natural protein by age; and any behavioural issues or difficulties with administration of weaning protein substitute, infant protein substitute formula or other foods. Data were collected from the time of weaning until the children's second birthday.

Statistical analysis

Descriptive statistics were used for quantitative outcome measures, as well as more detailed analysis using non parametric tests (Mann Whitney).

Results

Subjects

The dietetic case records of 31 subjects with PKU (16 male) given a second stage weaning protein substitute as a gel were identified. Sixteen were first born children, 11 were second born and four were third born. Thirteen subjects had a sibling(s) with PKU and one subject's mother had PKU.

Introduction of weaning protein substitute and solid foods

The median age of introducing weaning foods was 17 weeks (range 12 25 weeks) and the median age for commencing weaning protein substitute was 20 weeks (range 13 37 weeks). There was no difference between boys and girls and it was not influenced by birth order or older PKU siblings. However, children who were breast fed for a minimum of 4 weeks ($n = 14$) were more likely to wean at a later age (19 versus 15 weeks; $P = 0.009$; t test) and therefore commenced weaning protein substitute later (23 versus 19 weeks; $P = 0.04$; t test).

At weaning commencement, all children were given small amounts of unmeasured, low protein foods, such as puree fruit, suitable vegetables and low protein rusks. The primary Phe source was breast milk ($n = 6$) or standard infant formula ($n = 25$) and non Phe protein by infant protein substitute formula. The time of starting weaning was usually determined by the parents/caregivers.

The process of introducing weaning protein substitute was similar for all children. They usually started with 5 10 g day^{-1} of powder (providing 2 4 g day^{-1} protein equivalent) ($n = 29/31$), given once or twice daily. The weaning protein substitute was mixed with water to a paste (resembling baby rice texture) and was given prior to any solids or milk (i.e. breast milk, standard infant formula or infant protein substitute formula). It was administered from a weaning spoon ($n = 29/31$) but, in two children (aged 7 18 months and 8 10 months), it was given via a syringe as a result of temporary difficulties with its administration. When weaning protein substitute was commenced, breast feeds or standard infant formula were gradually replaced by weaning foods containing an equivalent Phe content (i.e. the introduction of a 50 mg Phe exchange system). Twenty nine percent of caregivers ($n = 9$) used a flavoured weaning protein substitute from a median age of 13 months (range 7 16 months).

Median protein equivalent from protein substitute and natural protein

For the first 2 years, protein equivalent from weaning protein substitute increased linearly, whereas the infant protein substitute formula decreased (Fig. 1 and Table 1). Protein equivalent from weaning protein substitute increased by a mean intake of 0.2 g kg^{-1} day^{-1} (2 g day^{-1}) each month from commencement until 12 months of age and then by 0.1 g kg^{-1} day^{-1} every 2 months (1 g day^{-1} monthly) to 24 months of age (Table 1). Infant weight gain and volume of daily infant protein substitute formula determined dosage.

By direct contrast, the median daily protein equivalent from the infant protein substitute formula gradually decreased by 0.1 g kg^{-1} day^{-1} each month from the start of introducing the weaning protein substitute until 15 months of age (Table 1). The infant protein substitute continued to provide a protein equivalent of 0.5 0.6 g kg^{-1} day^{-1} until 2 years, and by this time, most children ($n = 30/31$) had stopped taking it.

There was a trend toward boys consistently consuming more protein equivalent from the infant protein substitute formula at each age (additional 1.0 2.6 g day^{-1} protein or 55 130 mL day^{-1} of formula), although this only reached statistical significance at 14 months of age (additional 130 mL formula day^{-1} and 2.6 g/day protein; $P = 0.03$; t test).

Phenylalanine tolerance was similar for most children with an intake of natural protein of between 3 and 5 g day^{-1} throughout the study period, except for two children who tolerated a median of 8 g of natural protein per day. Natural protein intake remained consistent at a median of 4 g day^{-1} for the first 2 years (range 3 11 g day^{-1}) (Table 1). However, when expressed as g kg^{-1} day^{-1}, natural protein intake decreased from 0.6 g kg^{-1} day^{-1} at 3 months to 0.4 g kg^{-1} day^{-1} at

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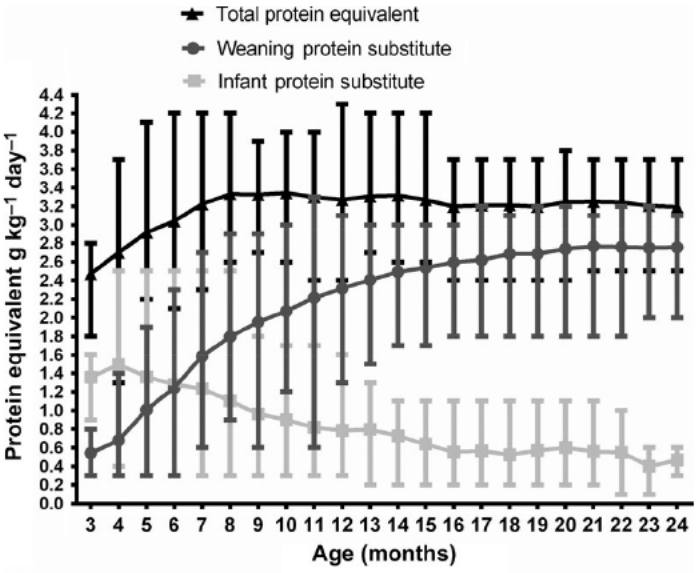

Figure 1 Protein equivalent (g kg ^{1} day ^{1}) by age for weaning protein substitute, infant protein substitute and total protein.

Table 1 Median protein equivalent from weaning protein substitute (PS), infant protein substitute, natural protein and total protein

Age (months)	*n*	Amount weaning PS powder g day ^{1}	Weaning PS protein equivalent (range)		Infant PS protein equivalent (range)		Natural protein (range)		Total protein (range)	
			g day ^{1}	g kg ^{1} day ^{1}	g day ^{1}	g kg ^{1} day ^{1}	g day ^{1}	g kg ^{1} day ^{1}	g day ^{1}	g kg ^{1} day ^{1}
3	7*	7 (5–12)	3 (2–5)	0.5 (0.3–0.8)	9 (7–10)	1.4 (0.9–1.6)	4 (3–5)	0.6 (0.4–0.8)	16 (15–18)	2.5 (1.8–2.8)
4	19*	11 (5–21)	4 (2–9)	0.7 (0.3–1.4)	10 (3–18)	1.5 (0.4–2.5)	4 (3–8)	0.6 (0.4–1.0)	19 (11–25)	2.7 (1.3–3.7)
5	24*	18 (5–36)	8 (2–15)	1.0 (0.3–1.9)	9 (2–15)	1.4 (0.3–2.5)	4 (3–8)	0.6 (0.4–0.9)	21 (17–27)	2.9 (2.2–4.1)
6	30*	23 (5–45)	10 (2–19)	1.2 (0.3–2.3)	10 (2–18)	1.3 (0.3–2.5)	4 (3–8)	0.5 (0.3–1.0)	23 (13–29)	3.0 (2.1–4.2)
7	31	29 (5–50)	12 (2–21)	1.6 (0.6–2.7)	10 (2–17)	1.2 (0.3–2.5)	4 (3–8)	0.5 (0.3–1.0)	26 (17–33)	3.2 (2.3–4.2)
8	31	35 (5–55)	15 (2–23)	1.8 (0.9–2.9)	9 (3–16)	1.1 (0.3–2.5)	4 (3–8)	0.5 (0.3–1.0)	27 (18–34)	3.3 (2.6–4.2)
9	31	40 (15–60)	17 (6–25)	2.0 (0.6–2.9)	8 (2–18)	1.0 (0.3–1.8)	4 (3–8)	0.5 (0.3–1.0)	29 (21–34)	3.3 (2.7–3.9)
10	31	44 (25–60)	19 (11–25)	2.1 (1.2–3.0)	8 (2–16)	0.9 (0.3–1.7)	4 (3–8)	0.4 (0.3–1.0)	30 (23–35)	3.3 (2.6–4.0)
11	31	49 (25–70)	21 (11–29)	2.2 (0.6–3.3)	7 (2–15)	0.8 (0.3–1.7)	4 (3–8)	0.4 (0.3–1.0)	30 (23–40)	3.3 (2.4–4.0)
12	31	53 (25–70)	22 (11–29)	2.3 (1.3–3.3)	8 (2–18)	0.8 (0.3–1.6)	4 (3–9)	0.4 (0.3–1.0)	31 (23–50)	3.3 (2.4–4.3)
13	31	56 (36–75)	24 (15–32)	2.4 (1.5–3.0)	8 (2–14)	0.8 (0.2–1.3)	4 (3–8)	0.4 (0.3–1.0)	32 (23–45)	3.3 (2.7–4.2)
14	31	59 (36–80)	25 (15–34)	2.5 (1.7–3.0)	7 (2–14)	0.7 (0.2–1.1)	4 (3–11)	0.4 (0.3–1.0)	33 (26–47)	3.3 (2.6–4.2)
15	31	62 (36–80)	26 (15–34)	2.5 (1.7–3.0)	8 (2–14)	0.6 (0.2–1.1)	4 (3–11)	0.4 (0.3–1.0)	34 (26–45)	3.3 (2.6–4.2)
16	31	65 (45–80)	27 (19–34)	2.6 (1.8–3.0)	6 (2–14)	0.6 (0.2–1.1)	4 (3–11)	0.4 (0.2–1.0)	34 (26–45)	3.2 (2.4–3.7)
17	31	67 (45–90)	28 (19–38)	2.6 (1.8–3.2)	6 (2–14)	0.6 (0.2–1.1)	4 (3–11)	0.4 (0.2–1.0)	34 (27–45)	3.2 (2.4–3.7)
18	31	69 (51–90)	29 (21–38)	2.7 (1.8–3.1)	6 (2–14)	0.5 (0.2–1.1)	4 (3–11)	0.4 (0.2–1.0)	35 (29–45)	3.2 (2.4–3.7)
19	31	70 (51–90)	30 (21–38)	2.7 (1.8–3.2)	7 (2–14)	0.6 (0.2–1.1)	4 (3–11)	0.4 (0.2–1.0)	35 (29–45)	3.2 (2.4–3.7)
20	30#	73 (51–90)	31 (21–38)	2.7 (1.8–3.2)	7 (2–14)	0.6 (0.2–1.1)	4 (3–11)	0.4 (0.2–1.0)	36 (29–46)	3.2 (2.4–3.8)
21	30#	75 (54–90)	31 (23–38)	2.8 (1.8–3.1)	6 (2–14)	0.6 (0.2–1.1)	4 (3–11)	0.4 (0.2–1.0)	37 (29–46)	3.3 (2.5–3.7)
22	29#	75 (54–90)	32 (23–38)	2.8 (1.8–3.2)	7 (2–14)	0.6 (0.1–1.0)	4 (3–11)	0.4 (0.2–1.0)	37 (29–46)	3.2 (2.5–3.7)
23	29#	77 (54–90)	32 (23–38)	2.8 (2.0–3.2)	5 (2–6)	0.4 (0.1–0.6)	4 (3–11)	0.4 (0.2–1.0)	37 (29–46)	3.2 (2.5–3.7)
24	29#	77 (54–100)	32 (23–42)	2.8 (2.0–3.1)	5 (4–6)	0.5 (0.3–0.6)	4 (3–11)	0.4 (0.2–0.9)	38 (29–46)	3.2 (2.5–3.7)

*Some children not commenced weaning.
#One or two children progressed onto post weaning protein substitute.

10 months and then remained stable at 0.4 g kg^{-1} day^{-1} until 2 years of age.

Overall, the total daily protein intake increased initially by 0.1 0 2 g kg^{-1} (2 3 g) each month and then stabilised at 3.2 3 3 g kg^{-1} day^{-1} from 8 months to 2 years (Table 1). Total protein intake remained consistently above 2 g kg^{-1} day^{-1} throughout. Gender, birth order, PKU siblings and breastfeeding did not influence daily total protein intake.

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Issues with administration of weaning protein substitute and food

Factors such as teething, illness and food refusal adversely affected both the administration of weaning protein substitute and food intake (Fig. 2 and Table 2). Common illnesses associated with refusal included: vomiting, diarrhoea or colds ($n = 15$; 10 on more than one occasion); sore mouth/ulcers/herpes/thrush ($n = 6$; 2 on more than one occasion); sore throat/tonsillitis ($n = 5$; 2 on more than one occasion); and ear infections ($n = 4$). Over one third of infants (35%; $n = 11$) also refused to take their infant protein substitute formula at some point during the weaning process, commonly on more than one occasion ($n = 7$) (Table 2). This occurred predominantly between 9 and 11 months of age.

Twenty three percent ($n = 7$) of caregivers had poor technique/routines for administration of weaning protein substitute. This included inconsistency of administration approach between parents/caregivers and inappropriate timing of administration (immediately after meal time when the child was full, at sleep times when they were tired, or inconsistent times each day) (Table 2). Some parents/caregivers (16%; $n = 5$) mixed the weaning protein substitute with food aiming (unsuccessfully) to entice their children to eat it.

Similarly, there were problems with eating solid foods. Almost one third (32%; $n = 10$) of children were delayed in their progression onto coarser food textures at some point in time (median age 9 months; range 7.3 20.9 months) (as reported by the dietitian), 19% ($n = 6$) of caregivers described their child as having a poor appetite and almost one third (29%) had low energy intakes and required glucose polymer or fat emulsions added to foods to ensure energy requirements were met (Table 2).

Children reported by the dietitian to have delayed introduction of textured (mashed) foods ($n = 10$) were more likely to be first born (80% versus 38%; $P = 0.05$), have no older siblings with PKU (0% versus 43%; $P = 0.03$) and had more food refusal (80% versus 29%; $P = 0.02$) when teething.

Similarly, although not statistically significant, those weaned <17 weeks of age ($n = 14$) compared to those weaned >17 weeks ($n = 17$) were less likely to have received breastfeeds (29% versus 59%), experienced more problems with food refusal with teething (57% versus 35%) or illness (64% versus 35%) and demonstrated

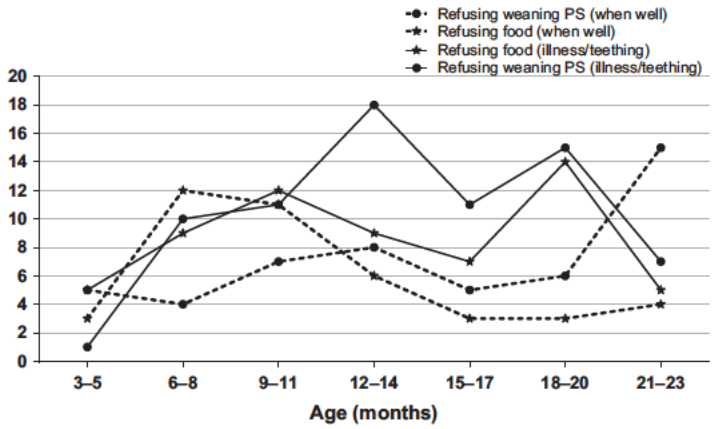

Figure 2 Number of individual incidences of weaning protein substitute (PS) and food refusal by age group (n 31).

Table 2 Issues with administration of weaning protein substitute and food/exchanges

Issues with weaning protein substitute	*n*	Issues with food/exchanges	*n*
Teething and refusing weaning protein substitute	14 (6)	Teething and refusing food/exchanges	14 (8)
Self induced vomiting	5 (2)	Self induced vomiting	2 (1)
Refusing weaning protein substitute (child well)	19 (15)	Refusing food/exchanges (child well)	19 (8)
Refusing weaning protein substitute (child unwell)	21 (16)	Refusing food/exchanges (child unwell)	16 (10)
Holding weaning protein substitute in the mouth	2	Delayed textures	10 (3)
Spitting weaning protein substitute out	4 (2)	Poor appetite	7 (3)
Poor routine for weaning protein substitute administration	7 (2)	Additional energy supplements required for low energy intake	9 (7)
Weaning protein substitute mixed with other foods	5	Nasogastric feeding due to illness leading to lack of fluids, protein substitute and food intake	3
Lengthy time for administration	2		

Values in parenthsis indicate the numbers of subjects experiencing issues on >1 occasion.

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more refusal of weaning protein substitute when unwell (79% versus 59%). Those introduced to weaning protein substitute >26 weeks of age ($n = 7$) experienced more weaning protein substitute refusal (71% versus 58%) and were more likely to take flavoured weaning protein sub stitute >1 year of age (57% versus 29%).

Anthropometry

Children had appropriate growth during their first 2 years of life. Median weight for age *z* scores were: 0.59 (range 2.6 to 2.0) at 6 months; 0.03 (range 2.6 to 1.8) at 12 months; and 0.20 (range 1.7 to 2.4) at 24 months. At 6 and 12 months of age, three children had a *z* score < 2, although, by 24 months, their *z* scores were > 2.

Blood phenylalanine levels

Median blood Phe levels were within reference range (120 360 μmol L^{-1}) from the start of weaning to 12 months of age: 175 μmol L^{-1} (range 90 310); and from 12 to 24 months of age they were: 180 μmol L^{-1} (range 100 400 μmol L^{-1}). No children had median blood levels above the reference range between weaning and 1 year of age and only one exceeded the reference range between 1 and 2 years.

Discussion

Weaning is a challenging time for parents/caregivers of any child, with or without PKU, and although the process of weaning onto solid foods is similar, specific challenges and issues differ [5]. In PKU infants, the demands of the dietary treatment commonly take priority over normal feeding development. The present study is one of the first to report on the practicalities and issues associated with weaning children with PKU. In this retrospective study, we describe the use of a protein substitute that had been developed to administer as a semi solid paste in the weaning period. It was well accepted when it was first administered at 17 25 weeks of age, although it was asso ciated with some infant refusal during late weaning, mainly accompanied by teething and intercurrent illness. In parallel, the same frequency of food refusal occurred with 'natural' foods. As reported previously in children without PKU [6,7], parental approach influenced the recovery from protein substitute and food difficulties, with poor routines and inconsistency exacerbating these issues. Breast feeding, usually associated with mothers of higher educational achievement, and/or previous experi ence of managing a PKU infant, was associated with fewer difficulties during the weaning process.

Weaning protein substitute was generally well tolerated and provided an appropriately textured product to progressively replace infant protein substitute. Initially, weaning protein substitute was given in small doses (equivalent to 5 g powder once or twice daily) from a median age of 20 weeks and was gradually increased according to weight gain and intake of infant protein substitute formula. At the same time, standard infant for mula or breast milk was gradually replaced by an equiva lent amount of protein from Phe containing exchange foods with the aim of maintaining a total protein intake of approximately 3 g kg^{-1} day^{-1}. By 2 years of age, most children had stopped infant protein substitute formula. Breast milk or standard infant formula was replaced by equivalent protein from solid foods by the age of 12 months. The total protein intake given to this group of children was consistent with previously reported intakes from Northern Europe [8]. Despite the lower energy content of weaning protein substitute compared to infant protein substitute, no child was reported to have growth issues. It is assumed that an increasing solid food intake will compensate for this energy discrepancy.

The WHO advocate weaning from 6 months of age [9] but in PKU, it is considered beneficial to introduce solids from 17 weeks [2]. Earlier exposure to foods has been shown to improve acceptance to a wider food variety [10–13], which is vital to establish in a low Phe diet that is already severely limited and where reliance on prescrib able low protein special foods is paramount. In this observational study, there was suggestion that very early weaning (<17 weeks of age) or later introduction of weaning protein substitute (>26 weeks of age) was associ ated with a lower acceptance of weaning protein substi tute and a higher incidence of refusal. Those individuals with a delayed introduction of textured foods also tended to have more problems with refusal of both food and weaning protein substitute, particularly when teething. This is likely to be associated with lack of parental encouragement and a tendency to continue offering foods that they know will be eaten rather than risking food refusal by offering something new.

Refusal of food is more predominant than weaning protein substitute refusal up until 12 months of age, after which the latter is more predominant. Weaning protein substitute refusal is most common between 12 and 14 months of age and this mainly coincides with teething and intercurrent illness. However, with perseverance and consistency, most caregivers could re establish good acceptance of the weaning protein substitute. There was a trend for children with a delayed introduction of more textured foods, as well as those weaned before 17 weeks of age and those commencing weaning protein substitute late (>26 weeks of age), to refuse weaning protein substi tute at some time. Overall, parents/caregivers of children with PKU are under pressure to administer the correct

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dose of protein substitute, three times daily. They are aware that failure to complete the prescribed dose may adversely affect blood Phe control and it is unsurprising that some rejection and self induced vomiting may occur. Previous studies have reported that, between the ages of 1 and 5 years, the administration of protein substitute can be problematic[14 16] and food neophobia is common in this group of children [16].

This was a retrospective, uncontrolled study, and so there were potentially several confounding factors that may have affected the results, including caregiver level of education, socio economic status and previous experience with weaning (in a child with or without PKU). In addi tion, because the data were retrospective and taken from patient notes, it may have been incomplete or remained unreported for some patients. Small subject numbers, particularly in subgroups, may have led to some differ ences not being statistically significant. Some children came from families with severe social problems that may have influenced the weaning experience.

The findings of the present study highlight the impor tance of timing of weaning protein substitute introduc tion, as well as appropriate progression onto more textured foods in infants with PKU. Much of the negative behaviour observed was associated with normal weaning behaviour, teething and intercurrent infections. Good and consistent feeding routines are required from the com mencement of weaning. It was clear that good parenting strategies overcame practical issues, but when parental skills were inconsistent and uncertain, feeding difficulties persisted. As a group, these families had received inten sive one on one practical dietetic support. However, the time and patience required for the successful administra tion of both weaning protein substitute and solid foods during periods of infant rejection is overwhelming for some vulnerable parents. The involvement of extended families, in addition to obtaining early nursery provision, attending parenting courses, and encouraging discussion with other parents of PKU children who have already been through this process, might provide additional hands on, practical and emotional support. Judging the right time to introduce weaning protein substitute is essential. If an infant is introduced to a weaning protein substitute several weeks after they have been accustomed to the taste of solids foods, establishing its successful acceptance may be challenging.

Transparency statement

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

Conflict of interests, source of funding and authorship

Anne Daly has received research funding from Vitaflo, as well as financial support from Nutricia and Vitaflo to attend study days and conferences. Sharon Evans has received research funding from Nutricia, as well as finan cial support from Nutricia and Vitaflo to attend study days and conferences. Alex Pinto has received an educa tional grant from Cambrooke Therapeutics and grants from Vitaflo, Nutricia, Merck Serono and Biomarin to attend scientific meetings. Anita MacDonald has received research funding and honoraria from Nutricia, Vitaflo International and Merck Serono, is a Member of the European Nutrition Expert Panel (Merck Serono interna tional), a Member of the Sapropterin Advisory Board (Merck Serono international) and a Member of the Advi sory Board Element (Danone Nutricia).

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All authors were involved in the initial conception and design, data collection, interpretation of the data and crit ical revision of the paper for important intellectual con tent and final approval of the version submitted for publication. AM and SE were additionally involved in the collation of data and drafting of the initial article.

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CHAPTER 2B: HOW DOES FEEDING DEVELOPMENT AND PROGRESSION ONTO SOLIDS IN PKU COMPARE WITH NON-PKU CHILDREN DURING WEANING?

nutrients

MDPI

Article

How Does Feeding Development and Progression onto Solid Foods in PKU Compare with Non-PKU Children During Weaning?

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Abstract: Weaning is complex for children with phenylketonuria (PKU). Breastmilk/infant formula and phenylalanine (Phe)-free infant protein-substitute (PS) are gradually replaced with equivalent amounts of Phe-containing food, a semi-solid/spoonable weaning PS and special low-protein foods. In PKU, feeding patterns/practices during weaning in PKU have not been formally evaluated. In this longitudinal, prospective, case-control study (n = 20) infants with PKU transitioning to a second-stage PS, were recruited at weaning (4–6 months) for a comparison of feeding practices and development with non-PKU infants. Subjects were monitored monthly to 12 months and at age 15 months, 18 months and 24 months for: feeding progression; food textures; motor skill development and self-feeding; feeding environment; gastrointestinal symptoms; and negative feeding behaviours. Children with PKU had comparable weaning progression to non-PKU infants including texture acceptance, infant formula volume and self-feeding skills. However, children with PKU had more prolonged Phe-free infant formula bottle-feeding and parental spoon feeding than controls; fewer meals/snacks per day; and experienced more flatulence (p = 0.0005), burping (p = 0.001), retching (p = 0.03); and less regurgitation (p = 0.003). Negative behaviours associated with PS at age 10–18 months, coincided with the age of teething. Use of semi-solid PS in PKU supports normal weaning development/progression but parents require support to manage the complexity of feeding and to normalise the social inclusivity of their child's family food environment. Further study regarding parental anxiety associated with mealtimes is required.

Keywords: Phenylketonuria; protein substitute; weaning; feeding development

1. Introduction

The dietary management of the inherited disorder of protein metabolism, phenylketonuria (PKU), particularly in infancy and childhood, is a critical component of care, and treatment is recommended to commence prior to 10 days of age [1]. The feeding of young children with PKU is complex, with most children requiring a severe to moderate natural protein restriction (commonly < 10 g/day), in order to control blood phenylalanine (Phe) levels within target ranges and prevent severe intellectual impairment [2]. In infancy, the diet is supplemented with a Phe-free protein substitute formula to meet energy and non-Phe protein requirements. Maintaining adequate intakes of energy, total protein

equivalent, and natural protein to individual tolerance in PKU is important for the promotion of normal growth and development and maintaining acceptable Phe control.

Weaning defined as the introduction of solid or more textured foods into an infant's liquid-based diet, is a crucial time as it has an impact on future food preferences and longer-term health status. Feeding patterns and practices during weaning in PKU have never been formally evaluated and compared with the normal population. Ideally weaning in PKU should reflect the weaning process of healthy non-PKU infants [3], aiming to transition from an exclusively liquid diet, to a mixed diet encompassing a wide variety of foods, tastes and textures; and encouraging development of independent self-feeding.

There is anecdotal information about how best weaning should be approached in this population: for example, administering a protein substitute in the form of a paste or spoonable protein substitute to young children appears to be well accepted, with fewer associated feeding problems [4], but no prospective, controlled study of the benefits of such products has been carried out. In the UK, it has become practice to introduce a Phe-free weaning protein substitute that is administered as a low volume semi-solid texture, given by spoon and suitable for use from weaning age. The amount of weaning protein substitute is gradually increased, and the volume of Phe-free infant formula reduced during the weaning period. Retrospective data suggests that most refusal of weaning protein substitute is generally associated with normal weaning behaviour, commonly occurring, alongside food refusal, during teething and illness [5]. Nevertheless, the timing of introduction of a second stage protein substitute is vital, with early (<17 weeks of age) or late (>26 weeks of age) weaning and delayed introduction of more textured foods increasing the likelihood of refusal of both weaning protein substitute and food in general [5].

Compared with the general population, there are additional issues and anxieties for parents/carers of children with PKU, particularly with respect to the acceptance and tolerance of Phe-free protein substitutes and special low protein foods. It is possible that earlier weaning (from 17 weeks) may reduce food neophobia and improve acceptance particularly of low protein foods but this has not been proven in PKU.

These disorder-specific challenges are an adjunct to the 'general' tasks of weaning infants such as: ensuring the safe and timely introduction of solid foods around key developmental milestones [6], supporting age appropriate growth rates [7], the support of oral motor and speech development and normal feeding and swallowing [8], maintaining nutritional adequacy [9], and supporting social and cultural competence and enjoyment of eating.

This multi-centre longitudinal, prospective case-control study aims to evaluate the weaning development of infants with PKU transitioning to a semi-solid Phe-free protein substitute and to compare the feeding patterns, practices and difficulties with those of non-PKU infants during weaning.

2. Materials and Methods

2.1. Subjects

In this study, all infants meeting the inclusion criteria (i.e., diagnosed with PKU via newborn screening with no illness or co-morbidities who were commencing weaning and required a second stage Phe-free protein substitute during the 2-year study period) were recruited from 3 UK inherited metabolic disease centres: Birmingham Children's Hospital, Bradford Teaching Hospitals and the Royal Hospital for Children, Glasgow. Subjects were recruited by the one dietitian in each centre.

Non-PKU control children who were commencing weaning, were matched with the PKU group for mother's educational level and birth order; they were recruited by one of two dietitians. Control children included infants from a local Birmingham community centre and siblings of children with inherited metabolic disorders. Control subjects proceeded with weaning according to their own experience, or advice from health visitors/health professionals as required. This was independent of the study procedures.

2.2. Study Design

Subjects were recruited when solid foods were introduced (age determined by parental discretion, usually between 4–6 months) and monitored monthly to 1 year of age and then at 15, 18 and 24 months of age (total of 10–12 visits).

2.3. Feeding Development Diary

Prior to each monitoring visit, all parents/carers completed a non-validated qualitative 3-day diary recording: stage of feeding progression with respect to food textures (thin puree, thick puree, weaning foods, or normal foods); motor skill development (ability to use utensils and drink from a cup/straw) and self-feeding; the feeding environment including where infants were fed (e.g., highchair, lap, table); and any gastrointestinal symptoms (mouth ulcers, constipation, diarrhoea, colic, retching, vomiting, flatulence, burping, abdominal pain/distention). For the PKU group, the frequency of taking protein substitute and the incidence of 10 different behaviours associated with administering protein substitute (spitting it out, closing mouth, crying at start, crying at end, turning head away, pushing spoon away, deliberately spilling, refusing more than they take or refusing as much as they take) was also recorded over 3 days at each monitoring visit. These records were cross checked with parents/carers by the dietetic researcher at each visit.

2.4. Weaning Protein Substitute and Weaning Diet

At each monitoring visit, daily intake of weaning protein substitute, infant protein substitute and natural protein (breastmilk, standard infant formula or solid food equivalent) were recorded (PKU group). The weaning protein substitute (Anamix First Spoon, Nutricia Ltd, Trowbridge, UK) was a powdered Phe-free supplement containing essential and non-essential amino acids, carbohydrate, fat, vitamins, minerals, trace elements and long chain polyunsaturated fatty acids. When mixed with water it produced a low volume, semi-solid spoonable paste. Dose and increments of weaning protein substitute, infant protein substitute and natural protein intake are described in another publication [10].

After the infants with PKU had been established on low protein weaning foods (such as puree fruit or vegetables), weaning protein substitute was introduced. Usually 5–10 g of weaning protein substitute powder (providing 2–4 g protein equivalent) was introduced once daily, and gradually built up to 3 doses per day. This coincided with a stepwise protein equivalent reduction in infant protein substitute as well as gradual introduction of Phe-containing weaning foods to replace the Phe equivalent from breast milk or standard infant formula. Although any change/decrease in breastmilk or standard infant formula was at the request of parents/carers, the nutritional aim was to provide a combined total of 3 g/kg/day of protein [2] from protein substitute (both infant and weaning) and natural protein exchanges (from food, breastmilk or standard infant formula).

2.5. Ethical Approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and favourable opinion was given by the local research ethics committee (West Midlands–South Birmingham). Written informed consent was obtained from parents/carers for all children.

2.6. Data Analysis

Subject numbers were based on the number of new PKU diagnoses meeting the study criteria across 3 centres during the 2 years study period. Descriptive statistics and quantitative outcome measures were summarised. Non-parametric tests (Wilcoxon signed rank and Mann Whitney tests) were used to compare PKU and control groups for differences in quantitative measures using GraphPad Prism version 6.01 for Windows, GraphPad Software, La Jolla California USA.

3. Results

3.1. Subjects

Twenty infants (diagnosed with PKU on newborn screening from Birmingham Children's Hospital (n = 17), Bradford Teaching Hospitals (n = 2) and the Royal Hospital for Children, Glasgow (n = 1) who were commencing weaning and required a second stage Phe-free protein substitute during the study period were recruited (Table 1).

Twenty control children commencing weaning and matched with the PKU group for mother's educational level (n = 18) and birth order (n = 18), were also recruited. Two control children were of Caucasian/Afro Caribbean origin, all other subjects were white Caucasian.

3.2. First Weaning Foods and Textures

Parents/carers in both PKU and control groups decided the age to commence weaning and this differed between groups (median PKU: 4.1 m (range: 2.8–5.0); control 5.0 m (range: 3.7–6.0); p = 0.001 (Mann Whitney)). Children with PKU commenced weaning on a narrow range of low protein foods –mostly puree fruit, vegetables or low protein rusks; whilst control children had a larger variety of first weaning foods with most consuming baby rice/porridge (Table 2). The texture of first weaning foods was similar between groups.

There was no significant difference between the groups in terms of age of progressing onto different textured foods other than the PKU group starting thin fluids at a younger age, but this is associated with the younger age of weaning in this group (Table 3).

3.3. Fluid Intake

Total fluid intake from breastmilk and formula (combined protein substitute and standard infant formula for PKU) was similar in both groups at all ages (Table 4). Breastmilk intake was estimated based on recommended quantities of infant formula for that age. Children with PKU consumed less fluid each day (mean difference 82 mL/day; range 13–184 mL) than did control children up to 12 months of age, thereafter, control children consumed less (mean difference 72 mL/day; range 35–113), but this was not statistically significant (Mann-Whitney). Fewer children with PKU were breastfed (n = 3) compared with control children (n = 9) during the study period. Children with PKU also stopped infant formula (Phe-free) on average later than control children. By 12 months of age all children with PKU had fully transitioned to eating solid foods as the source of their phenylalanine/protein exchanges in place of breastmilk or standard infant formula, with most (n = 18) completing this transition by 8 months of age. However, protein substitute formula continued to provide a significant portion of energy, protein and nutrient intake during the gradual transition onto the weaning protein substitute. During this transition period, low protein milk replacements were introduced to meet energy requirements. In comparison, nearly half (45%; n = 9) of the control children had transitioned from breastmilk or standard infant formula to cow's milk by 12 months of age, and all but one (95%; n = 19) by 24 months of age.

Table 1. Subject demographics.

	PKU				Control			
Subject No.	**Sex**	**Age at Weaning (months)**	**PKU Siblings**	**Mother's marital Status**	**Age Stopped Breast Feeding (months) ***	**Sex**	**Age at Weaning (months)**	**PKU Siblings**	**Mother's marital Status**	**Age Stopped Breast Feeding (months) ***
1	M	4.1		Married		M	4.6	3	Married	
2	M	5.0		Married		F	5.0		Married	10
3	F	3.3		Single		M	5.5		Married	9
4	M	3.4	1	Married		F	4.4		Married	7
5	F	5.0		Partner		M	5.7		Married	8
6	F	3.7		Partner		M	5.8		Married	
7	M	3.9		Married		M	6.0		Single	
8	F	5.0		Married		M	3.8		Partner	
9	F	4.5		Single		M	5.7		Partner	
10	M	2.8		Single		F	3.7		Single	
11	M	3.7		Married	5	F	4.5		Single	
12	F	3.7		Married		M	4.0		Partner	12
13	M	3.3		Married		F	5.0		Partner	
14	M	4.7		Married	8	F	4.5		Partner	18
15	M	4.4	2	Married		F	5.0		Married	
16	M	4.4	2	Married		M	4.5		Married	
17	M	4.5		Married		M	5.0		Partner	
18	M	5.0		Partner		M	4.4		Married	7
19	M	4.0		Married		F	5.0		Married	10
20	M	4.0		Married	8	M	5.0		Married	18

* age stopped breast feeding for those still breast feeding at point of weaning (3–6 months)

Table 2. First weaning foods and textures.

	PKU %	Control %
1st Weaning Foods		
Baby rice/porridge	0	75
Puree fruit	85	55
Puree vegetables	65	30
Rusks- regular	0	25
Low protein rusks	45	0
Mashed potato	5	20
Stage 1 baby food jar	0	20
Weaning Textures		
Thin puree	70	70
Thick puree	60	45
Mashed	15	25
Finger food	5	15

Table 3. Average age for progression onto different textured foods.

	Mean Age in Months (Range)		
	PKU	**Control**	***p* value ***
Thin puree	4.5 (3–6)	5.0 (3–8)	0.03
Thick puree	5.8 (3–7)	5.4 (3–8)	0.29
Weaning foods	5.2 (4–8)	5.3 (4–7)	0.82
Normal family foods	7.6 (5–11)	7.1 (4–10)	0.46

* Mann-Whitney.

Table 4. Total fluid intake (mL) per day; PKU vs. control.

	Mean Volume ml (Range)						
Age (m)	**PKU Phe-Free Infant Formula**	***n***	**PKU Standard Infant Formula**	***n***	**PKU Total Volume**	**Control Total Volume**	***n***
4	493 (160–870)	11 *	304 (170–540)	11 *	796 (400–1170)	981 (750–1260)	9 *
5	510 (190–900)	20	260 (90–480)	20	744 (435–1200)	776 (550–1110)	18 *
6	509 (210–720)	20	181 (60–340)	20	623 (450–750)	663 (450–840)	20
7	567 (300–840)	19	126 (600–380)	11	641 (360–930)	653 (240–1000)	20
8	511 (200–810)	19	90 (90–90)	2	521 (200–810)	614 (270–900)	20
9	498 (250–840)	19	90	1	503 (250–930)	559 (285–1010)	20
10	397 (240–600)	19	90	1	403 (240–600)	485 (120–890)	20
11	387 (110–640)	18	90	1	393 (110–640)	501 (180–900)	17
12	347 (80–840)	18	0	0	347 (80–840)	502 (240–1160)	11
15	448 (210–840)	13	0	0	448 (210–840)	488 (270–600)	6
18	311 (120–840)	10	0	0	311 (120–840)	383 (300–450)	4
24	420 (240–750)	4	0	0	420 (240–750)	540	1

* not all subjects had commenced weaning at this age; m: months.

3.4. Independent Self-Feeding Skills

Children with PKU were more likely to be spoon fed by the parent and drink from a bottle compared with control children and this was still apparent at 2 years of age. They were also slower to start drinking from a cup or straw than control children (Table 5). In terms of age of developing specific self-feeding skills, there was no significant difference between the groups other than for self-finger feeding which commenced at a younger age in control children (Table 6).

Table 5. Age of subjects for attaining feeding skills (%) (n = 20 PKU; n = 20 Control).

	PKU Control	4 m n = 13 n = 11	5 m n = 19 n = 20	6 m n = 20 n = 20	7 m n = 20 n = 20	8 m n = 20 n = 20	9 m n = 20 n = 20	10 m n = 20 n = 20	11 m n = 20 n = 20	12 m n = 20 n = 20	15 m n = 20 n = 20	18 m n = 20 n = 20	24 m n = 20 n = 20	p value #
Spoon fed by parent	PKU	100	100	100	100	100	100	100	100	100	95	90	85	0.008
	Control	89	100	100	100	100	100	95	95	85	80	55	25	
Spoon feeds self	PKU	8	11	10	15	5	20	10	30	50	65	80	100	0.29
	Control	0	6	5	15	10	5	30	25	40	55	85	90	
Fork feeds self	PKU	0	0	0	0	0	0	0	0	15	10	40	80	0.25
	Control	0	0	0	0	0	5	5	5	5	40	60	85	
Finger fed by parent	PKU	0	21	40	65	55	60	70	70	65	50	60	35	0.78
	Control	44	39	50	40	50	70	75	85	70	65	25	5	
Finger feeds self	PKU	5	15	45	90	100	100	100	95	100	100	100	100	0.76
	Control	15	40	80	85	90	95	95	100	100	100	100	90	
Drinks from bottle	PKU	100	95	100	95	100	100	100	90	85	90	100	85	0.006
	Control	67 *	61	75	70	100	100	85	90	95	80	65	30	
Drinks from cup	PKU	8	16	30	40	45	55	60	65	65	80	85	85	0.004
	Control	0	17	35	55	70	75	85	85	90	95	95	90	
Drinks from straw	PKU	0	0	0	0	0	10	10	15	30	30	45	70	0.002
	Control	11	6	5	5	10	20	25	25	30	40	50	70	

* n = 3 breastfed only (no bottles); # Wilcoxon signed rank test; m: months.

Table 6. Mean age (months) of feeding skills development.

	Mean Age in Months (Range)		
	PKU	Control	p value *
Self finger-feeding	6.5 (4–8)	5.7 (4–9)	0.01
Self spoon-feeding	12.8 (5-24)	12.6 (5–18) 1 still not	0.99
Self fork-feeding	19.2 (12–24) 3 still not	18.1 (12–24) 2 still not	0.41
Drinking from a cup	8.8 (4–15) 2 still not	7.8 (5–15)	0.43
Drinking from a straw	14.7 (9–24) 6 still not	14.9 (4–24) 4 still not	0.94

* Mann-Whitney.

3.5. Feeding Environment

At 4 months of age, both groups were more likely to feed children in a bouncer followed by a highchair or their parent's lap (Table 7). Feeding in a bouncer was more common in the control group from 4–7 months than in the PKU group. From 6 months of age, a highchair was the most common place to feed for both groups until 24 months when the control group were more likely to be fed at the family table.

Table 7. Percentage of children fed in different environments at different ages.

		4 m	5 m	6 m	7 m	8 m	9 m	10 m	11 m	12 m	15 m	18 m	24 m
Highchair	PKU	38	53	70	80	85	85	80	90	100	80	70	50
	Control	11	33	60	90	85	90	95	100	100	80	75	45
Lap	PKU	31	26	30	15	10	10	15	15	5	10	15	5
	Control	56	28	25	20	10	20	20	20	0	5	0	0
Bouncer *	PKU	38	26	15	0	5	5	5	0	5	5	5	0
	Control	67	67	40	20	5	5	5	5	0	0	0	0
Table/Booster **	PKU	0	0	0	0	5	0	0	0	0	5	15	30
	Control	0	0	0	0	10	5	0	0	0	25	40	85
Other (sofa, cot, pushchair, floor)	PKU	8	21	0	10	10	15	15	5	5	5	10	15
	Control	11	6	10	15	5	10	10	0	0	10	10	5

* bouncer = a low-lying chair that can be used to lightly bounce babies, it is designed to fully support a baby in a semi reclined position; ** booster = a seat that can be placed on an existing seat to 'boost' the height of the child at a normal table; m: months.

3.6. Frequency of Meals and Snacks

Children with PKU generally had one less meal or snack of solid food per day compared to control children and sometimes an additional meal of infant (Phe-free) formula; although by 2 years of age this difference was not evident (Table 8).

Table 8. Median number of meals or snacks per day.

	4 m	5 m	6 m	7 m	8 m	9 m	10 m	11 m	12 m	15 m	18 m	24 m
Total Number of Meals/Snacks												
PKU	6	6.5	6	6	6	6	6	6	5	6	5	6
Control	9	8	7	8	7.5	7	7	7	7	7	6	6
p value *	0.01	0.07	0.37	0.01	0.02	0.07	0.02	0.06	0.04	0.03	0.12	0.80
Number of Solid Meals/Snacks												
PKU	2	3	3	3.5	3	3.5	4	4	4	4	4	4
Control	1	2	3	4	5	5	5	5	5	5	5	5
p value *	0.05	0.27	0.57	0.38	0.30	0.01	0.02	0.04	0.09	0.02	0.03	0.16
Number Fluid Breastmilk/Formula Feeds												
PKU	6	6	5	5	4	4	3	3	2	2	1	0
Control	7.5	5	4	4	4	3	2.5	3	2	0	0	0
p value *	0.005	0.22	0.04	0.33	0.80	0.12	0.05	0.76	0.12	0.06	0.04	0.25

* Mann-Whitney; m: months.

3.7. Negative Behaviour with Protein Substitute (PKU Group)

The most frequent negative behaviours associated with taking weaning protein substitute were closing the mouth or turning the head away when offered the protein substitute; occurring in at least 5 children at each monitoring visit. These negative behaviours were most common at the age of 10–18 months coinciding also with the age for teething (Figure 1).

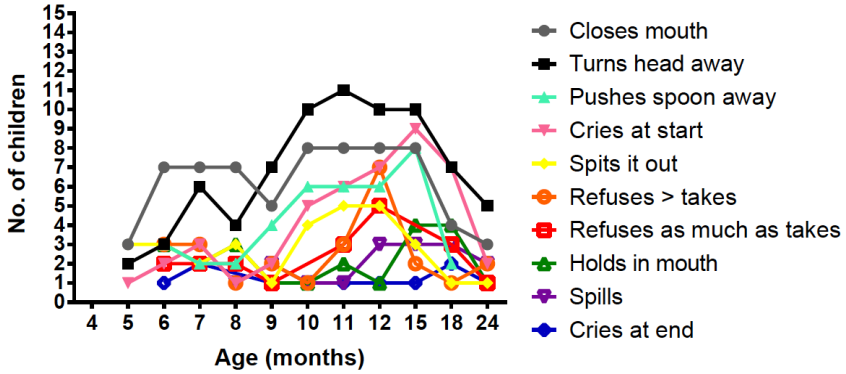

Figure 1. Number of children displaying different negative behaviours associated with protein substitute administration at each age.

3.8. Gastrointestinal Symptoms

There was no significant difference between the two groups for total number of incidences or number of subjects experiencing vomiting, diarrhoea, bloating, constipation or colic at any age. However, more children with PKU reported problems with flatulence (0.0005), burping ($p = 0.001$) and retching ($p = 0.03$) at all ages; and more controls reported regurgitation ($p = 0.003$) (Wilcoxon signed rank test). Symptoms generally declined with increasing age although vomiting and diarrhoea were variable, most likely associated with illness (Figure 2).

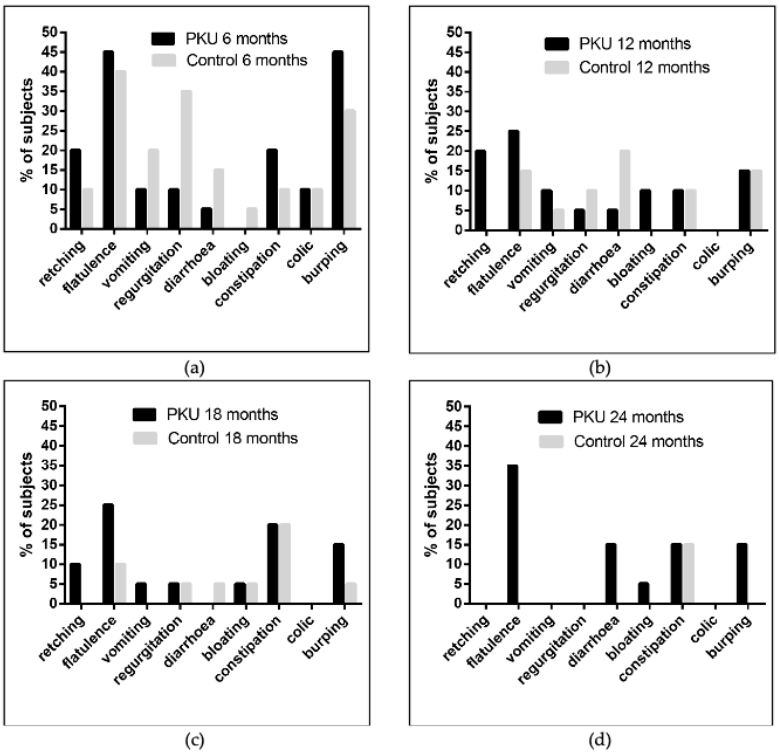

Figure 2. Percentage of subjects reporting gastrointestinal symptoms at (**a**) 6 months, (**b**) 12 months, (**c**) 18 month and (**d**) 24 months of age.

4. Discussion

Children with PKU in this cohort generally experienced very similar weaning experiences to their non-PKU peers. Progression onto more textured foods, reduction in fluid (breastmilk/infant formula) intake, self-feeding skills, the feeding environment and gastrointestinal symptoms such as diarrhoea, vomiting, constipation and colic were similar across the two groups and where differences did occur, most had resolved by 2 years of age.

There may be a critical period between 6–7 months when infants are developmentally ready for the introduction of more textured foods, and delaying this beyond 10 months of age may increase the risk of food refusal, fussy eating, delayed self-feeding, poor routines, unhealthy eating, and limited variety in the diet [6,11]. This is particularly important in PKU where the diet is already quite limited and feeding problems are known to be more common [12]. The stepwise introduction of a semi-solid Phe-free protein substitute and the stepwise decrease in liquid protein substitute during weaning enabled age-appropriate progression with respect to food textures, in line with the general aims of weaning. This permitted children to become accustomed to solid foods with less dependence on high volumes of infant protein substitute; and it appeared to maintain the appetite for solid foods by reducing energy intake from protein substitutes.

Typically, during weaning, non-PKU children gradually increase solid food intake and concomitantly reduce breastmilk/formula intake, generally replacing it with cow's milk at 12 months of age. For children with PKU the process is more complex; reducing infant protein substitute formula results in a reduction in energy intake that is not compensated for by the lower energy weaning protein substitute. This energy deficit is made up by the introduction of a low protein milk replacement and low protein special foods, and these are generally introduced before 12 months of age.

Total volume of fluid intake was similar in both groups up to 11 months of age (except at 4 months when subject numbers were much smaller as some had not commenced solids). By 12 months, half the

control children had fully transitioned to cow's milk, but it was another 6 months before half the PKU group had fully transitioned to weaning protein substitute and low protein milk replacement. Children with PKU relied on their infant protein substitute and subsequently continued to drink from a bottle for longer in order to meet energy requirements. However, by 2 years of age most children in both groups had stopped both Phe-free infant formula and breastmilk/standard infant formula. Fewer infants with PKU were breastfeeding (in combination with infant formula (Phe-free) than controls at weaning commencement, but it has been established in other studies that the duration of breastfeeding is short in PKU, and this is an area that warrants further research to examine any barriers [13]. Breastfeeding can successfully be incorporated into the PKU diet, but a normal intake of breastmilk would exceed the Phe requirements of infants, and thereby lead to poor blood Phe control. Therefore the volume is limited, carefully controlled, and given in combination with a Phe-free infant formula. During weaning, any breastmilk is gradually replaced with the equivalent quantity of Phe/protein from a food source. Although the timing of this process is led by parents, the gradual introduction of protein containing foods is important to help the feeding development of a child particularly with respect to adjusting to new tastes and textures. This process requires close supervision by the metabolic dietitian.

Children with PKU were given first non-milk foods 1 month earlier than their non-PKU peers and the range of first weaning foods was unsurprisingly less varied than in control children, due to the protein content of some of the more common first weaning foods, such as baby rice, other cereals and mashed potato. However, the age for moving onto more textured foods and developing self-feeding skills was similar. Children with PKU were more likely to be spoon fed by a parent for longer than controls although this may reflect the need to give the weaning protein substitute and to ensure the full amount of prescribed natural protein, given as measured Phe exchanges, are consumed.

Self-finger feeding occurred at a later age in children with PKU possibly associated with longer spoon feeding by parents, but they also generally consume one less meal or snack per day than controls. However, children with PKU were less likely to be offered snacks between meals in case this decreased appetite for their Phe-free weaning protein substitute.

Control children were more likely to be fed at the family table from 15 months of age compared with children with PKU. This is consistent with previous reports that suggest children with PKU are more likely to be fed separately from the rest of the family [14]. Parents of children with PKU may feel the need to be more in control of their child's mealtime due to fears of poor metabolic control; so, eating at a separate time to the family and spoon-feeding may help parents to concentrate and focus more on their child's eating. In addition, there may be an element of guilt surrounding the different foods offered to their child with PKU compared with the rest of the family, and this requires further study.

Gastrointestinal symptoms were different in both groups with flatulence, retching and burping more common in the PKU group and regurgitation in controls. Parents perceived flatulence as being directly associated with weaning protein substitute and this may be associated with its low volume and high osmolality. Negative behaviours associated with weaning protein substitute also occurred during food consumption around the age of 10-18 months and coinciding with teething. The problems observed were comparable to a previous retrospective report [5].

5. Conclusions

Weaning development and progression in children with PKU is comparable to that of control children, despite the additional changes required in terms of introducing protein exchange foods, and weaning protein substitute. Where differences occur, they are likely to be associated with additional parental anxiety, as food intake needs to be controlled and closely monitored. This may have led to extended spoon-feeding, earlier cessation of breastfeeding, prolonged bottle feeding with Phe-free infant protein substitute and separate mealtimes to the rest of the family; which may in turn delay independent self-feeding. Parents require support to manage the complexity of feeding times, and to normalise the social inclusivity of their child's food environment. Further study about parental anxiety associated with mealtimes is required.

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CHAPTER 2C: MEALTIME ANXIETY AND COPING BEHAVIOUR IN PARENTS AND CHILDREN DURING WEANING IN PKU: A CASE-CONTROL STUDY

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Mealtime Anxiety and Coping Behaviour in Parents and Children during Weaning in PKU: A Case-Control Study

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Abstract: Solid food introduction may create anxiety for parents of children with phenylketonuria (PKU) due to the burden associated with protein substitute (PS) administration and natural protein restriction. In a longitudinal, prospective study, 20 mothers of children with PKU and 20 non-PKU control mothers completed 4 questionnaires (mealtime emotions, feed-time, Beck's anxiety inventory and the coping health inventory for parents), examining parent/child mealtime emotions, anxiety, stress and coping strategies at child ages: weaning start, 8 months (m), 12 m, 15 m, 18 m and 24 m. Overall, mothers of children with PKU cope well with solid food introduction when applying a low-phenylalanine diet, with comparable low levels of stress and anxiety reported in both PKU and non-PKU groups. However, mothers of children with PKU reported peak scores in anxiety for emotive/cognitive symptoms at a child age of 15 m, and higher use of coping strategies at 15 m and 24 m ($p < 0.05$) of age. Generally, there was a trend that maternal anxiety regarding child rejection of PS increased with time, peaking between 12–24 m. In PKU, a child age of 12–18 m is identified as a key period when mothers feel most anxious/stressed with feeding, coinciding with raised blood phenylalanine levels probably associated with teething, illness and developing independence. Health professionals should be conscious of this vulnerable period and be prepared to offer more directional support as required.

Keywords: Phenylketonuria (PKU); weaning; mothers; anxiety; stress; coping; Child Health Inventory for Parents (CHIP); Beck Anxiety Inventory (BAI)

1. Introduction

A diagnosis of phenylketonuria (PKU), an inherited metabolic amino acid disorder, is a life-changing event for parents [1]. Treatment involves strict adherence to a controlled, very low-protein diet, consisting of a limited range of special low-protein foods in order to maintain blood phenylalanine (Phe) within the target range and prevent neurological sequelae. Parents must ensure that their children consume a non-negotiable and weighed amount of natural protein together with a bitter tasting protein substitute in prescribed amounts each day. Parents understand that the neurological outcome of their

child depends on their ability to successfully implement diet therapy, and this can have a significant impact on stress and quality of life [1–4].

The process of weaning a child with PKU is likely to enhance parental anxiety [3], and whilst anxiety is a normal emotion that prepares the mind and body to respond to a threat, when it persists over long periods, it can lead to a number of negative medical and psychological outcomes including the development of anxiety disorders [5]. The guilt, grief and disappointment of not having a healthy baby is prominent, and they must face administering a severely restrictive diet in order to control their child's blood Phe levels at a time when they are still emotionally vulnerable [3,5]. There are many milestones that must be reached (e.g., introduction of a measured quantity of Phe from solid food each day, tolerance of a second-stage spoonable protein substitute and acceptance of special low-protein foods), which require extensive parental organisational skills and tenacity. Any food refusal, rejection of protein substitute, child illness, inability to access special dietary products, social situations involving food (parties, eating out) and sub-optimal blood Phe control is likely to heighten parental anxiety [1,3]. Consequently, there may be conflict between maintaining blood Phe levels within the target range and allowing dietary deviations aimed at meeting child and parent psychosocial needs and the desire for family normality [5].

Thereby, parents of children with PKU require a diverse range of complex skills and additional time in order to manage and control dietary treatment, as well as the stress associated with adherence, social isolation and regular blood Phe monitoring [1,3,6,7]. Metabolic and psychological outcomes are likely to be influenced by parental coping strategies and the ability of the family to alter their lifestyle to accommodate the demands of a complex dietary regimen [2–5]. However, it is uncertain what coping mechanisms exist in the parents of children with PKU, and how this might compare with parents of children with other health conditions or those of normal healthy children.

The aim of this study was to compare maternal and child anxiety and coping mechanisms in PKU with that of mothers of non-PKU children during the first two years of life whilst transitioning onto a solid food diet. This is to determine the effect of the additional burdens of protein substitute administration and natural protein prescription adherence.

2. Materials and Methods

2.1. Subjects

2.1.1. Phenylketonuria (PKU) Group

Twenty children (14 male) diagnosed with PKU at newborn screening and commencing weaning onto solid foods during the 2 year study period were recruited prospectively from three UK specialist PKU centres (Birmingham Children's Hospital (n = 17), Bradford Teaching Hospitals NHS Trust (n = 2) and the Royal Hospital for Children, Glasgow (n = 1)). Subjects were recruited and reviewed by the same dietitian at each centre. Data was recorded from 4 months for 16 subjects, from 5 months for 19 subjects and from 6 months for all 20 subjects.

2.1.2. Control Group

Twenty control children who were weaning were recruited prospectively (12 male, 18 matched with the PKU group for mother's educational level and birth order). They included siblings of inherited metabolic disorder (IMD) patients, or children recruited from a local community centre. The caregivers of control subjects received no dietary advice from one of two research dietitians. Data was recorded from 4 months for 9 subjects, from 5 months for 19 subjects and from 6 months for all 20 subjects.

2.1.3. Demographics

All subjects were white Caucasian except for 2 control children of mixed 50% Caucasian, 50% Afro-Caribbean decent. Five children from the PKU group and one from the control group had a

sibling with PKU and three carers in both groups were single mothers (note: the control child with a PKU sibling had much older adult siblings so the time lapse since they experienced weaning a child with PKU was several years).

Median age of PKU diagnosis was 10 days (range: 2–16 days). Median age of weaning was 4.3 m (range: 2.9–6.6 m) for PKU and 5.1 m (range: 3.7–6.5 m) for controls ($p = 0.04$). The decision to start introduction of solid foods was initiated by parents/caregivers in both groups. For children with PKU, the prescribed target total protein intake was 3 g/kg/day [8] and the natural protein intake at weaning was a median of 5 g/day (range: 3–7 g/day).

PKU group respondents utilised support from variable and multiple sources: 100% ($n = 20$) reported the help of a dietitian, 30% ($n = 6$) a health visitor, 25% ($n = 5$) a General Practitioner (GP) or paediatrician, 20% ($n = 4$) a specialist nurse, 50% ($n = 10$) relied on support from their own parents (children's grandparents), 35% ($n = 7$) from other family members, 50% ($n = 10$) accessed the patient support group (National Society for PKU (NSPKU)) and 65% ($n = 13$) sited the internet (websites/social media).

2.2. Study Procedures

This was a longitudinal, prospective, observational case-control study investigating anxiety and stress during mealtimes from age of weaning to 2 years.

Following introduction and establishment of low Phe (protein) solids at 4–6 months of age, children with PKU were gradually weaned from a phenylalanine (Phe)-free liquid infant protein substitute onto a semi-solid spoonable Phe-free weaning protein substitute (PKU Anamix First Spoon; Nutricia), providing 2 g of protein equivalent and 16 kcal per 5 g serving. In parallel, and under the supervision of a clinical dietitian, Phe allocation from breastmilk or infant formula was replaced with equivalent Phe, from weaning foods in the form of a 50 mg exchange system. Parents of both PKU and control children determined the age for weaning based on their assessment of readiness. However, infants with PKU were regularly monitored so there were opportunities for dietitians to discuss and advise about weaning.

Four questionnaires were completed by mothers of children with PKU and control mothers: (1) mealtime emotions, (2) feed-time, (3) Beck's anxiety inventory and (4) the child health inventory for parents (CHIP). Mealtime emotions was completed monthly from weaning until 12 months of age, then at 15, 18 and 24 months. This non-validated questionnaire comprised 5 statements with a 5-point Likert scale response, addressing maternal experiences during their child's mealtimes with respect to how stressed (1)/relaxed (5), and rushed (1)/unrushed (5) they felt, how noisy (1)/quiet(5) mealtimes were and if mealtimes were mostly tearful (1) or happy (5) for both themselves and their child.

The remaining three questionnaires were completed by mothers at weaning commencement, 8, 12, 15, 18 and 24 months of age. The non-validated feed-time questionnaire comprised 9 questions (8 for controls) with a 7-point Likert scale response. Questions addressed maternal feelings before giving their child a bottle feed and before giving solids. For mothers of PKU children, additional questions addressed feelings around administering a weaning Phe-free spoonable protein substitute, including how they felt: immediately before administration, if their child refused, when their child had finished and compared to how they felt three months ago. They were also asked if they ever delayed protein substitute administration because of the way it made them feel and how much they would prefer someone else to administer it to their child. Control mothers were asked the same questions but with reference to how they felt when giving solid foods.

The Beck anxiety inventory (BAI) [9,10] is a validated, 21-item, multiple-choice self-report questionnaire that measures the severity of anxiety, with each question describing emotional, physiological or cognitive symptoms of anxiety, and mothers reported the extent to which they had been concerned by each of the symptoms in the previous week (scoring: not at all, mildly, moderately or severely). Two thirds of the questionnaire ($n = 14/21$) focuses on physiological symptoms

of anxiety such as trembling, numbness, dizziness and sweating. A total score of 0–7 indicates minimal anxiety, 8–15 mild anxiety, 16–25 moderate anxiety and 26–63 severe anxiety.

The coping health inventory for parents (CHIP) is a validated questionnaire designed for use with families of children with a serious and/or chronic illness [11]. CHIP lists 45 coping behaviours, each scored from 0 (not helpful) to 3 (extremely helpful). Coping behaviours are divided into 3 subsets that identify different coping patterns: (a) maintaining family integration, cooperation and an optimistic definition of the situation (19 items), (b) maintaining social support, self-esteem and psychological stability (18 items) and (c) understanding the medical situation through communication with other parents and consultation with the medical staff (8 items). The sum/total of each item within subsets provides a measure of the usefulness of that coping strategy in the management of the condition. A high score represents a higher degree of coping. The same questionnaire was used for both mothers of PKU and control children, but during analysis, questions specifically relating to medical care ($n = 6$ from subset 1) were removed from both groups to enable comparison. Data was also compared with normative data provided by the questionnaire authors based on the results of 308 mothers with a chronically ill child [11].

Weekly blood spots for Phe were taken by parents on standard filter cards, Perkin Elmer 226 (UK Standard Newborn Screening (NBS), Public Health England, London, UK). Parents had received training on blood spot collection prior to study commencement. Blood spots were returned to the subject's hospital laboratories. Blood Phe was analysed by MS/MS tandem mass spectrometry.

Data on growth, qualitative aspects of feeding development, gastro-intestinal symptoms, the feeding environment, feeding practices and progression, and weaning problems, are reported in separate publications [12,13].

2.3. Ethical Approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and a favourable ethical opinion was obtained from West Midlands–South Birmingham National Research Ethics Service (NRES) Committee. Written informed consent was obtained from the mothers of all participants.

2.4. Data Analysis

As an observational study, there was no formal hypothesis comparing the two treatment groups and therefore, no formal power calculation to determine the study size. The number of subjects recruited was determined by the number of subjects diagnosed with PKU per year in the study centres (approximately 4–6 per centre). Quantitative outcome measures are summarised, and descriptive statistics of the data are presented. Between-group differences for questionnaire responses were analysed using suitable non-parametric tests (paired/unpaired *t*-tests and Mann–Whitney tests) using GraphPad Prism version 6.01 for Windows, GraphPad Software, La Jolla, CA, USA. Correlations between blood Phe results and questionnaire responses were calculated using Spearman's rank correlation. In addition, data was analysed using longitudinal regression techniques using a linear mixed-models approach, including subject ID as a random intercept, time as a categorical factor and, where appropriate, treatment group (PKU versus control). These were analysed using R version 3 computer software.

3. Results

3.1. Mealtime Emotions Questionnaire

For both PKU and non-PKU groups, levels of stress and mood reported at mealtimes were generally not statistically different over time and were mainly positive (score between 4 to 5 out of 5). However, there was a trend for mealtime mood to be less enjoyable for both parent and child with PKU (Figure 1) between the age of 8 m and 24 m, compared to before 8 m and compared to controls.

Children with PKU were reported by mothers to be "less happy" than their control peers, although this only reached significance at 12 m ($p = 0.04$; Mann–Whitney test) (Figure 1b). Longitudinal regression analysis demonstrated that there was a significant difference ($p = 0.002$) between children with PKU and control children in terms of how happy they were at mealtimes, and this difference remained unchanged overtime between the two groups.

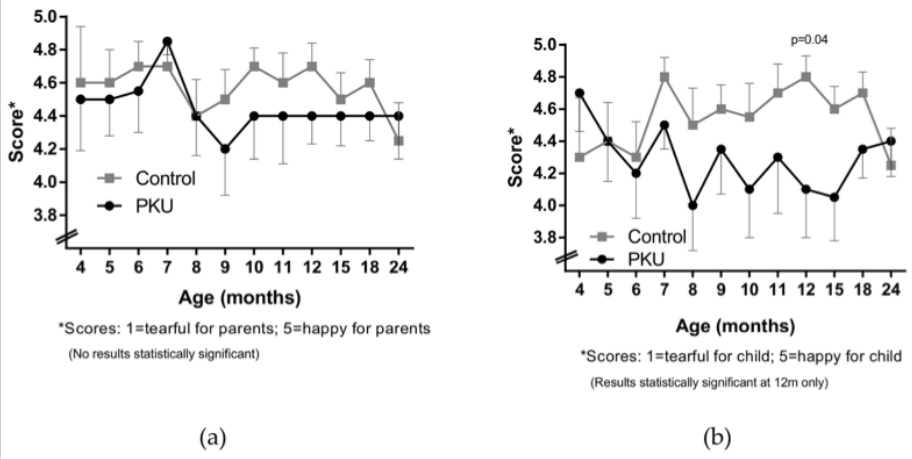


Figure 1. Mean (**a**) maternal and (**b**) child mood during children's mealtime.

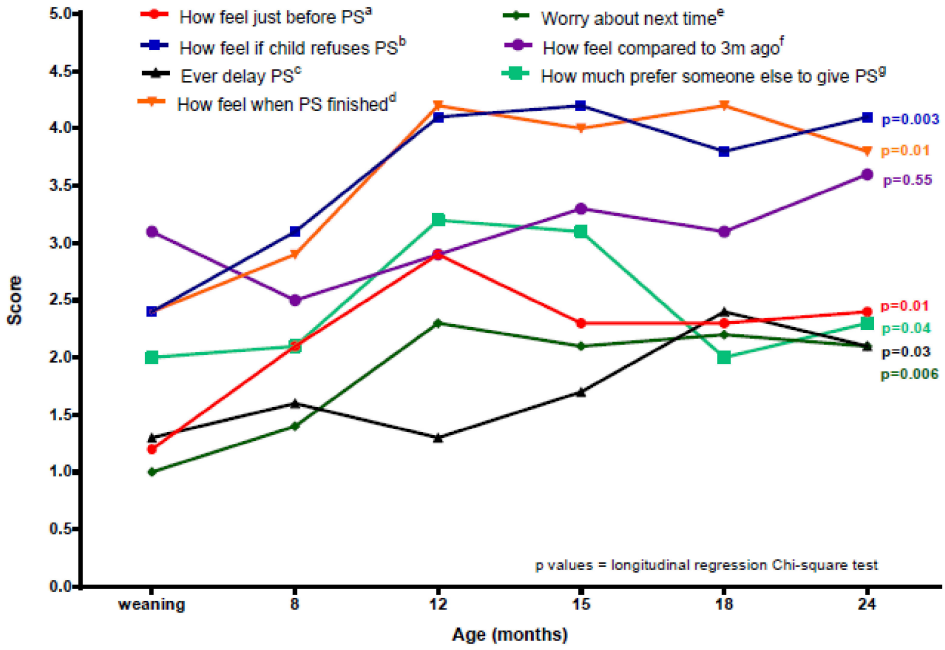
Both groups of parents reported similar levels of anxiety with respect to how rushed they felt during children's mealtimes, and control parents often reported noisier mealtimes than parents of PKU children, although no differences were statistically significant between groups or over time.

3.2. Feed-Time Questionnaire

When asked how they felt before giving their child a solid meal during weaning, both groups scored low (between 1.4–2.0 out of 7), suggesting few negative feelings about solid food administration. Mothers of children with PKU, were well supported and at weaning were less anxious than controls (score 1.4 versus 2.0), but after an initial increase at eight months, their concerns had little change until a peak at two years of age. Control mothers became more confident about giving solid meals with increasing age of their children, although there was a peak again at two years of age. However, differences between PKU and control groups did not reach statistical significance, nor did differences within groups over time.

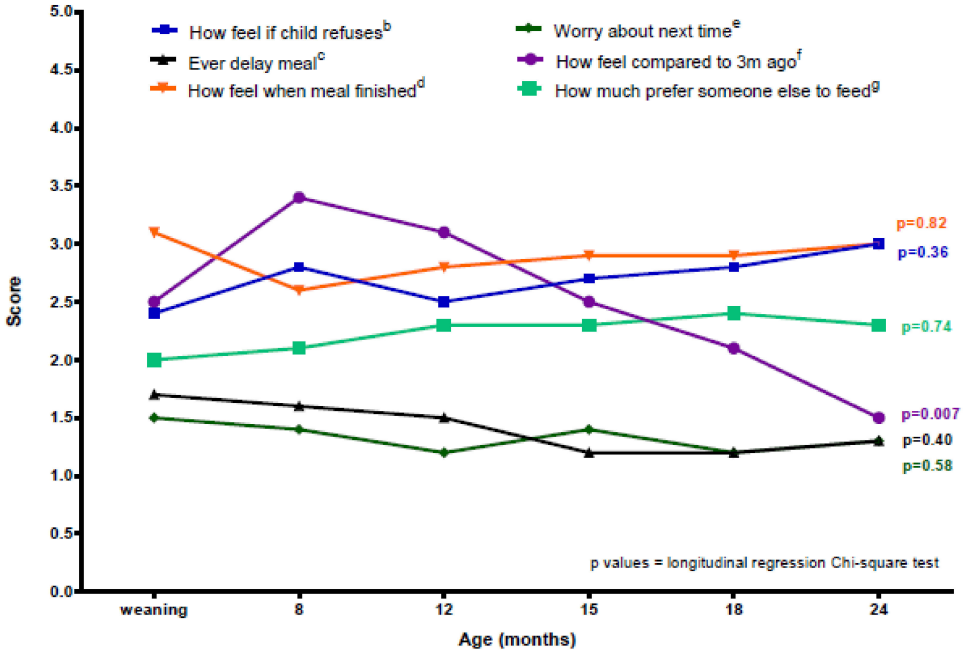
When mothers of children with PKU were asked how they felt about various aspects of giving the protein substitute, concerns were generally low (scoring between 1 and 4.5 out of 7) but peaked at around 12 months of child age, then mostly plateauing between 12–24 months (Figure 2). When analysed using longitudinal regression, all questions showed a significant difference over time, with the exception of how they felt compared to three months ago. In particular, over time, child refusal of the protein substitute caused the most concern ($p = 0.003$), and mothers expressed relief post protein substitute administration ($p = 0.01$) (Figure 2).

However, when control mothers were asked the same questions but with respect to feeding their child solid foods, there was less change over time, with mean scores for each question remaining comparable across all assessment ages (Figure 3) and lower (scores between 1 and 3.5) than in the PKU group. The exception being the question about how they felt compared to three months ago ($p = 0.007$), which had a sharp increase in anxiety score between weaning commencement and eight months, followed by a gradual decline, suggesting that they were coping better with weaning over time.



SCORES:
[a] 1 = no change in the way I feel; 7 = the most I worry about anything
[b] 1 = no difference to how I feel; 7 = makes me worry more than anything else
[c] 1 = I never think about doing this; 7 = delay giving at every feed
[d] 1 = feel no different to normal; 7 = this is the most relieved I ever feel
[e] 1 = never; 7 = always
[f] 1 = no different; 7 = feel 100% more confident
[g] 1 = would not like at all; 7 = this would be ideal

Figure 2. Mean maternal feelings about giving protein substitute to their child with phenylketonuria (PKU) at different child ages in the first two years.



SCORES:
[b] 1 = makes no difference to how I feel; 7 = makes me worry more than anything else
[c] 1 = I never think about doing this; 7 = delay giving at every feed
[d] 1 = feel no different to normal; 7 = this is the most relieved I ever feel
[e] 1 = never; 7 = always
[f] 1 = no different; 7 = feel 100% more confident
[g] 1 = would not like at all; 7 = this would be ideal

Figure 3. Mean control maternal feelings about feeding their child solid foods at different child ages in the first two years.

3.3. Beck Anxiety Inventory (BAI)

Mothers of both groups of children demonstrated low levels of anxiety with mean scores below 7 (minimal anxiety) at all ages (Figure 4). However, whilst there was a gradual decline in mean anxiety in the control group with a spike again at two years, the mothers of children with PKU showed more variable anxiety (larger standard deviations), peaking at 15 months and remaining higher than control mothers until two years, when both groups had equivalent levels of anxiety. Differences were not statistically significant between groups or over time.

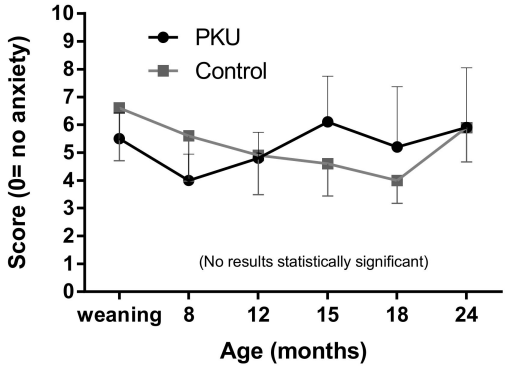


Figure 4. Mean anxiety scores for mothers of children with PKU and control mothers.

Due to the predominantly physiological focus of symptoms in the Beck's questionnaire, emotional/cognitive symptoms (nervous, terrified/afraid, fear of losing control, fear of dying, fear of the worst happening and inability to relax) were separated for analysis. When grouped together, and individual scores totalled (1 = mildly, 2 = moderately, 3 = severely) for all emotive symptoms, there was no significant difference between the two study groups (Figure 5). However, mothers of children with PKU had a peak score at 15 months of age, and this was notably higher than observed in controls, although not statistically significant. Conversely, mothers of control children had peak scores at 24 months.

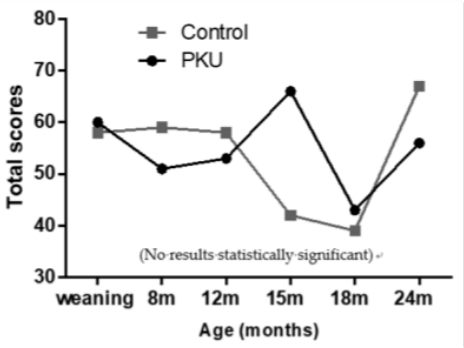


Figure 5. Total group scores for emotive symptoms experienced by mothers of PKU children and control mothers.

3.4. The Coping Health Inventory for Parents (CHIP) Questionnaire

When compared with normative mean data [11], results on the CHIP questionnaire showed that mothers of children with PKU scored, on average, higher in all three subsets (Table 1), meaning that they utilise coping behaviours more so than the average mother of a child with a chronic illness. Highest scores were at weaning, 15 and 24 m in all three subsets. Coping strategies were significantly higher at both 15 and 24 m, compared with 18 m in subset B (15 versus 18 m, $p = 0.04$ and 18 versus 24 m, $p = 0.02$; paired *t*-test) and at 15 m compared with 8 m in subset C ($p = 0.05$; paired *t*-test). Frequency of using the three different coping strategies/subsets varied, with mothers of children with PKU using family integration, cooperation and optimism (subset A), and medical communication and consultation (subset C) significantly more often than social support, self-esteem and psychological stability (subset B). Out of a total score of 3, individual mean scores for subset A were 2.3, for subset C were 2.2 and subset B was significantly lower at 1.8 (Table 1).

Table 1. Means and standard deviations for phenylketonuria (PKU) coping health inventory for parents (CHIP) subsets compared with normative data.

	A. Family Integration, Cooperation, Optimism		**B. Social Support, Self-Esteem, Psychological Stability**		**C. Medical, Communication, Consultation**	
No. of Items	**19**		**18**		**8**	
	Mean Score (SD)	**Mean Score per Item ***	**Mean (SD)**	**Mean Score per Item ***	**Mean (SD)**	**Mean Score per Item ***
Normative data [11]	40 (15)		28 (12)		15 (7)	
PKU weaning	45 (9.0)	2.4	34 (8.6)	1.8	19 (4.6)	2.4
PKU 8 m	42 (7.6)	2.2	29 (11)	1.6	16 (5.7)	2.0
PKU 12 m	44 (8.0)	2.3	31 (11)	1.7	17 (4.5)	2.1
PKU 15 m	44 (6.5)	2.3	33 (8.6)	1.9	18 (4.5)	2.3
PKU 18 m	42 (9.6)	2.2	30 (8.6)	1.7	17 (4.8)	2.1
PKU 24 m	44 (7.3)	2.3	33 (9.7)	1.8	18 (4.5)	2.3
Mean	43 (8.0)	2.3	32 (9.6)	1.8	18 (4.8)	2.2

* Unpaired t-tests: A versus B $p < 0.0001$ for all ages; A versus C p = NS (not significant) for all ages; B versus C $p = 0.0002$ (weaning), $p = 0.003$ (8 m), $p = 0.004$ (12 m), $p = 0.001$ (15 m), $p < 0.0001$ (18 m), $p = 0.003$ (24 m). SD = standard deviation.

Control mothers in comparison scored lower on subsets A and B than mothers of children with PKU (subset C was not compared as it was largely based on behaviours related to medical care; questions directly relating to medical conditions in subset A were also removed for comparative analysis) (Table 2). Differences were statistically significant at 15 and 24 months of age for subset A, and at weaning and 15 months for subset B. Longitudinal regression analysis demonstrated that there was a significant difference between groups for both subset A and subset B ($p < 0.0001$), but this difference did not change overtime between the PKU and control groups.

Table 2. Means and standard deviation scores for PKU CHIP subsets compared with control mothers.

Infant Age at Questionnaire Completion	A. Family Integration, Cooperation, Optimism			B. Social Support, Self-Esteem, Psychological Stability		
No. of Items	**13**			**18**		
	Mean (SD)			**Mean (SD)**		
	PKU **	**Control**	***p* Value ***	**PKU**	**Control**	***p* Value ***
Weaning	30 (6.4)	27 (6.9)	0.17	34 (8.6)	24 (10)	0.006
8 m	29 (5.5)	26 (7.0)	0.20	29 (11)	26 (11)	0.38
12 m	30 (6.4)	27 (6.5)	0.10	31 (11)	29 (11)	0.61
15 m	30 (4.9)	25 (7.9)	0.03	33 (8.6)	26 (11)	0.03
18 m	28 (7.0)	25 (7.3)	0.19	30 (8.6)	29 (10)	0.89
24 m	30 (5.8)	25 (6.7)	0.02	33 (9.7)	27 (9.4)	0.07

* unpaired *t*-test. ** Phenylketonuria.

When considering individual coping behaviours, a large percentage (95%) of mothers of children with PKU reported behaviours that related to medical care as being most helpful, particularly at weaning age. For example, the most helpful coping behaviour in subset A for the PKU group was believing that their child was getting the best medical care (95% scoring it a 3), followed by doing things with their children, talking to medical staff, ensuring medical treatments are carried out at home and trusting in their spouse for support. Whilst for the control group, doing things with their children was reported as the most helpful (65% scoring it a 3), followed by trusting their spouse for support, doing things together as a family and building a closer relationship with their spouse. In subset B, the most helpful behaviour in both groups was sleeping (70% of PKU and 50% of controls scoring it a 3). Other behaviours that scored highly were similar across both PKU and control groups, although the PKU group generally had a greater number of respondents scoring 3 for all behaviours. In contrast, the coping behaviours considered least helpful (score of 0) by the largest percentage of mothers (up to 50%) were similar across groups, generally from subset B, and focused on working and outside employment and doing things for themselves (hobbies, entertaining, getting away on their own).

3.5. Blood Phenylalanine Levels

Mean blood phenylalanine levels were highest and more variable between 13 to 18 months of age ($p = 0.04$, analysis of variance (ANOVA)) (Figure 6) in line with levels of maternal stress, anxiety and coping behaviours. Longitudinal regression also demonstrated a significant change in Phe values over time ($p = 0.0003$).

In addition, there were correlations between higher blood Phe and lower scores for mealtime maternal stress (i.e., more stress) at 15 months ($p = 0.01$; $r = -0.56$; Spearman, 90% confidence interval (CI), 2-tailed) and 18 months ($p = 0.03$; $r = -0.51$) but not at other ages. Similarly, mothers' mealtime mood was negatively correlated with blood Phe levels at 15 and 18 months ($p = 0.04$; $r = -0.48$) only. There was also a correlation between higher Phe levels and concern about child refusal of protein substitute at 15 months of age ($p = 0.05$; $r = 0.44$).

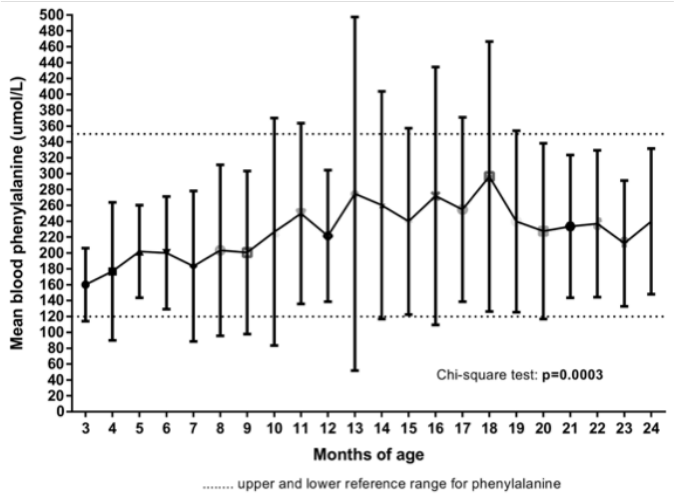


Figure 6. Mean child blood phenylalanine levels by age.

4. Discussion

This is one of the first longitudinal feeding studies in PKU to report maternal anxiety and changes in coping strategies during the introduction of solid foods and second-stage protein substitute. It is important that this is documented. The same longitudinal study has already reported on the challenges of feeding children with PKU [13,14]. Before 12 months of age, weaning was associated with low levels of anxiety in both mothers of children with PKU and mothers of normal healthy children. When weaning first commenced, mothers of children with PKU were relaxed at mealtimes in contrast to control mothers who were more stressed at the start of weaning but became more confident after this initial feeding milestone had been reached. It was evident that support from family and specialist dietitians and confidence in their child's medical care helped mothers cope with the demands of PKU management. However, as infants approach teething age and encounter minor illness and growing independence, feeding in PKU becomes more challenging and parents worry about the neurological consequences of poor dietary control. Overall, mothers of children with PKU were more variable with their concerns about feeding. This became particularly evident from 12 months of age, when their there were more issues, particularly with protein substitute administration. Results of four different study questionnaires consistently demonstrated that at around 15 months of child age, there were peaks in maternal stress and anxiety in mothers of children with PKU, and they had increased reliance on coping mechanisms. Conversely, control parents generally became more confident and less anxious with time but had peak concerns at two years of age, perhaps coinciding with growing child independence.

In PKU, there is an association between parent stress, anxiety and depression [3]. Some studies report a higher incidence in parents (particularly mothers) of children with PKU compared with parents of healthy controls, negatively impacting on quality of life and psychosocial wellbeing [15], others report no significant difference [16,17]. Whilst this study found no overall differences in anxiety, there was a suggestion that mothers of children with PKU experienced more emotive or subjective symptoms (as opposed to physiological symptoms), specifically at 15 months of age, compared to controls. This is consistent with quality of life studies in PKU that report a greater emotional impact on parents [18]. Fear of neurological damage in their children dominates their thoughts [1,3], and it is established that average blood phenylalanine levels rise in the second year of life [19], associated with teething and intercurrent illnesses. In this study, blood phenylalanine levels were highest between 13 to 18 months of age, correlating with increased levels of maternal stress, particularly with respect to protein substitute refusal. This lends further support to the previously acknowledged age of feeding difficulty of 12–18 months in children with PKU [13]. It was reassuring in this study that by the age of two years, anxiety associated with feeding children with PKU was similar to control mothers. Throughout

this time period, intense dietetic support and proactive management strategies to counteract feeding problems as they arise may have helped lessen parental anxiety.

Mothers of children with PKU frequently carry the responsibility for managing the low Phe diet, including meal preparation and dietary supervision. This requires an additional time burden (an average of 19 h/week extra) [15], which may result in a loss of earnings [1,6]. Fathers, in contrast, have been reported to feel less disease-specific parenting stress as they may be less directly involved in dietary management [15], and this disparity can affect relationships within the family [1,5]. Furthermore, low educational levels in parents have been reported to compound levels of stress and depression [2,15].

Mothers of children with PKU demonstrated significantly higher coping scores than control mothers at weaning, 15 and 24 months of age, higher scores compared to normative data for mothers of chronically ill children and similar scores to that previously reported for parents of children with asthma [20]. In theory, the more coping behaviours used by parents, the less stress they will experience, so higher CHIP scores reflect more adaptive styles of coping [11]. Therefore, in line with previous research into parental anxiety in PKU [17], mothers of children with PKU seem to adapt to the additional stresses that they experience during the weaning period. They are most likely to cope by relying on family (including grandparents) [4], being optimistic [3] and gaining understanding of PKU through communication with their specialist dietitians. This involved believing their children were receiving the best medical care, spending quality time with their children, receiving support from their spouse and trying to ensure prescribed protein substitute and protein exchanges were taken. They were less likely to use strategies that involved social support outside of the family or caring for their own self-esteem and psychological wellbeing by pursuing hobbies [1]. Whilst all mothers in this study had good support from a specialist dietitian, two thirds of mothers with PKU (65%) used the internet for support compared with 50% using their own family support network (e.g., grandparents) and 35% using other health professionals (health visitors, GPs, nurses). This is surprising but may reflect a need to talk to other mothers who have similar experiences.

Therefore, following a diagnosis of PKU and early months of weaning, families need to redefine their way of life. Family unity, optimism in the face of stress, developing positive relationships with their health care team and gaining a good understanding of the treatment required are important coping mechanisms [3,4]. Regular weekly contact with a consistent and experienced dietitian for the first two years of life is central to this support. Maintaining weekly blood phenylalanine monitoring will provide knowledge and reassurance about blood Phe control. Encouraging guilt-free downtime for mothers to do things for themselves is important in improving self-confidence, enabling adaption to the changing family dynamics and ultimately, to cope better with their situation. However, it is acknowledged that this is difficult as mothers may fear relinquishing control and allowing others to care for their child, who they may not trust to administer the diet as prescribed [3,8]. Providing formal training about PKU, protein substitute preparation and food suitability to grandparents and other family members is important. Anxiety and stress may lead to parents using overprotective parenting strategies that restrict the child's emotional or social development [3]. Assisting parents to maintain a balance of different coping behaviours is imperative.

There were some study limitations. While consistent trends were identified between PKU and control groups with respect to anxiety and coping, results in this study were often not statistically significant, probably associated with the small numbers of patients available for recruitment. A larger cohort of subjects may have led to more feasible statistical comparisons and more significant differences, and this is an area that warrants further study. Furthermore, the Beck's anxiety inventory focused predominantly on physiological symptoms. In keeping with previous reports in parents of children with PKU, this study identified a more significant impact from emotional symptoms [3]. In addition, only mothers completed questionnaires, so coping strategies and anxiety may manifest in different ways in fathers. They may also differ according to the severity of PKU, the age of the child and country of origin. How tearful or happy children were during mealtimes was also a subjective measure that was likely influenced by the mother's feelings at the time. It also must be noted that one parent in

the control group had experienced weaning both a PKU and a non-PKU child (although they were only included in the control group and the PKU siblings were adults) and this may have influenced their responses. In addition, the feed-time questionnaire looked at feelings around protein substitute administration for parents of children with PKU, but for the control children, it looked at administration of solid food. It might have been useful to look at maternal feelings around solid food administration in the PKU group as well, to ascertain whether it is mostly administering the protein substitute that is causing anxiety or whether feeding solid food also causes additional stress.

5. Conclusions

Mothers of children with PKU appear to cope well with weaning, with low levels of stress and anxiety comparable to control mothers. Administration of a protein substitute is challenging, particularly between 12–18 months of age. This is a key period during weaning when mothers of children with PKU feel most anxious and stressed, coinciding with raised blood phenylalanine levels, teething, illness and children developing their own independence. Health professionals need to be cognisant of this vulnerable period and be prepared to offer more directional support as required. Periodic psychological reviews of parental stress levels, anxiety and coping strategies would enable health professionals to better identify those parents of children with PKU most at risk of depression or negative psychosocial outcomes. However, it is reassuring to report that by two years of child age, with health professional support, this group of mothers of children with PKU reported similar levels of anxiety and stress to that of control mothers.

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CHAPTER 2D: GROWTH, PROTEIN AND ENERGY INTAKE IN CHILDREN WITH PKU TAKING A WEANING PROTEIN SUBSTITUTE IN THE FIRST TWO YEARS OF LIFE: A CASE-CONTROL STUDY

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Growth, Protein and Energy Intake in Children with PKU Taking a Weaning Protein Substitute in the First Two Years of Life: A Case-Control Study

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Abstract: Growth issues have been observed in young children with phenylketonuria (PKU), but studies are conflicting. In infancy, there is an increasing trend to introduce a second-stage semi-solid weaning protein substitute (WPS) but there is concern that this may not meet energy requirements. In this longitudinal, prospective study, 20 children with PKU transitioning to a WPS, and 20 non-PKU controls were observed monthly from weaning commencement (4–6 months) to 12 m and at 15, 18 and 24 months of age for: weight, length, head circumference, body mass index (BMI), energy and macronutrient intake. Growth parameters were within normal range at all ages in both groups with no significant difference in mean z-scores except for accelerated length in the PKU group. No child with PKU had z-scores < −2 for any growth parameter at age 2 years. Total protein and energy intake in both groups were similar at all ages; however, from 12–24 months in the PKU group, the percentage of energy intake from carbohydrate increased (60%) but from fat decreased (25%) and inversely for controls (48% and 36%). In PKU, use of low volume WPS meets Phe-free protein requirements, facilitates transition to solid foods and supports normal growth. Further longitudinal study of growth, body composition and energy/nutrient intakes in early childhood are required to identify any changing trends.

Keywords: Phenylketonuria (PKU); growth; protein substitute; weaning

1. Introduction

Phenylketonuria (PKU) is an inherited metabolic disorder caused by a deficiency of the enzyme phenylalanine hydroxylase resulting in neurotoxic accumulation of the amino acid phenylalanine (Phe). The central focus of treatment is dietary management from diagnosis (<10 days of age), comprising a very low natural protein (low phenylalanine) diet (commonly <10 g/day) supplemented with a synthetic Phe-free amino acid substitute formula to meet the energy and protein requirements for growth and to maintain acceptable blood Phe control [1,2].

Since the 1950s, although low Phe dietary treatment has been known to prevent severe neurological impairment [3], several studies have suggested that early growth may be suboptimal in young children [4–11] compared with reference populations, particularly with respect to reduced height and head circumference. It is postulated that this might be attributed to a lack of total protein, natural protein and/or energy intake. Other studies have found little or no difference in growth [12–17].

However, disparities in study findings might be attributed to geographical area, small subject numbers, retrospective or uncontrolled data and changes in dietary practices over time.

As with all children, introduction of solid foods (weaning) is recommended from 17 to 26 weeks of age [18,19]. However, solid foods can suppress the appetite for liquids, potentially lowering intake of infant protein substitute, thereby affecting the ability to achieve total protein requirements. As a consequence, it often becomes necessary to introduce a second stage, more concentrated protein substitute [20] at around 6 months of age. However, there is concern that the low energy density of weaning protein substitute (8 kcal/g protein) might not compensate for the energy content of infant protein substitute (33.5 kcal/g protein) and this may impact on growth and weight gain. Whilst evidence is limited, previous retrospective data has demonstrated that a weaning protein substitute can be introduced without adverse effects on appetite or growth [21]. However, no studies have prospectively studied growth, protein and energy intake of children during the weaning period and early years.

Weaning in PKU is particularly complex and challenging as parents try to coordinate the normal transition from liquid formula to solid foods with the additional challenges of introducing a second stage protein substitute. They also must introduce special low protein foods in addition to the natural protein containing foods that will eventually replace normal infant formula or breast milk. Feeding problems are known to be more common in young children with PKU compared to non-PKU controls [22] and so the weaning period may be a particularly vulnerable period for optimal growth attainment.

2. Materials and Methods

This was an open label, longitudinal prospective case-control study in 20 children with PKU and 20 healthy controls. Subjects were recruited to assess their growth, energy and macronutrient (protein, fat and carbohydrate) intake from the introduction of a second stage, low volume, Phe-free protein substitute (3–6 months of age) to the age of 2 years (total of 17–20 months follow-up).

2.1. Anthropometry

Body weight, length, head circumference and Body Mass Index (BMI) were measured and recorded and z-scores calculated at each monthly review (from weaning until 12 months), then at 15, 18 and 24 months of age for all subjects. Weight was measured to the nearest 10 g using Seca (Seca, Hamburg, Germany) electronic infant scales; length was recorded to the nearest 1 mm using a Seca mobile measuring mat; and head circumference was measured to the nearest 1mm using a flexible, non-stretch head circumference tape. Self-reported paternal and maternal heights were also recorded for both groups. Length-for-age-difference, the difference between actual length (cm) and WHO standard length (cm) for that age at the 50th percentile [23], was also calculated as a more appropriate measure of change in linear growth over time than z-scores as they do not rely on standard deviations from cross-sectional data.

2.2. Dietary Records

A 24 h dietary record (including intake of weaning protein substitute, infant protein substitute and prescribed natural protein intake for PKU subjects) was completed by parents/carers monthly from weaning commencement to 12 months of age and then at 15, 18 and 24 months of age for all subjects. These were then cross checked by the research dietitian. Nutrient intake from diet records were assessed using the computer software nutritional analysis program Nutritics (Nutritics Professional Premium v5.09, Libro v0.9, Dublin, 2018) (food composition data from: 2015 COFIDS including McCance and Widdowson 7th edition, 2015).

2.3. Study Product (PKU Subjects)

The weaning protein substitute (PKU Anamix First Spoon; Nutricia Ltd, Trowbridge, UK) was a powdered Phe-free protein substitute supplemented with long chain polyunsaturated fatty acids

(LCP's), containing essential and non-essential amino acids, carbohydrate, fat, vitamins, minerals and trace elements. The product was mixed with water to produce a semi-solid spoonable protein substitute and 5 g of protein substitute powder provided 2 g of protein and 16 kcal.

2.4. Introduction of Weaning Protein Substitute (PKU Subjects)

The weaning protein substitute was gradually introduced into the diet after low protein solids were established. This was at the same time that the Phe in breastmilk or standard infant formula was replaced with Phe from food sources. Subjects continued to drink Phe-free infant formula as their main source of Phe-free L-amino acids during the early stages of weaning. The clinical dietitian determined the dose and increments of the weaning protein substitute.

Data on qualitative aspects of feeding development, gastro-intestinal symptoms, the feeding environment, feeding practices and progression (texture of foods, self-feeding, where meals are consumed), and weaning problems (e.g., teething/illness) are reported in a separate publication.

2.5. Ethical Approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and favourable opinion was given by the local research ethics committee (West Midlands–South Birmingham). Written informed consent was obtained from parents/carers of all children.

2.6. Statistics

As an observational study, no formal hypothesis comparing the two treatment groups and no formal power calculation was used to determine the sample size. The number of patients chosen was a pragmatic choice allowing for reliable estimation of continuous covariates and deliverable within a reasonable timeframe based on the average number of subjects diagnosed with PKU per year (approximately 4–6 per centre).

Quantitative outcome measures were summarised, and descriptive statistics of the data presented. The growth of infants was compared with standard growth curves for healthy infants [23]. The change in subject measures over time were analysed using longitudinal modelling techniques including time as a continuous covariate and allowing for random intercept and slopes. Models were adjusted for gender with gender-by-time and treatment-by-time interactions to assess different trends between subject groups. Between-group differences for growth, energy, protein, carbohydrate and fat intake at defined time-points were analysed using unpaired t-tests. Statistics were analysed using R version 3 computer software.

3. Results

3.1. Subjects

3.1.1. PKU Group

Twenty children (14 male) with PKU diagnosed by newborn screening, commencing weaning and requiring a concentrated phenylalanine-free protein substitute were recruited from three specialist PKU centres prospectively (Birmingham Children's Hospital (n = 17), Bradford Teaching Hospitals NHS Trust (n = 2), and the Royal Hospital for Children, Glasgow (n = 1)). Subjects were recruited and reviewed by the same dietitian at each centre.

3.1.2. Control Group

Twenty control children (12 male; 18 matched with the PKU group for mother's educational level and 18 matched for birth order) also commencing weaning were recruited from the local community. They included siblings of inherited metabolic disorder (IMD) patients, or children recruited from

a local community centre. The caregivers of control subjects received no dietary advice from one of two research dietitians.

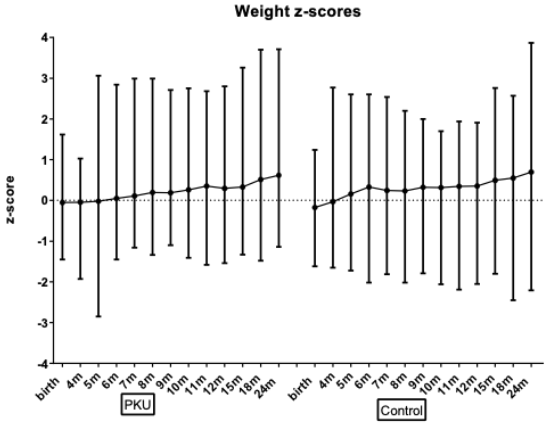
All subjects were white Caucasian except for two control children of mixed 50% Caucasian, 50% Afro-Caribbean decent. Five children from the PKU group and one from the control group had a sibling with PKU and three carers in both groups were single parents.

Median age for diagnosis of PKU was 10 days (range: 2–16 days). Median age of weaning was 4.3 m (range: 2.9–6.6 m) for PKU and 5.1 m (range: 3.7–6.5 m) for controls ($p = 0.04$). The decision to commence weaning (introduction of solid foods) was initiated by parents/caregivers in both groups. For children with PKU, the prescribed target total protein intake was 3 g/kg/day (2) and median natural protein intake at weaning was 5 g/day (range: 3–7 g/day). Two children were born prematurely at 33 weeks; corrected age (calculated by subtracting the number of weeks of prematurity from the chronological age) was used for calculating anthropometry.

3.2. Growth

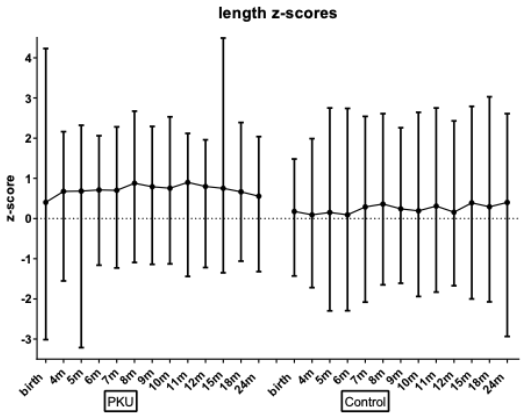
Longitudinal modelling showed that there was no significant difference between the PKU and control group for mean z-scores for (weight, head circumference or BMI) with both groups within normal range at all ages (Figure 1; Tables S1–S3). There was a trend toward slightly larger z-score length measurements (average increase of 0.73; $p = 0.003$; Table S4) for children with PKU; and consequently, slightly lower BMI, although not significantly ($p = 0.08$). Similarly, when length-for-age-difference was analysed using longitudinal modelling, children with PKU demonstrated significantly larger differences (mean difference between groups of 1.0 cm (0.7–1.6); $p = 0.02$; Table S5). No children with PKU had weight, length or BMI z-scores below −2 at 12 months or 24 months of age and only one control child had a weight and length z-score below −2 at 12 m and 24 months of age. Conversely, two control children and 1 child with PKU were overweight (BMI z-score > 2) at 12 months and 24 months.

There were some gender differences. Boys had lower mean BMI z-scores than girls across both groups ($p = 0.006$). In addition, whilst not statistically significant, there was a trend for boys with PKU toward lower BMI z-scores than controls (Figure 2); girls with PKU had length z-scores higher than control girls (Figure 3); and girls with PKU had higher weight, and subsequently higher BMI z-scores (Figure 4) than boys with PKU. Differences in length z-scores were not associated with genetic height potential as parental heights did not differ significantly between PKU and control groups.

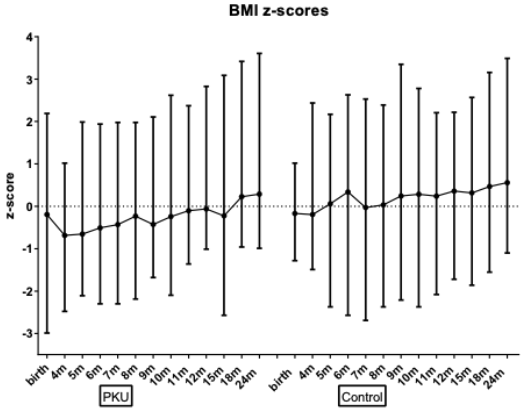


(a)

Figure 1. *Cont.*



(b)



(c)

Figure 1. Mean weight, length and body mass index (BMI) z-scores for PKU and control groups by age. (**a**) Mean weight z-scores for PKU and control groups by age; (**b**) Mean length z-scores for PKU and control groups by age; (**c**) Mean BMI z-scores for PKU and control groups by age.

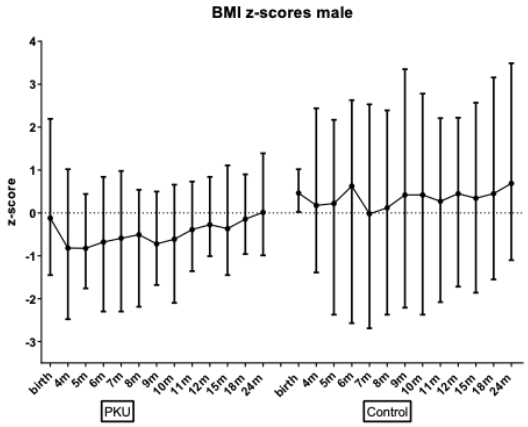


Figure 2. Mean **male** BMI z-scores for PKU vs. control groups by age.

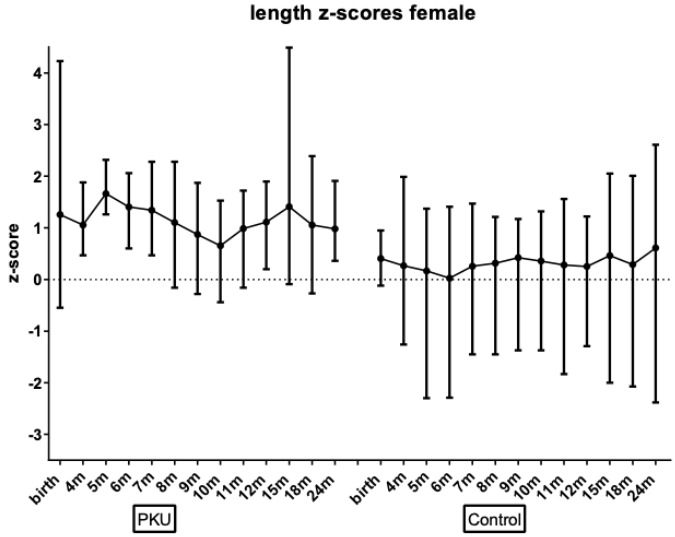


Figure 3. Mean female length z-scores for PKU vs. control groups by age.

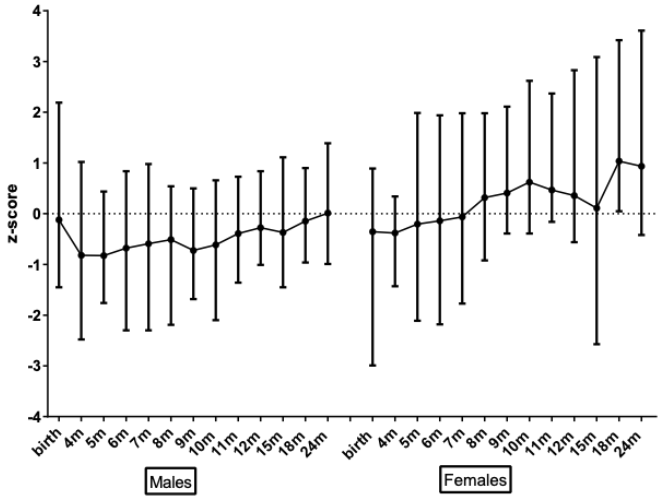


Figure 4. Mean PKU BMI z-scores for male vs. female by age.

3.3. Protein Intake from Protein Substitutes (PKU Group Only) (Excluding Natural Protein Intake)

As protein intake from weaning protein substitute increased in an almost linear fashion, Phe-free infant formula intake decreased, but mean total protein intake from protein substitutes remained relatively constant at around 2.8 g/kg/day (2.7–2.9 g/kg/day) from 7 months of age (Figure 5). Phe-free infant formula intake continued to make a significant contribution to protein intake (mean of 500–600 mL/day) until 7 months of age, then gradually declined so that by 24 months of age, 75% (n = 15) of subjects had stopped Phe-free infant formula and the remaining five subjects had a mean of 407 mL/day (200–820 mL). By 12 months of age weaning protein substitute contributed a total protein intake of 60% and by 24 months 74%. Natural protein intake remained relatively constant at all ages at a mean of 0.7–0.8 g/kg/day and 20–23% of total protein intake from 6 months of age.

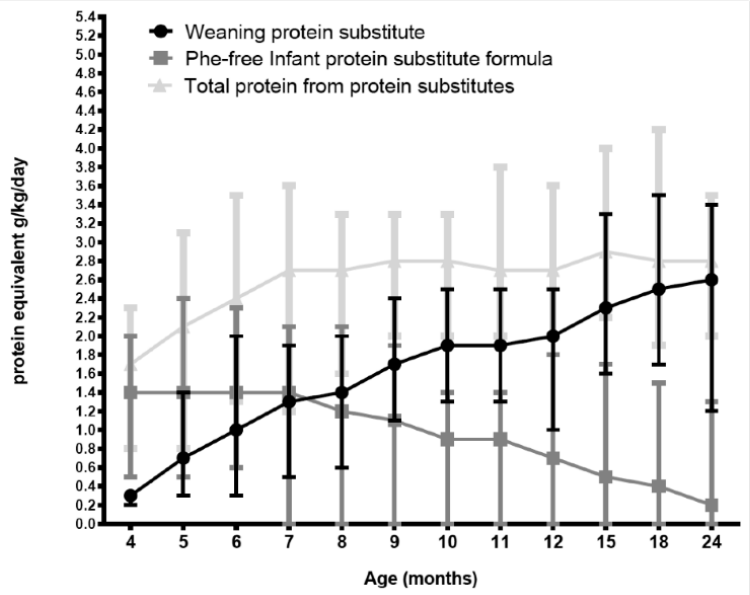


Figure 5. Mean protein intake g/kg/day from total protein substitute, weaning protein substitute, and Phe-free infant formula.

3.4. Total Protein Intake PKU vs. Control (Includes Natural Protein Intake)

At all ages, children in both groups exceeded 100% of WHO/FAO/UNU safe protein intakes [24] and the safe-levels plus 40% (mean PKU: 194%, range: 141–251%; mean control 188%, range: 133–272%) recommended for PKU (1). Average total protein intake (g/kg/day) increased slowly over time (mean 0.07; $p < 0.0001$) although this increase was greater in the control group ($p = 0.02$; Table S6). Whilst there was a trend towards higher total protein intakes in the PKU group compared to the controls aged up to 10 months, the reverse was true from 11 months of age. Mean protein intakes ranged from 2.5–3.6 g/kg/day for children with PKU and 2.4–4.0 g/kg/day for controls with both groups achieving $\geq$ 3.0 g/kg/day from 6 months of age in the PKU group, and from 7 months in the control group.

When protein was expressed as a ratio of energy intake (g protein/100 kcal), from 6 months of age children with PKU consumed a consistent 3.4–3.7 g/100 kcal whilst controls had a larger range of 2.7–4.2 g/100 kcal ($p = 0.001$; Table S7).

3.5. Total Carbohydrate and Fat Intake PKU vs. Control

Until 12 months of age, carbohydrate and fat intake was similar in both groups. However, from 1–2 years of age, children with PKU had significantly higher intakes of carbohydrate than controls and longitudinal modelling showed a statistically significant difference in the change in carbohydrate intake over time between PKU and control groups ($p < 0.01$; Table S8) (Figure 6). Conversely, after 12 months of age, fat intake was higher for control children although this only reached significance at 15 months ((g/day) 36 vs. 44, $p = 0.02$).

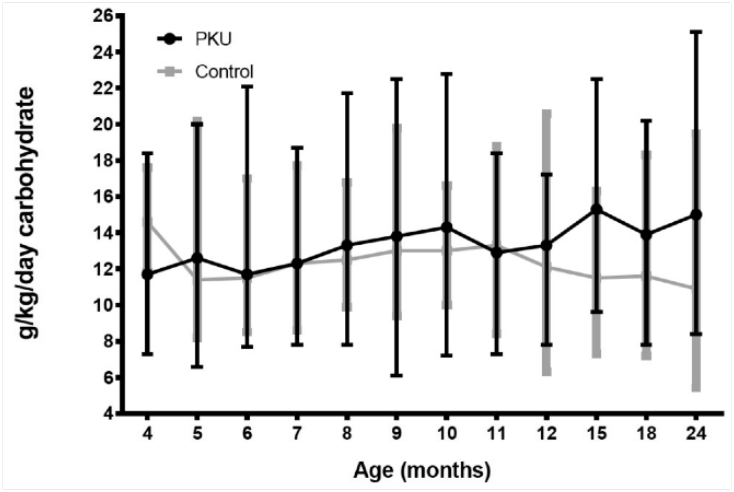


Figure 6. Mean carbohydrate intake (g/kg/day) for PKU and control groups by age.

3.6. Energy Intake from Protein Substitutes (PKU Group Only) (Excludes Energy Contribution from Food)

Energy intake from prescribed protein substitutes remained relatively constant at a mean of 423 kcal/day (349–486 kcal/day) (Table 1, Figure 7).

Table 1. Total energy intake (kcal/day) and percentage of total energy intake attributed to protein substitutes (infant and weaning) and food (mean (range)).

Age Number Subjects	% from Weaning Protein Substitute (WPS)	% from Phe-Free Infant Formula	% from Food	Total kcal/day
4 months	3	49	48	653
n = 10	(2–4)	(31–62)	(35–65)	(395–831)
5 months	6	52	43	709
n = 20	(2–11)	(30–74)	(18–67)	(482–1082)
6 months	9	55	37	716
n = 19	(3–19)	(25–75)	(16–60)	(529–1020)
7 months	11	49	40	802
n = 20	(5–19)	(0–69)	(17–85)	(583–1187)
8 months	13	44	44	864
n = 20	(5–22)	(0–68)	(21–86)	(643–1375)
9 months	14	39	47	939
n = 19	(7–25)	(0–69)	(21–82)	(592–1320)
10 months	16	30	55	983
n = 18	(7–24)	(0–61)	(26–84)	(610–1558)
11 months	17	29	54	970
n = 17	(10–26)	(0–50)	(27–84)	(703–1478)
12 months	18	23	59	978
n = 19	(7–29)	(0–49)	(35–83)	(621–1420)
15 months	18	17	65	1160
n = 19	(14–28)	(0–55)	(27–85)	(768–1509)
18 months	21	11	67	1155
n = 20	(14–40)	(0–44)	(42–85)	(595–1511)
24 months	22	5	73	1320
n = 19	(8–38)	(0–32)	(55–84)	(682–1910)

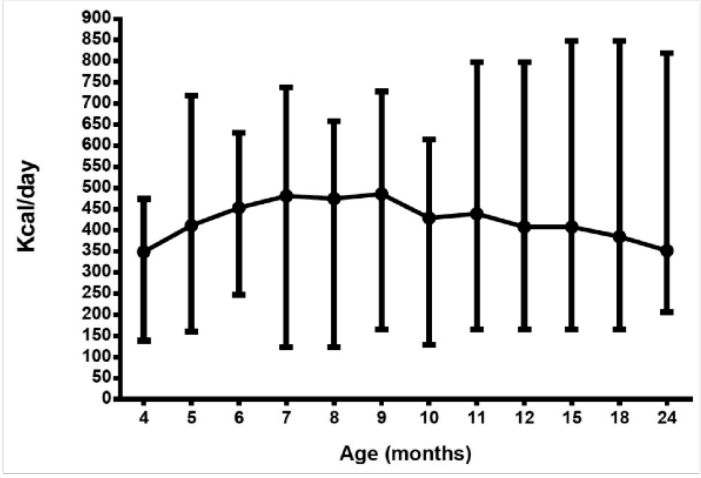


Figure 7. Mean energy intake (kcal) from both protein substitutes (infant and weaning) by age.

3.7. Total Energy Intake PKU vs. Control (Includes Energy Intake from Food)

There was no significant difference between PKU and control groups for total energy intake or total energy intake as a percentage of dietary reference values (DRV) [25] longitudinally or at any age (Figure 8; Tables S9 and S10). There were also no gender differences either within or between groups.

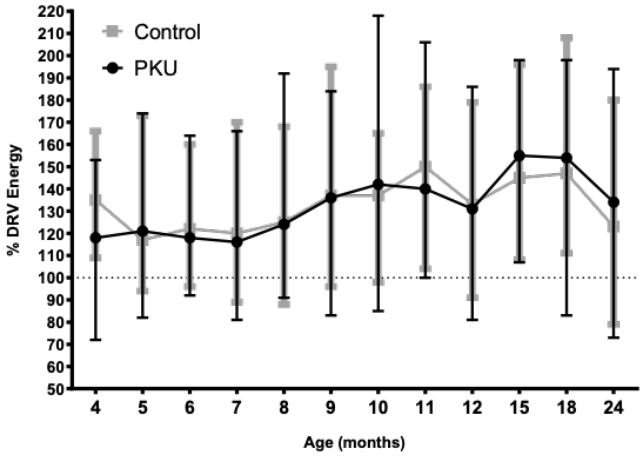


Figure 8. Mean % dietary reference values (DRV) for Energy for PKU and control groups by age.

3.8. Percentage of Energy from Carbohydrate, Fat and Protein PKU vs. Control (Includes Energy Intake from Food)

From 6 months of age the mean percentage of energy from protein was consistent at 14–15% for PKU, whilst in control children it gradually increased from 8% (at 4 months) to 17% (at 24 months). Carbohydrate as a percentage of energy steadily increased in the PKU group from 49% (at 4 months) to 60% (at 24 months) whilst the control group had a more variable and lower intake of energy (47–54%) supplied by carbohydrate. Similarly, fat as a percentage of energy was 34–38% for the control group from 6 months of age but in the PKU group energy from fat declined from 40% (at 4 months) to 25% (at 24 months) in line with the increase in energy from carbohydrate.

4. Discussion

Children with PKU had comparable growth patterns and similar energy intakes to control children during their early years with all growth parameters within normal range for age. There was

a non-significant trend for boys with PKU to grow at a slower rate than control boys and girls with PKU as has previously been seen [4], although still within normal growth parameters. In addition, children with PKU exhibited higher linear growth compared with controls. It has previously been reported that girls with PKU initially show accelerated growth up to 8 years of age followed by slower height progression and a slightly lower final height than the general population [4,15]. In addition, there is some evidence in PKU that girls are more prone to overweight and obesity in the teenage and adult years than boys [26–29], although it is unclear whether this is associated with early nutrition. Whilst girls with PKU did appear to be taller and had a higher weight and BMI than boys in this study, results did not reach significance possibly due to small subject numbers.

Children with PKU were weaned on average one month earlier than control children. Whilst some studies have linked early weaning to rate of weight gain, a recent systematic review found that when adjusted for type of milk/formula consumed, there was no significant difference [30]. Certainly there was no evidence that the children with PKU, who were weaned earlier than controls in this study, gained weight (or BMI) any faster.

Energy and protein intake from protein substitutes for the PKU group remained relatively constant at each age. By 2 years of age, weaning protein substitute contributed approximately 75% of protein intake and 20% of energy intake whilst natural protein from food contributed a mean of 20% of protein intake and 75% of energy intake, with Phe-free infant formula making up the deficit. Timely introduction of appropriate low Phe weaning foods was important to ensure any energy deficit was met.

In the 1990s and early 2000s despite improvements in dietary treatment and improved protein intake, studies from the USA, Germany, France, the Netherlands, Spain and Australia reported impaired linear growth in PKU [4,5,7,31–35]. One study identified adequate weight gain but moderate growth retardation in infants with PKU in the first 2 years of life compared with the normal population. This was more pronounced in boys but catch-up occurred later [4]. They suggested it might be due to vitamin or trace element intake or adaptation to supplementary feeding during weaning. More recently, a retrospective Spanish study identified inadequate weight and height from 0–2 years of age [10] and a retrospective German study also reported divergent growth in PKU although they acknowledged that in the last 20 years this problem had lessened [11]. It is difficult to compare results of early studies with current data as the protein source (casein hydrolysate vs amino acid) and dose differed.

In contrast, our prospective study demonstrated that subjects with PKU had higher linear growth z-scores than controls, possibly more so in girls. This gender difference is not uncommon in humans and in other animals. There is evidence to suggest that girls are less likely to deviate from their growth curves under adverse nutritional or environmental conditions than boys [4]. Why infants with PKU might grow faster than control children is unclear, although our study suggests that protein intakes in these children with PKU were adequate. Parental heights were not a confounding factor as these were similar across both groups. It may be that different international therapeutic approaches to treatment in PKU may account for some of the differences in growth identified in earlier studies, as well as improvements in the micronutrient content of protein substitutes [12].

There have been concerns in PKU about the absorption and utilisation of artificial sources of protein compared with natural protein and the effect this might have on growth [8,36]. Some studies have suggested that there is no correlation between Phe intake, blood Phe levels and growth deficits in PKU [4,5,7] whilst other studies have eliminated protein or energy malnutrition and endocrine causes [7,8,10]. One study demonstrated that natural protein intake rather than total protein intake was an important factor in head circumference growth in PKU but did not influence linear growth [8]. Certainly, natural protein intakes in our study group at a mean of 0.7–0.8 g/kg (20–23% of protein intake), exceeded those in other prospective studies, which demonstrated normal growth [17].

In PKU, as lean body mass is correlated with natural protein intake it is important to maximize natural protein intake to tolerance, although this is sometimes challenging in toddlers [13,17] with or without a metabolic condition. Similarly, an apparent 'safe' total protein to energy ratio of 3.0–4.5 g/100 kcal has

been reported as contributing to a more favourable body composition [13], and this is consistent with our children with PKU who had a ratio of 3.4–3.7 g/100 kcal.

Total energy intake as a percentage of DRVs was in fact remarkably similar at all ages in both study groups although, from 12 months of age the balance of carbohydrate and fat as a percentage of energy deviated, with the PKU group consuming a higher carbohydrate intake and the control group a higher fat intake. An increased consumption of carbohydrate compared with children without PKU has previously been reported associated with the high intake of energy-dense, nutrient-poor Phe-free special foods (necessary to meet the energy requirements for metabolic stability) [26,37–38]; althoughpreviously it was unclear at what time this deviation might occur. There are concerns that this mayimpact on body composition compared with regular diets [36]; although data around this topic in non-PKU infants are currently too limited to draw conclusions [39].

There were some study limitations. Due to the rarity of PKU and the random nature of any new diagnoses during the study period, gender groups were not equally divided such that some gender differences, whilst not statistically significant, were based on smaller subject numbers, particularly at 4 months of age, thereby making statistical comparisons unreliable. In addition, self-reported dietary data are renowned for under-reporting food intake, although the errors associated with this were the same for both groups. As dietary intake data was based on one 24 h record for each child at each age, it may not represent the norm for that child, although caregivers were advised to record food intakes on days without any illness or abnormal food intake. This method of dietary intake collection also enabled a high rate of return. For the PKU group, food intake generally matched the daily prescribed intake for protein substitute and natural protein (50 mg Phe exchanges); this was probably associated with the intensity of dietary counselling and interventions, the ability to control intake in children and highly motivated caregivers [17,40]. Differences in data collection and reporting were minimised by having the same dietitian follow up most of the subjects in each group. Much of the data on growth in PKU in large cohorts has been retrospective data spanning many years. Whilst this study included fewer subjects, data was collected systematically and prospectively.

This group of children with PKU, who were weaned onto a second-stage semi-solid protein substitute, demonstrated normal growth compared with their non-PKU peers. It is probable that developments in dietary treatment have led to significant improvements in early growth in PKU. Further work looking at longitudinal growth, body composition, nutrient intake and dietary patterns of children with PKU in early childhood and adolescence would be beneficial in establishing if trends in growth, energy and protein intake continue to follow that of control children and what effect, if any, this may have on their long-term health outcomes.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/11/3/552/s1, Table S1: Longitudinal modelling results for Weight z-scores; Table S2: Longitudinal modelling results for Head Circumference z-scores; Table S3: Longitudinal modelling results for BMI z-scores; Table S4: Longitudinal modelling results for Length z-scores; Table S5: Longitudinal modelling results for Length-for-age-difference; Table S6: Longitudinal modelling results for total Protein Equivalent g/kg/day; Table S7: Longitudinal modelling results for Protein Intake g/100 kcal; Table S8: Longitudinal modelling results for Carbohydrates g/kg/day; Table S9: Longitudinal modelling results for Total Energy kcal/day; Table S10: Total energy intake as a percentage of DRV(25) for PKU and control groups and males vs females (mean (range)).

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CHAPTER 3: FOOD PREFERENCES, FOOD ACCEPTANCE AND NEOPHOBIA IN PHENYLKETONURIA

CHAPTER 3A: VALIDATION OF A LOW PROTEIN SEMI-QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

nutrients

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Article

Validation of a Low-protein Semi-Quantitative Food Frequency Questionnaire

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Abstract: Analysis of dietary patterns and their role in long-term health is limited in phenylketonuria (PKU). Food frequency questionnaires (FFQ) are commonly used to assess habitual intake. A semi-quantitative 89-item FFQ with a portion size photographic booklet was developed for children with PKU as a tool for collecting data on habitual intake of foods, food groups, energy and macronutrient intake. Twenty children with PKU aged 11–16 years, 30 parents of children with PKU aged 4–10 years, and 50 age/gender-matched control children were recruited. To test reproducibility, FFQs were completed twice with a mean interval of 5 weeks (range: 4–10). In order to test validity, FFQs were compared with five 24-h dietary recalls with a mean interval of 10 days (range: 6–18). Energy and macronutrient intake and quantity/week of individual food items were calculated and compared. There was good reproducibility for the FFQ with macronutrient correlations $r > 0.6$ and good validity data with most correlations $r > 0.5$. Bland–Altman plots for reproducibility and validity showed mean levels close to 0 and usually within 2 standard deviations. FFQ comparisons of PKU and control groups identified expected differences in % energy from macronutrients (PKU vs. control: carbohydrate 59% vs. 51%, fat 26% vs. 33%, protein 15% vs. 16%). This FFQ for PKU produced comparable data to repeated dietary recalls and is a valid tool for collecting data on habitual food and nutrient intake. It will be useful in assessing changes in dietary phenylalanine tolerance of new pharmacological treatments for PKU.

Keywords: phenylketonuria (PKU); dietary patterns; food frequency questionnaire; validation; reproducibility

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1. Introduction

Phenylketonuria (PKU) is a rare genetic condition, resulting in the failure to metabolise the amino acid phenylalanine, resulting in severe neurocognitive disability if untreated. It is managed with a low phenylalanine diet supplemented with a protein substitute (either phenylalanine-free L-amino acids or glycomacropeptide (GMP), typically with additional micronutrients), and special low-protein foods (SLPFs). The remaining diet consists of food starches, sugars, fruit and low-protein vegetables.

Dietary pattern analysis is increasingly used to examine food intake and the synergistic effect of food and nutrients [1,2], but this is unreported in PKU. Conceptually, dietary patterns provide a broad picture of food and nutrient consumption and may be more predictive of disease risk than individual foods or nutrients. In dietary pattern analysis, food consumption patterns are characterised by habitual intake [3]. With PKU, although much is known about the dietary prescription, little is known about what is consumed, including food preferences, range of meal choices and food patterns. Whilst it is assumed that patients eat plentiful amounts of low-phenylalanine fruit and vegetables, evidence suggests the converse position [4–6]. Furthermore, food neophobia appears to be more

prevalent in children with PKU [5,7,8], and they appear reluctant to eat a wide range of fruits and vegetables [6].

Food frequency questionnaires (FFQ) are common tools used to measure dietary patterns. Respondents are given a list of foods and they describe how often each is eaten, e.g., how many times per day/per week/per month [9]. Compared with traditional dietary assessment methods, such as food diaries or 24-h recalls, FFQs require limited health professional time (both in data collection and analysis), low participant commitment, and may be completed by individuals with lower education or motivation [9,10]. They can be completed on paper or electronically in hospital clinics or the home environment. The results obtained by FFQs represent usual intakes over time and are suitable for ranking subjects into low, medium or high intake groups for individual foods or nutrients.

A FFQ should be tailored to each diet therapy. In PKU, portraying the full range of diverse foods permitted in a phenylalanine-restricted diet is challenging. This includes SLPFs and differing food types and quantities based on natural protein tolerance. Patients with classical phenotypes may tolerate only 3 g/day of natural protein (150 mg/day phenylalanine) but mild phenotypes tolerate ≥25 g/day (1250 mg/day phenylalanine), resulting in varying dependencies on SLPFs and protein substitutes. Pharmaceutical treatments, such as sapropterin dihydrochloride (BH4), may increase natural protein intake and the types of foods consumed in a subset of patients with PKU [11].

Ideally, a FFQ should contain no more than 100 commonly eaten foods grouped into sections, as only marginal gain is associated with more detailed questionnaires [12]. All FFQs should be validated to ensure that they measure what is intended and that they yield consistent results from repeated samples over time. This in turn improves the quality of the data collected and enables comparisons between studies using the same tool. There are different types of validity, meaning that a questionnaire is never fully validated but is valid for certain populations under specified conditions [13]. Validation of FFQs can be achieved in various ways and it is suggested that a combination of methods should be used to assess reproducibility and validity [14]. Checking that the questionnaire content is relevant and valid (content validity), that it can differentiate between different subject groups (construct/discriminative validity), that it produces reliable/reproducible results (reproducibility) and compares well with an existing standard (criterion validity), provides more credibility to the resulting data. Similarly, reporting that experts established questionnaire face validity, that the questionnaire was pilot tested on a subset of participants for understanding and relevance, and that appropriate statistical tests were used, also improve the integrity of the data [15].

Any FFQ designed for PKU should be validated by comparing it with a control group population to demonstrate that it is able to distinguish between the variations in macronutrient intake associated with the different food items eaten in a phenylalanine-restricted diet. Food intake will also vary according to the age, ethnic, social, educational, and economic background of the study population. Thus far, only one PKU-specific FFQ has been validated from the USA; 29 adults/adolescents were studied, and they compared the results of a FFQ with a 3-day food diary [10]. Whilst this study found good agreement between the different dietary methods and between repeated measures of the FFQ for protein intake, it was not validated in children, is likely to be specific to the USA population for food types and portion sizes, and it did not report on the validity of energy or other macronutrient intake such as carbohydrates, fat or fibre.

The aim of this study was to develop and validate a semi-quantitative FFQ for use in children with PKU, providing a tool that collects data on habitual intake for foods, food groups, energy and macronutrient intake, which can be utilised for dietary pattern and lifestyle analysis nationally.

2. Materials and Methods

2.1. Construct Validity (Ability to Differentiate between Different Subjects)—Study Subjects

Fifty children with PKU and 50 age and gender-matched healthy control children were recruited to test the FFQ for construct validity, which is the ability to differentiate between the dietary patterns for different groups. For children aged 4–10 years, data was completed by a parent/carer with assistance from an inherited metabolic disorder (IMD) dietitian; and for children aged 11–16 years, data was completed by children with assistance from the parent/carer and IMD dietitian. Inclusion criteria for subjects with PKU comprised the following: diagnosed by newborn screening; dietary treatment only (i.e., not prescribed sapropterin), supplemented with a prescribed free/low phenylalanine protein substitute from diagnosis and SLPFs; and no co-existing medical conditions, other special dietary requirements or intercurrent infection. All subjects with PKU were recruited from Birmingham Children's Hospital over a 30-month period (2018–2020). For control subjects, inclusion criteria comprised the following: age (within 6 months) and gender-matched to subjects with PKU; and on a regular diet (special diets, including vegan, vegetarian, and dairy-free were excluded). Control subjects were recruited from siblings of other children with inherited metabolic disorders, friends, or family of Birmingham Children's Hospital staff during the same time period as PKU subjects. The average nutrient and individual food intake from the 2 FFQs for each group were compared to establish construct validity.

2.2. Content Validity (Checked by Experts in the Field)—Food Frequency Questionnaire Development

Other UK and European PKU centres who were members of the SSIEM (Society for the Study of Inborn Errors of Metabolism) or BIMDG (British Inherited Metabolic Diseases Group) were invited to share their food frequency questionnaires. Five questionnaires were received (2 from England, 2 Scotland and 1 Germany). From these, a draft FFQ was developed for PKU and then adapted for control children (low-protein meal choices were matched with regular foods in the control FFQ, and SPLFs were also added to the PKU FFQ). The FFQs were reviewed by 5 IMD dietitians and piloted on 5 children with PKU and 5 control children to assess content validity. Following minor modifications, an 89-item PKU FFQ (+11 general dietary pattern questions) and a 69-item control FFQ (+5 general dietary pattern questions) were produced, including portion sizes for each item. The difference in the number of food items on the 2 questionnaires was a result of the additional SLPFs on the PKU version. General dietary pattern questions focused on meal frequency, missed meals, addition of salt to food, vitamin and mineral supplements and frequency of eating out (restaurants/cafes). The PKU FFQ also included 6 questions about quantity and frequency of protein substitute dosage and the number and amount of protein exchanges (the weight of food/drink that yields 1 g protein or 50 mg phenylalanine is one exchange).

2.3. Reproducibility—Food Frequency Questionnaire

The FFQ was completed at recruitment and at the end of the study (1–2 months apart) to test for reproducibility (test/retest reliability). This length of time was chosen in order to minimise changes over time but also to minimise recall of previous answers. The questionnaires were administered by one of four trained IMD dietitians using a standard script (see Supplementary Materials Supplementaries S1–S4). The same dietitian completed both questionnaires with each subject. For each food item on the FFQ, both the number of daily and weekly food portions consumed were recorded. Items consumed less than once a week were omitted.

2.4. Portion Size Booklet

Photographic portion size booklets were designed to accompany each FFQ, with pictures of the portion sizes specified in the questionnaire. Of 425 food photographs (captured from a range of distances and angles for each food), 65 were selected for the PKU

FFQ and 58 for the control FFQ (24 foods were the same; 21 were the same but for the PKU FFQ were SLPFs instead of regular foods, e.g., low-protein pasta, bread, burgers; 8 were the same food but differed in portion sizes to show the amount that was equivalent to a 1 g protein/50 mg phenylalanine exchange; the remaining were diet specific foods, e.g., SLPFs with no regular comparative, or meat products with no low-protein comparative). Foods were prepared and presented on a plain white plate or bowl or transparent glass, on a neutral background and photographed immediately to maximise the aesthetic appearance of the food. Foods that were pre-packaged in standard portion sizes or were equivalent in size to another food did not have photographic representation. Portion sizes for all foods (including SLPFs) were described by weight (g), volume (mL) (if applicable), and household measurements, e.g., tablespoons, teaspoons, glasses, slices, packets/sachets, whole items, or a combination of these. Portion sizes for protein/phenylalanine containing exchange foods were usually described as the amount yielding 1 g protein or 50 mg/phenylalanine, often described as a weight or volume range, e.g., 50–70 g to allow for small variations in intake. Average portion sizes were generally used, these could then be multiplied up (e.g., double) or down (e.g., halved) if a larger or smaller portion size was consumed. For dietary analysis purposes, the smaller value was used to calculate nutrient intake.

2.5. FFQ Database

In order to analyse energy and macronutrient intake (protein, fat, carbohydrate and fibre) from the FFQ, food items were assigned a nutrient content based on composition data compiled from *McCance and Widdowson, The Composition of Foods* [16] supermarket nutrient analysis data (Tesco website accessed May 2017) and SLPF nutrient composition data from manufacturers. For each FFQ item, the nutrient analyses were selected from one or more of these sources and the nutrient contents were averaged to obtain a single value for each nutrient. These values were then entered in the *Nutritics* [17] software computer analysis program as 'new foods', including portion sizes, and data from the FFQs were then analysed using the items as entered in *Nutritics*. Data entry was completed by the same dietitian and cross-checked for accuracy by a second dietitian. Nutrient intakes for each of the 2 FFQs for each subject were obtained and converted into average daily intakes for energy (kJ), carbohydrate, protein, fat, dietary fibre, starch and sugars. The 2 FFQs were then compared to establish reproducibility, and the average of the 2 FFQs compared with the average of the 24-h recalls to establish criterion validity.

2.6. Criterion Validity (Comparison with an Existing Standard)—24-h Dietary Recalls

As a means of comparison and to test criterion validity (comparison with an existing standard), five 24-h dietary recalls were completed with subjects by one of 4 trained IMD dietitians experienced in taking diet diaries and using the same standard script for asking questions. All food and drink consumed the day before were recorded including type and quantity, and time of day consumed. All 5 dietary recalls for each subject were completed by the same dietitian during the same time period as the FFQs (over a 1–2-month period) and with a minimum of 5 days between each one including at least 1 weekend day and no more than 2 of the same weekdays, to ensure a representative intake (Figure 1).

Dietary recalls were all analysed by the same dietitian using the *Nutritics* [17] computer analysis program, and results were averaged to obtain an average daily intake. These were then compared with the average of the 2 FFQs to establish criterion validity.

2.7. Anthropometry

Body weight, height and BMI (body mass index) were measured and z-scores calculated at recruitment. Weight was measured to the nearest 10 g using Seca electronic scales; length was recorded to the nearest 1 mm using a Seca 213 portable stadiometer (Seca, Hamburg, Germany).

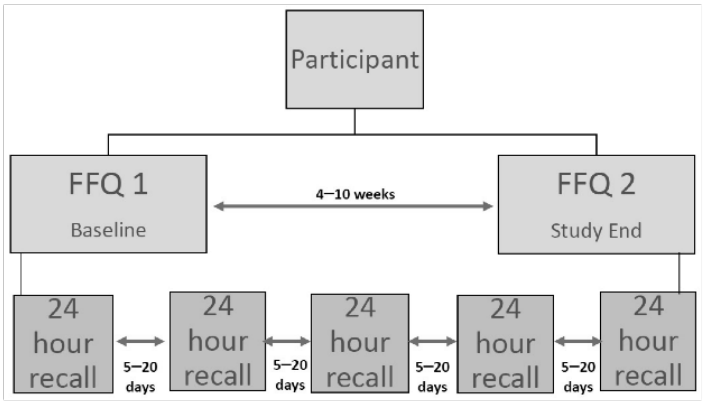


Figure 1. Study design. FFQ: Food frequency questionnaires.

2.8. Statistics—Sample Size

Data from a previous study [5] in a subset of children with PKU suggested that for a high fat, high carbohydrate food such as potato fries, power to detect a clinically relevant difference in mean intake of 1.7 days/week (the difference between a PKU group mean, μ_1, of 3.2 days/week and a control group mean, μ_2, of 1.5 days/week). Assuming a common standard deviation (SD) of 2.08 and a two-sided significance level of 0.05, a sample size of 33 in each group will have a power of 90%. Sample size calculations were performed using nQuery Advisor.

2.9. Data Analysis

Analyses were performed to evaluate the differences between the FFQs for PKU and control groups, as well as between the FFQ and dietary recalls data using GraphPad Prism version 6.01 for Windows, GraphPad Software, La Jolla California, USA. Continuous data were summarised as median (IQR) and categorical data were summarised as frequencies of counts with associated percentages. The strengths of association between dietary components were estimated using Spearman's correlation coefficients with Wilcoxon signed rank tests used to evaluate any differences between PKU and control groups. Comparisons of continuous data were performed using Wilcoxon sign rank for paired data and rank sum test for unpaired data. Categorical data were compared between groups using a Fisher test. Bland–Altman methods were used to assess the agreement between the FFQ and the 24-h dietary recall data. A *p*-value of 0.05 was used to determine statistical significance throughout.

2.10. Ethical Approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and a favourable ethical opinion was obtained from the London—Queens Square National Research Ethics Service (NRES) Committee (REC reference: 15/LO/1463 and IRAS ID: 185896). Written informed consent was obtained from the parent/carer of all subjects, and assent from children was obtained where appropriate, according to their level of understanding.

3. Results

3.1. Subjects

3.1.1. PKU Group

Fifty children (24 male) with PKU, (mean age 9.3 years; range: 4–16 years) on a phenylalanine-restricted diet only, were recruited from one specialist PKU centre (Birmingham Children's Hospital, UK). No changes were made to dietary intake during the study period. For 30 of the children aged 4–10 years, their mothers completed the questionnaires

with a dietitian, and for the remaining 20 children, (aged 11–16 years) they self-completed the questionnaires with assistance from parents/carers and a dietitian.

3.1.2. Control Group

Fifty control children were age (within 6 months) and gender matched to the subjects with PKU. Questionnaires were either completed by parents/carers or by teenagers, following the same criteria as in the PKU group.

3.2. Demographics and Anthropometry

Most children were white UK/European origin (n = 45 subjects with PKU and n = 47 controls), with the remaining being of either Asian (n = 3 PKU, n = 2 control) or mixed-race (n = 2 PKU, n = 1 control) origin. There was no significant difference between mean z-scores for BMI, weight or height between PKU and control groups (see Supplementary Materials Table S1).

3.3. Meal Patterns—FFQ 1 vs. FFQ 2 (Reproducibility) vs. Dietary Recalls (Criterion Validity-Comparison with an Existing Standard)

The mean time between the two FFQs was 5 weeks (range: 4–10). Recalls were completed at a mean interval of 10 days (range: 6–18).

For meal patterns, there was little difference between the two FFQs, or between the FFQ and dietary recalls (Table 1). For the PKU group, there was a difference in the median number of meals that were consumed for FFQ 2 (4 meals/day) compared with FFQ 1 and the dietary recalls (5 meals/day). The PKU group varied across assessment methods in the percentage consuming mid-morning snacks, and this group was also less likely to consume a mid-morning snack compared with controls (FFQ 2 $p = 0.0005$). However, some children took their protein substitute at this time.

Table 1. Meal patterns—percentage of subjects consuming meals and snacks, or missing meals for FFQ 1, FFQ 2 and dietary recalls (PKU and control groups).

	PKU				CONTROL			
	FFQ 1 n = 50	**FFQ 2 n = 50**	**24-h Dietary Recalls ** n = 50**	***p* Value**	**FFQ 1 n = 50**	**FFQ 2 n = 50**	**24-h Dietary Recalls ** n = 50**	***p* Value**
Median no. meals eaten/day	5	4	5	0.01 #	5	5	5	0.37 #
% eating breakfast (n)	92 (46)	94 (47)	100 (50)	1 *	100 (50)	100 (50)	100 (50)	1 *
% eating midday meal (n)	100 (50)	98 (49)	100 (50)	1 *	100 (50)	100 (50)	100 (50)	1 *
% eating evening meal (n)	100 (50)	100 (50)	100 (50)	1 *	100 (50)	100 (50)	100 (50)	1 *
% eating mid-morning snack (n)	62 (31)	38 (19)	48 (24)	0.027 *	78 (39)	74 (37)	60 (30)	0.815 *
% eating afternoon snack (n)	70 (35)	52 (26)	70 (35)	0.100 *	55 (28)	62 (31)	74 (37)	0.685 *
% eating bedtime snack (n)	40 (20)	46 (23)	52 (26)	0.686 *	46 (23)	54 (27)	58 (29)	0.549 *
% miss meals 1 x/week (n)	10 (5)	6 (3)	8 (4)	0.715 *	10 (5)	8 (4)	8 (4)	1 *
% miss meals > 1 x/week (n)	12 (6)	8 (4)	2 (1)	0.741 *	2 (1)	0 (0)	0 (0)	1 *

** average of 5-day dietary recalls; * Fisher test; # Wilcoxon signed rank; (n) = number of children; FFQ = Food Frequency Questionnaire; PKU = Phenylketonuria

3.4. Protein Exchanges and Protein Substitute Intake

Dietary patterns related to intake of protein substitute and natural protein exchanges were comparable between repeated FFQs and between FFQ and dietary recall data (Table 2). The median daily number of 1 g protein exchanges (50 mg phenylalanine) from the 24-h dietary recalls was 0.5 g protein (25 mg phenylalanine) less than prescribed (5.0 g vs. 5.5 g). Dietary recall data showed that 70% (n = 35) of children with PKU were taking their daily number of 1 g protein exchanges to within 0.5 exchanges of prescribed amounts. Of the remaining 30%, 6% (n = 4) were allocated $\geq$ 10 g/day of protein and 18% (n = 9) were prescribed $\geq$5 g/day, indicating that these patients were less protein restricted.

Table 2. Percentage of subjects consuming natural protein exchanges (1 g protein = 50 mg phenylalanine) and protein substitute at meals and mid meals (PKU group only).

	FFQ 1 n = 50	**FFQ 2 n = 50**	**24-h Dietary Recalls ** n = 50**	***p* Value**
Median no. 1 g natural protein (50 mg phenylalanine) exchanges/day (range)	5.5 (3–25)	5.5 (3–25)	5.0 (2–23.5)	0.02 #
% eating prescribed protein exchanges at every meal (n)	62 (31)	60 (30)	66 (33)	1 *
% actually eating prescribed protein exchanges at every main meal (n)	74 (37)	60 (30)	46 (23)	0.202 *
Median no. meals/snacks per day that prescribed protein exchanges are consumed (range)	3 (1–6)	3 (1–6)	3 (1–5)	1 *
% eating prescribed protein exchanges at breakfast (n)	78 (39)	72 (36)	68 (34)	0.645 *
% eating prescribed protein exchanges at midday meal (n)	94 (47)	86 (43)	88 (44)	0.318 *
% eating prescribed protein exchanges at evening meal (n)	96 (48)	100 (50)	82 (41)	0.495 *
% eating prescribed protein exchanges at mid-morning snack (n)	4 (2)	2 (1)	4 (2)	1 *
% eating prescribed protein exchanges at mid-afternoon snack (n)	14 (7)	14 (7)	14 (7)	1 *
% eating prescribed protein exchanges at bedtime snack (n)	10 (5)	4 (2)	20 (10)	0.436 *
Median no. times/day protein substitute dose taken (range)	3 (3–5)	3 (3–5)	3 (3–5)	1 *
% taking protein substitute dose at breakfast (n)	100 (50)	100 (50)	100 (50)	1 *
% taking protein substitute dose at midday meal (n)	78 (39)	72 (36)	78 (39)	0.645 *
% taking protein substitute dose at evening meal (n)	74 (37)	68 (34)	62 (31)	0.660 *
% taking protein substitute with morning snack (n)	10 (5)	12 (6)	10 (5)	1 *
% taking protein substitute with afternoon snack (n)	36 (18)	34 (17)	26 (13)	1 *
% taking protein substitute with bedtime snack (n)	48 (24)	56 (28)	66 (33)	0.548 *

** average of 5 days; * Fisher test; (n) = number of children; # Wilcoxon signed rank.

3.5. Macronutrient Intake

3.5.1. Reproducibility (A Measure of Whether the FFQ Produces the Same Results at Different Times)—FFQ 1 vs. FFQ 2

There was no significant difference between the two FFQs for PKU for any nutrients or between the two control FFQs except for protein and starch ($p = 0.05$) in control children, with FFQ 1 reporting values slightly higher than FFQ 2 (Table 3). Similarly, correlation *r* values all exceeded 0.5 for nutrients in both PKU and control groups, showing good correlation between FFQs taken at different intervals. Bland–Altman plots also demonstrated no clinically significant differences with mean levels close to 0 and homogeneous data mostly within the upper and lower levels of agreement (2 standard deviations—SD) (Figure 2).

Table 3. Nutrients—median (IQR) intakes FFQ 1 vs. FFQ 2 and FFQ vs. Dietary Recalls (DR) for PKU and control groups; and FFQ PKU vs. control.

		PKU				CONTROL				Spearman Rank Correlation (*r*)				Wilcoxon Signed Rank (*p* value)				
		FFQ 1	FFQ 2	FFQ Average	Diet Recalls	FFQ 1	FFQ 2	FFQ Average	Diet Recalls	PKU FFQ 1 vs. 2	PKU FFQ vs. DR	Ctrl FFQ 1 vs. 2	Ctrl FFQ vs. DR	PKU FFQ 1 vs. 2	PKU FFQ vs. DR	Ctrl FFQ 1 vs. 2	Ctrl FFQ vs. DR	PKU FFQ vs. Ctrl FFQ
KJ/day	median (IQR)	8116.8 (7078–11,288)	8591.1 (6824–9446)	8259.7 (7312–10,064)	7851.8 (6806–8927)	7011.7 (6008–8678)	6779.4 (6274–7804)	7144.2 (6157–8216)	7163.0 (6318–7878)	0.723	0.500	0.633	0.334	0.19	0.005	0.10	0.57	<0.0001
CHO g/day	median (IQR)	277.3 (229–394)	293.7 (237–343)	287.8 (241–356)	267.1 (231–315)	226.9 (191–271)	215.4 (194–247)	221.0 (186–262)	208.0 (183–233)	0.682	0.601	0.651	0.408	0.62	0.01	0.17	0.02	<0.0001
Sugars g/day	median (IQR)	135.7 (105–172)	128.8 (103–173)	131.2 (108–178)	107.0 (92–134)	112.2 (92–138)	111.6 (97–130)	115.7 (94–135)	95.7 (77–108)	0.78	0.464	0.719	0.550	0.78	0.0001	0.38	<0.0001	0.01
Starch g/day	median (IQR)	136.3 (114–183)	146.8 (105–187)	144.2 (114–195)	140.5 (117–167)	102.8 (81–132)	96.7 (79–115)	100.1 (82–124)	116.8 (93–133)	0.731	0.600	0.742	0.493	0.51	0.25	0.05	0.004	<0.0001
Fat g/day	median (IQR)	57.4 (49–76)	55.7 (48–68)	58.2 (49–69)	52.0 (43–61)	61.1 (54–77)	59.4 (54–69)	60.7 (54–73)	65.7 (57–75)	0.691	0.271	0.614	0.381	0.07	0.04	0.11	0.11	0.23
Total protein equivalent g/day	median (IQR)	72.9 (65–84)	73.2 (66–82)	72.6 (65–83)	71.8 (68–80)	66.7 (55–77)	63.4 (55–73)	65.6 (57–74)	64.6 (56–76)	0.913	0.848	0.523	0.344	0.37	0.08	0.04	0.91	0.0009
Fibre g/day	median (IQR)	20.4 (16–27)	22.6 (18–25)	21.5 (19–26)	18.1 (14–22)	18.0 (15–33)	17.4 (15–20)	17.8 (15–21)	17.0 (14–20)	0.673	0.445	0.514	0.427	0.70	0.001	0.26	0.02	0.0005
% Energy CHO	median (IQR)	58.8 (55–62)	58.6 (55–61)	59.3 (56–62)	59.0 (55–64)	51.3 (48–54)	51.1 (48–53)	50.7 (49–53)	49.0 (47–52)	0.768	0.689	0.492	0.414	0.35	0.94	0.85	0.02	<0.0001
% Energy Fat	median (IQR)	26.6 (23–29)	25.4 (23–29)	26.1 (24–29)	25.1 (22–29)	33.1 (31–36)	33.6 (32–35)	33.3 (32–35)	35.7 (32–37)	0.628	0.333	0.422	0.402	0.04	0.44	0.56	0.005	<0.0001
% Energy Protein	median (IQR)	14.5 (12–18)	15.4 (13–17)	14.3 (13–17)	15.3 (14–18)	15.4 (15–17)	15.5 (14–17)	15.5 (15–16)	15.2 (11–15)	0.777	0.729	0.622	0.290	0.34	0.21	0.45	0.89	0.12

DR = Dietary recall, IQR = Interquartile range.

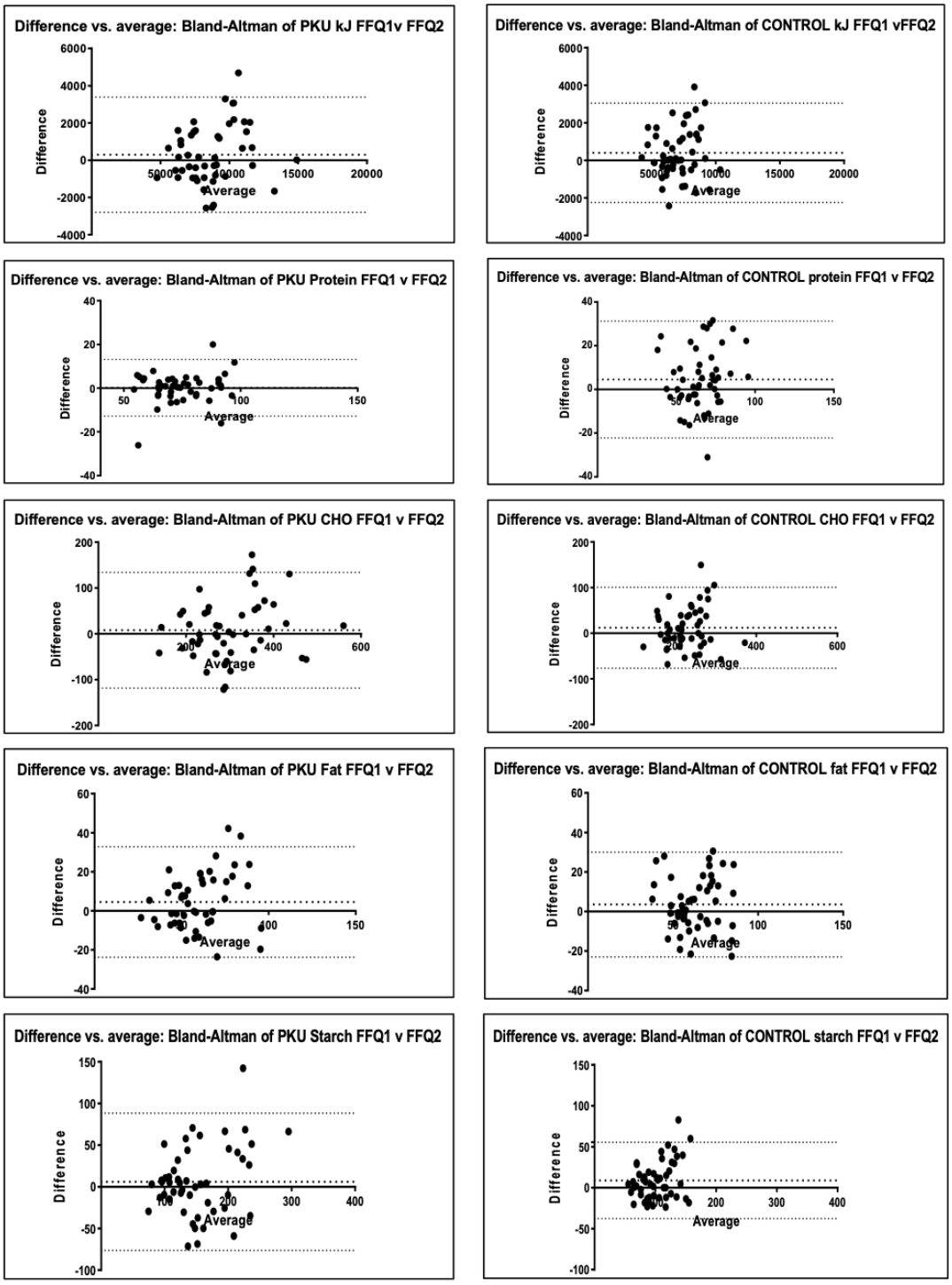


Figure 2. *Cont.*

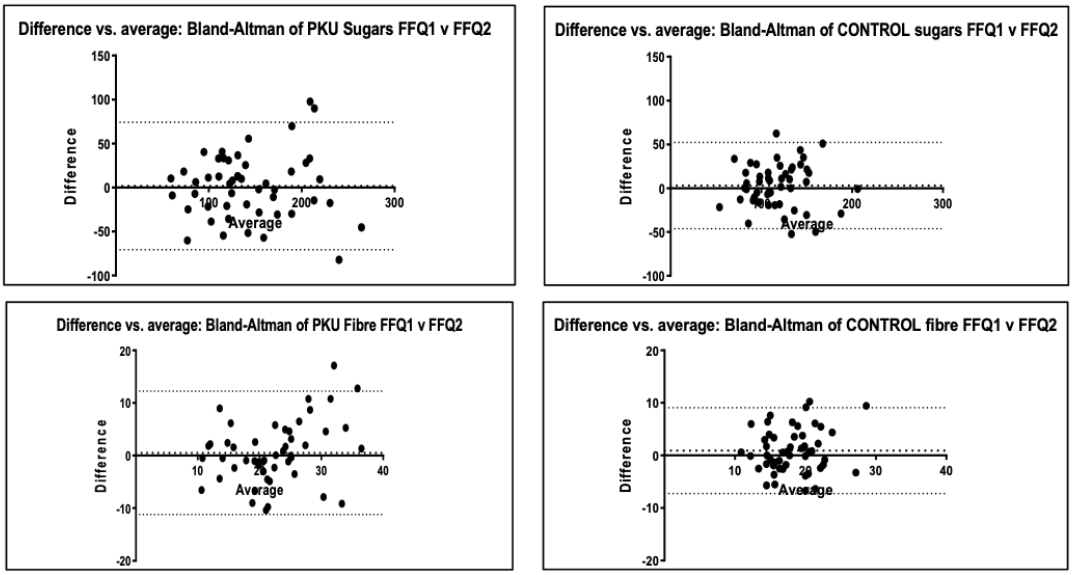
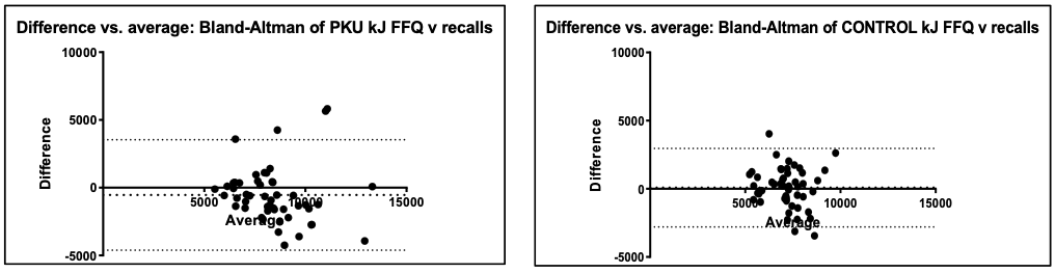


Figure 2. Bland–Altman plots for PKU and control group macronutrient intake FFQ 1 vs. FFQ 2. bias line (mean); upper and lower levels of agreement 95% confidence (2 SD). CHO = carbohydrate.

3.5.2. Criterion Validity (Comparison with an Existing Standard)—FFQ vs. Dietary recalls

For the PKU group, there was a trend for the FFQ to report higher intakes of all nutrients compared to the dietary recalls (Table 3). In the control group, the same was observed except for energy, starch and fat. Nutrient correlations for the PKU group were close to or above 0.5 (r) except for fat. For the control group, correlations were less strong, ranging from 0.33 to 0.55. Conversely, most Wilcoxon *p* values for the PKU group were significant except for protein and starch, whilst in the control group fewer nutrients showed statistical differences, with only protein, fat and energy not showing a difference. However, from a clinical perspective, differences were not of relevance. For example, the difference in sugar intake between FFQ and dietary recalls for the PKU group was around 25 g or approximately 1 tablespoon per day, whilst the difference in fat intake was around 5 g or 1 teaspoon of fat per day. The Bland–Altman plots show homogeneous data with most values falling within the upper and lower levels of agreement (2 SD) and mean values close to 0 (Figure 3). The exceptions to this were sugar and fibre for the PKU group, and sugar for the control group.



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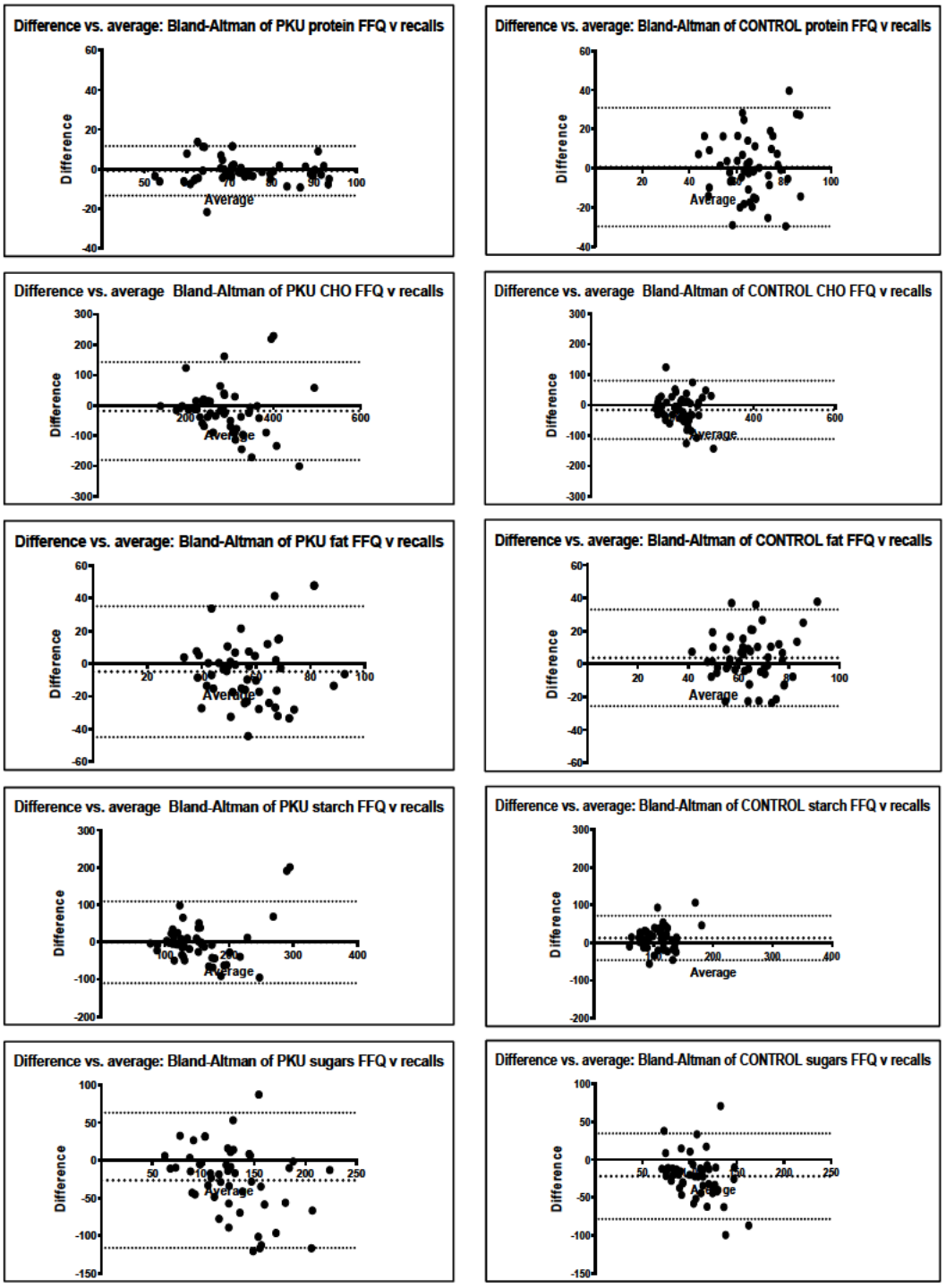

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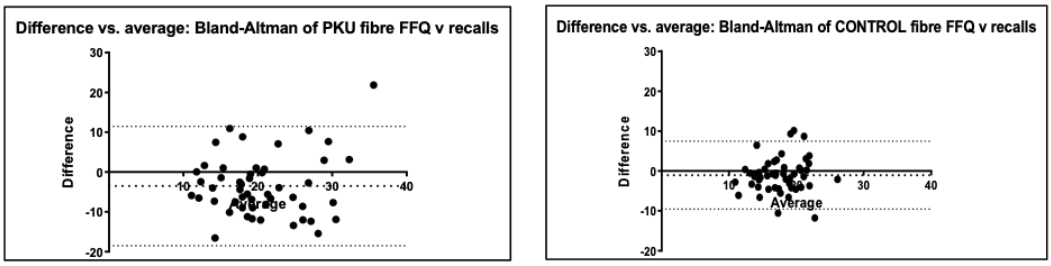

Figure 3. Bland–Altman plots for PKU and control group macronutrient intake FFQ vs. dietary recalls. Bias line (mean); upper and lower levels of agreement 95% confidence (2 SD); CHO = carbohydrate.

3.5.3. Construct Validity (Ability to Distinguish between Different Groups)—PKU FFQ vs. Control FFQ

As expected, and in agreement with previous research, due to the composition of a phenylalanine restricted diet there were significant differences in macronutrient intake between the PKU and control groups when using the FFQ (Table 3). The PKU group had significantly higher carbohydrate and starch intakes, and a higher percentage of energy from carbohydrate and a lower percentage of energy intake from fat compared to controls.

3.6. FFQ Individual Food Items

3.6.1. Reproducibility (A Measure of Whether the FFQ Produces the Same Results at Different Times)—FFQ 1 vs. FFQ 2

Most food items for both PKU and controls showed good correlation between FFQ 1 and 2 ($r > 0.40$), demonstrating good reproducibility (see Supplementary Materials Table S2). Foods with a lower correlation coefficient were usually consumed by fewer than 10 subjects. The exceptions for the PKU group were vegetarian gummy sweets, pasta sauce, dried fruit and regular biscuits. For the control group, exceptions were meat pie, meat curry and butter/margarine.

Similarly, for commonly eaten foods (> 10 subjects) there was no significant difference ($p > 0.05$) between FFQ 1 and 2 for most food items in either group. Exceptions in the PKU group included: corn/rice/oat-based breakfast cereal, sweet drinks and vegetables containing phenylalanine < 75 mg /100 g and those with >100 mg/100 g. Exceptions in the control group included the following: dairy desserts, wheat-based breakfast cereals, mayonnaise/dressings, pizza and crackers. No items with a low r value (<0.40) were significantly different ($p < 0.05$).

3.6.2. Criterion Validity (Comparison with an Existing Standard)—FFQ vs. Dietary Recalls

There was a trend for the FFQ to report higher intakes compared with the dietary recalls for just over half the items (n = 54/89, 61% PKU; n = 35/69, 51% control) (See Supplementary Materials Table S3). Similarly, for most foods, the FFQ reported more people consuming individual foods than the dietary recalls (FFQ 85%, n = 76/89 foods vs. recalls 15%, n = 13/79 foods for PKU; FFQ 83%, n = 57/69 foods vs. recalls 17%, n = 12/69 foods for controls).

Most food items for both PKU and control groups showed good correlation between the FFQ and the dietary recalls ($r > 0.40$), demonstrating satisfactory criterion validity except for items consumed less often (<10 subjects). The exceptions for the PKU group were as follows: vegetarian gummy sweets, chips, vegetarian burgers and low-protein biscuits. For the control group, exceptions were greater in number and similar to those that varied between the 2 FFQ: meat pie, meat curry and butter/margarine, in addition to chips, processed meats, ice cream, cheese, cake, pizza, pasta and chocolate.

Similarly, for commonly eaten foods (>10 subjects) there was no significant difference between the FFQ and dietary recalls for most food items in either group. Those that did tended to be different than the items that had low r values (<0.40). Exceptions in the PKU

group included vegetarian gummy sweets, and in the control group cake, gummy sweets and table sauces—which had low r values (<0.40) and were significantly different ($p < 0.05$).

There were some commonly eaten foods (>10 subjects consuming) that showed significant differences in the mean g/week consumed between the FFQ and dietary recalls in both control and PKU groups. These included the following: boiled, mashed and jacket potato, table sauce, crisps and vegetables containing phenylalanine >75 mg /100 g. These foods tended to be considerably higher in the FFQ, except for crisps which were lower compared with the dietary recalls.

3.6.3. Construct Validity (Ability to Distinguish between Different Groups)—PKU FFQ vs. Control FFQ

There were significant differences between the intake of PKU and control groups using the FFQ, particularly in the foods expected to be different (see Supplementary Materials Table S4). This included higher protein foods that were consumed in greater quantities by controls: milk, cheese, soft cheese, dairy desserts, cream, wheat-based breakfast cereal, sandwich spreads, milk sauces, legumes, vegetables containing phenylalanine >75 mg/100 g, eggs, meat pies, meat curries, sugar-free drinks (usually containing aspartame), hot chocolate powder, nuts/seeds, and regular varieties of bread, bread rolls, pasta, pizza, biscuits, cakes, puddings, jelly, chocolate, gummy sweets and crackers. In addition, the higher carbohydrate/fat foods allowed since they are low protein/aspartame-free, were higher in the PKU group; these included: sweet spreads, mayonnaise/dressings, sweetened drinks (aspartame free), sugar, other sweets and butter, in addition to vegetarian varieties of foods such as burgers, pies and curries. Additionally, some foods commonly used as protein exchange foods were higher in the PKU group: tinned pasta, processed potato and potato or corn-based crisps.

4. Discussion

This is the first FFQ validated for children with PKU, with data suggesting that it is an effective, accurate and practical tool for estimating energy and macronutrient intake as an alternative method to dietary recalls. It identified dietary patterns, the quality of natural protein consumed, and adherence with protein prescription.

This FFQ demonstrated excellent reproducibility when administered at a mean time interval of 5 weeks. PKU group meal patterns were similar, and all nutrients showed good correlations ($r > 0.6$). The protein amounts in the PKU group had a correlation of 0.91, demonstrating that the FFQ reliably estimated usual intakes with similar accuracy to repeated 24-h dietary recalls. In addition, individual foods generally showed good correlation ($r > 0.4$) if they were commonly consumed items (eaten by >10 individuals). Discrepancies between FFQ 1 and 2 for individual foods may be explained by differences in interpretation between the two questionnaires or in participant memory of the types of food consumed at the various time points. For example, parents were sometimes ambivalent about the sugar content of drinks their children consumed, particularly if drinks were consumed at school/nursery or outside of the home. However, this could equally vary across the dietary recalls. Furthermore, some foods on the FFQ were rarely consumed (by <3 individuals). In order for a food itemisied on a FFQ to contribute to absolute intake or differentiate between individuals, it should be eaten regularly and by a significant number of the study population [9]. Therefore, some foods were removed from the study FFQ following analysis.

A minimum correlation coefficient of 0.3 to 0.4 has been suggested to detect associations when validating FFQs [9]. In this study, all nutrient correlation coefficients were above 0.5 for the comparison of the 2 FFQs in both groups, and above 0.4 for the comparison of FFQ and dietary recalls, except for fat in both groups and energy and protein for the control group only. Similar correlation results were shown in other validation studies [18–23].

Bland–Altman plots were used to display the stability and direction of the bias across levels of intake [19]. Agreement was considered reliable if the difference between the two

measures for reproducibility (FFQ 1 vs. FFQ 2) or validity (FFQ vs. recalls) was within 2 standard deviations (SD) of the mean [10]; the mean was close to 0; and demonstrated homogeneous data. Expert consensus suggests a combination of correlation or regression statistical methods together with Bland–Altman analysis should be used to assess reproducibility and validity of a FFQ, rather than any one single method [14].

To be truly valid, reported dietary intake from any assessment method should not be significantly different to actual intake, however there are practical difficulties with measuring 'absolute validity'; thus alternatively, 'comparative validity' (comparing with an alternative or 'reference method') is reported [24]. There is no gold standard method for recording dietary intake, all have limitations: weighed food records require a high level of subject commitment, adherence and understanding that would have excluded some recruits from this study; 3-day food diaries represent the current diet, rather than typical or usual intake over time; doubly labeled water, a more accurate method for comparison of energy intake, is expensive and requires specialised equipment that may be intimidating to children. Repeated dietary recalls have been previously used for validating FFQs [22,23,25]; whilst single day recalls do not account for day-to-day variability in food intake or episodically consumed foods [19], we chose to complete multiple 24-h recalls over a 4–10-week period to capture a more realistic picture of usual intake over time. This approach is supported by a systematic review of the validity of different dietary assessment methods compared with doubly labeled water, suggesting that multiple 24-h dietary recalls conducted over at least 3 days and using parents as proxy reporters was the most accurate method for children aged 4–11 years [25]. Neither the FFQ nor the multiple 24-h dietary recalls are likely to measure actual macronutrient intake with precision, as both are subject to recall bias; however, both methods produced a similar picture of intake.

Our FFQ designed for PKU demonstrated acceptable criterion validity when compared with the chosen reference standard, repeated 24-h dietary recalls. Total natural protein intake only varied by 0.5 g (25 mg phenylalanine) per day between methods, which is a very good correlation. There was some variability between assessment methods for the percentages of children reporting that they consumed food at mid meals; however, this is likely to be something that varies in individuals from day to day. Some individuals with PKU may also choose to consume their protein substitute in place of a snack between meals so as not to reduce appetite at main meals.

In keeping with other validation studies comparing FFQs with other methods [18–20,22,23] there was a tendency for the FFQ to report higher nutrient and individual food intakes than the dietary recalls. FFQs have been reported to overestimate dietary intake in children resulting from the use of adult portion sizes [10]; however, we overcame this by developing pictorial child-size portions. The main difference between the FFQ and dietary recalls was not so much the quantity of a food consumed, but less variation in the types of foods consumed for the dietary recalls. This reflects one of the limitations of dietary recalls in that they only capture recent intake rather than habitual food intake.

Consistent with previous studies looking at the macronutrient content of the PKU diet [26–28] our results demonstrated that the FFQ can differentiate the differences in macronutrient and individual food intake between children with PKU and children in the general population that would be expected. This substantiates good construct validity.

FFQs rely on recall over a longer assessment period than other methods and hence are associated with less accurate quantification. It is suggested that children under the age of 8 years may have difficulty recalling food intake, estimating portion size and conceptualizing frequency of food consumption [24,25]. The ability to cognitively self-report dietary intake accurately is commonly given as approximately 12 years [24,25]. Previous research has shown that when older children complete a FFQ, they receive less assistance from parents, and this can result in a greater number of inaccuracies [2]. There may also be anomalies in data (from both dietary assessment methods) for adolescents due to inaccurate self-reporting and the highly variable food patterns commonly seen in this age group [19]. In this study, children aged 11–16 years completed the FFQ and

recalls themselves which may have led to misreporting, although parents were able to assist. Furthermore, children with PKU have a more repetitive food pattern, receive dietary education and are accustomed to measuring portion sizes and completing dietary assessments. Correlations for the FFQ compared with the dietary recalls were stronger for the PKU subjects than for controls, suggesting that PKU subjects or their parents may have had better dietary recall than the control group [14,29].

Completion of any dietary assessment method may draw participants' attention to their diets [9], and there is also the risk of subjects responding in a way that demonstrates good adherence only in the presence of a dietitian. FFQs were administered by an IMD dietitian trained and experienced in dietary assessment rather than self-completion due to anticipated initial difficulties of comprehension and interpretation. As four different dietitians were involved in administration, there may have been some degree of inter-rater reliability. However, a standard script was used to administer questionnaires and food recalls to minimise this. It is anticipated that with repeated use, parents/carers and adolescents (>12 years) would be able to self-administer the FFQ independently. Recent studies have demonstrated that technology-assisted methods, such as an online FFQs, performed equally as well in estimating intakes as doubly labelled water and other methods [23,24]. As such, further analysis of this tool after regular use and with an online version may be warranted.

5. Conclusions

A FFQ can simplify dietary data collection in PKU, particularly if patients are familiar with the tool and can complete it electronically before clinic appointments. This low-protein FFQ designed for use in patients with PKU yielded comparable data to repeated dietary recalls, and can be validly used to collect data on usual food and nutrient intake in place of other dietary assessment methods. It will also enable assessment of the dietary patterns that may lead to lifestyle diseases, such as obesity in PKU, and in turn will facilitate tailored health messages to the PKU population that will help to reduce the incidence of health-related illness. It could also be particularly important in assessing the impact of dietary changes associated with pharmaceutical treatments in PKU. Further testing of an online version of the FFQ is warranted.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/nu14081595/s1: Table S1: Mean z-scores for BMI, weight and height for PKU and control children; Table S2: FFQ food items mean g/week FFQ 1 vs. FFQ 2 (PKU and Control); Table S3: FFQ items mean g/week FFQ vs. Dietary recalls (PKU and Control); Table S4 Kapp coefficient statistics; Supplementary S1: Script for FFQ (PKU); Supplementary S2: Script for FFQ (Control); Supplementary S3: Script for 24-h dietary recall (PKU); Supplementary S4: Script for 24-h dietary recall (Control).

Author Contributions: Conceptualization, S.E. and A.M.; data curation, S.E., R.J. and A.M.; formal analysis, S.E., R.J. and A.M.; funding acquisition, S.E.; investigation, S.E., C.A., A.D., A.P. and A.M.; methodology, S.E., C.A., A.D., A.P. and A.M.; project administration, S.E. and A.M.; supervision, A.M.; validation, S.E., R.J. and A.M.; visualization, S.E. and A.M.; writing—original draft, S.E. and A.M.; writing—review & editing, S.E., C.A., A.D., R.J., A.P. and A.M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the London—Queens Square National Ethics Research Service Committee, UK (protocol version 3, 18 April 2019, approved 16 May 2019; REC reference: 15/LO/1463; IRAS ID: 185896).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

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Conflicts of Interest: S.E. has received research funding from Nutricia, financial support from Nutricia and Vitaflo to attend study days and conferences; C.A. received honoraria from Nutricia and Vitaflo International to attend study days and conferences; A.D. research funding from Vitaflo, financial support from Nutricia and Vitaflo to attend study days and conferences; A.P. has received an educational grant from Cambrooke Therapeutics and grants from Vitaflo International, Nutricia, Merck Serono, Biomarin and Mevalia to attend scientific meetings; A.M. has received research funding and honoraria from Nutricia, Vitaflo International, and Biomarin. She is a Member of the Advisory Board Element (Danone-Nutricia). The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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CHAPTER 3B: FOOD ACCEPTANCE AND NEOPHOBIA IN CHILDREN WITH PHENYLKETONURIA: A PROSPECTIVE CONTROLLED STUDY

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RESEARCH PAPER

Food acceptance and neophobia in children with phenylketonuria: a prospective controlled study

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food frequency, metabolic disorders, neophobia, phenylketonuria, taste preferences.

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Abstract

Background: In phenylketonuria (PKU), little is known about the effect of bitter-tasting phenylalanine-free L-amino acid exposure on taste preference development. The present prospective study aimed to determine the flavour preferences of children with PKU versus healthy control children.

Methods: Thirty-five children with PKU and 35 age/gender-matched controls, aged 4–13 years, tasted 10 blinded puree foods in random order. They were rated using a seven-point pictorial hedonic scale (super yummy to super yucky) and ranked in preferential order. Caregivers completed a neophobia and food frequency questionnaire on behalf of their children.

Results: Both PKU and control groups rated sweet foods higher than savoury, bitter and sour foods. However, control children ranked fruits as a group higher than PKU children (mean 3.7 versus 4.6; $P = 0.03$), whereas PKU children ranked vegetables as a group higher than controls (mean 5.6 versus 6.3; $P = 0.05$). Children with PKU had more neophobia and were untrusting/fearful of new foods.

Conclusions: Although there was some evidence to suggest that children with PKU aged ≥4 years prefer savoury foods (vegetables) more than control children, they did not prefer bitter-tasting foods, and so early and persistent administration of bitter-tasting L-amino acids was not associated with apparent taste imprinting. Neophobia appears to play significant part in food refusal in PKU, perhaps more so than taste preferences.

Introduction

In phenylketonuria (PKU), the principal treatment is a low phenylalanine diet (commonly permitting <10 g/day^{-1} natural protein), supplemented with a phenylalanine-free L-amino acid supplement. Only a limited range of natural foods, mainly fruits and some vegetables, are given without restriction. Within the confines of this dietary restriction, it is important to support the development of normal feeding behaviour encouraging a wide range of flavours to maximise food choices. Little is known about the impact of these dietary restrictions on taste preferences of young children with PKU, although there is limited evidence that savoury and bitter foods may be preferred by young children[1].

Among the basic taste perceptions [sweet, bitter, salty, sour and umami (savoury)], bitterness and sweetness are largely innate, genetically linked and are evident at birth[2]. In PKU, it is commonly suggested that an important influence on food preference development is the early administration of bitter-tasting phenylalanine (phe)-free L-amino acid supplements from diagnosis (<14 days of age), potentially 'imprinting' a preference for bitter flavours and altering food preferences and food acceptance.

It is well established that, during the first few months of life, there is a sensitive learning period during which unpalatable flavours can become more acceptable[3]. In PKU, phe-free L-amino acid supplements are given at least 3 times/day^{-1} in children aged >1 year but every 3–4 h in early infancy. Many L-forms of amino acids used in phe-free amino acid supplements taste bitter (L-isoleucine, leucine, tryptophan and histidine). Similarly, infants with cow's milk protein intolerance may be treated with extensively hydrolysed formulas. These contain free amino

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acids, di and tripeptides and have similar bitter flavour notes (e.g. sulphur volatiles) to vegetables such as broccoli[4]. Children given bitter tasting protein hydrolysate formula during infancy are more likely to prefer broccoli and other savoury, bitter and sour tasting foods than those children who had received sweeter milk based formulas[3,5–7]. There is also evidence that these early flavour experiences influence subsequent flavour preferences several years after infancy[3,5,6]. It is possible that the introduction of L amino acid supplements may have a similar effect in PKU.

Food neophobia, an unwillingness to eat novel foods[8], may also affect children's food choices, with some preferring the familiar to the unfamiliar. There is evidence that young children with PKU eat a limited range of foods even within the confines of their dietary restriction[9], although little has been documented about its impact on food choices and food preferences in children with PKU aged >5 years.

In PKU, it is unknown whether 'flavour imprinting' occurs because of the early introduction of L amino acids and, if so, how long this effect may last and how it may affect food preferences. The aim of the present controlled, prospective study was to determine the flavour preferences of children with PKU and to compare these with a group of healthy age matched control children.

Materials and methods

Subjects

Thirty five children, aged 4 13 years (median 8.5 years; 17 male), with PKU diagnosed on newborn screening, were recruited from one specialist PKU centre. They were following a low phenylalanine diet providing ≤10 g/day^{-1} of natural protein allocated from 50 mg phenylalanine exchanges (each exchange equivalent to 1 g of natural protein) from diagnosis (<14 days of life). All were taking a phe free L amino acid supplement [PKU Cooler (n = 19), PKU Gel (n = 4) (Vitaflo International, Liverpool, UK), PKU Lophlex LQ (n = 11) and Anamix First Spoon (n = 1) (Nutricia, Trowbridge, UK)]. Thirty five age (within 6 months) and gender matched control children on a normal diet and with no existing health conditions that might influence taste perception were also recruited from a local community centre. The age group of subjects studied was determined by the minimum age at which children are able to comprehend and comply with tasting procedures.

The present study was conducted in accordance with guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the local research ethics committee. Written informed consent was obtained from all subjects/patients.

Study design

This was a single centre prospective controlled trial. Children were asked to taste 10 blinded puree foods in a computer generated random order, rating them using a seven point pictorial hedonic scale and then ranking them in preferential order.

Categorisation and ranking of foods

Hedonic categorisation and ranking methods[10,11] were used to assess taste preferences in PKU children on a low protein diet and control children on a normal diet. Children attended the hospital play centre for 60 min. Assisted by a hospital play therapist and following a practice session to check understanding, each child (after assent) was blindfolded (using a child friendly blindfold) to avoid any influence of colour on food preference[12]. Using a standard script, the play therapist presented each child with a spoonful of each of 10 foods in a random, pre selected order (to eliminate order effects) and asked them to categorise each food using a seven point hedonic scale; '*super yummy*', '*really yummy*', '*yummy*', '*just okay*', '*yucky*', '*really yucky*' or '*super yucky*' using images of faces[13]. Foods were presented in small, opaque, plastic containers so that the colour and appearance of the food was hidden. To validate responses, children were then asked to feed the foods that they liked to a friendly puppet and the foods they disliked to a monster puppet that 'liked yucky foods'. Water was offered between each tasting. Once all foods had been categorised and fed to a puppet, children then rank ordered the foods from most liked to least liked.

An initial pilot study on 12 children of varying age was conducted to confirm that the study methods and procedures were achievable.

Study foods

The foods were chosen to represent a range of foods that could be eaten by both groups of children and to represent a broad range of flavour sensations including bitter, sour, sweet and savoury. Eight foods (strawberry, apple, banana, low protein custard, carrot, sweet potato, broccoli and cauliflower) were blended (using the same equipment by the same researcher) to an equivalent pureed texture and 'spoon dropping' consistency using only the addition of a measured amount of water. Two liquids (50% lemon juice/water and decaffeinated instant coffee 1 g to 80 ml water) were also used. All were served at room temperature, with the exception of the four vegetables, which were steamed, pureed and served at normal serving temperature for cooked vegetables (50 60 °C). The food temperature was checked prior to serving.

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Food frequency questionnaire

Caregivers completed a food frequency questionnaire. This indicated the number of times (per week^{-1}) that children consumed each of 60 commonly eaten food items from major food groups, including milk and milk replacements; dairy products; fruit and vegetables; cereals, pasta and bread; meat and meat alternatives; ready meals; snack foods; fats and oils; and drinks. Caregivers also completed a question naire on how often children consumed each of the 10 test foods using a seven point scale (from daily to never).

Neophobia ccale

Caregivers completed a neophobia questionnaire using a seven item scale (ranging from 'always' to 'never') to measure their child's variety seeking tendency with respect to foods, food neophobia and general neophobia. This was modified from an existing questionnaire for adults[14]. This included nine questions specifically related to food and five questions on general neophobia.

Statistical analysis

Flavour preferences were compared using the categorisa tion and ranking of the 10 food items for the PKU and con trol groups. Neophobia and food frequency data were similarly compared between PKU and control groups. Descriptive statistics were used for quantitative outcome measures and a more detailed analysis of the food ranking/ rating scores was conducted using nonparametric tests.

Results

Rating of foods

Overall, both groups of children preferred sweet foods to savoury, sour and bitter. The three most liked foods by PKU children irrespective of age were custard, banana and apple, whereas, for control children, the top three foods were the same but in reverse order. PKU children preferred custard, sweet potato and carrot significantly more than control children (Table 1). Most foods (except apple and lemon) were rated higher by the PKU children compared to the control subjects. There was no signifi cant difference in responses by gender, although younger children (4 8 years) with PKU significantly preferred sweet potato and custard and older children (9 13 years) with PKU significantly preferred carrot and custard com pared to controls (Table 1).

Food ranking

The three most highly ranked foods by PKU children were custard, banana and strawberry, and, for control, children banana, apple and custard (Table 2). This differed little with age for the PKU children but younger control children ranked custard highest, whereas older control children pre ferred strawberry to custard. When foods were considered in food groups, control children ranked fruits as a group higher than PKU children (mean ranking: PKU 4.6 versus 3.7; $P = 0.03$), whereas PKU children ranked vegetables as a group higher than controls (mean ranking: PKU 5.6 versus 6.3; $P = 0.05$). However, when foods were grouped as bitter (broccoli, cauliflower, coffee) and sweet (apple, banana, cus tard), there was no significant difference between PKU and control children.

Food frequency questionnaire

Children with PKU consumed 50 100% more than con trol children of high energy, sugar containing drinks, sweets, chips, sweet biscuits and crisps (Table 3).

Table 1 Mean values for categorisation of foods by age group

	All ages			4 8 years			9 13 years		
	PKU	Control	*P* value*	PKU	Control	*P* value*	PKU	Control	*P* value*
Apple	4.7	5.1	0.53	4.4	5.2	0.27	4.9	4.9	0.99
Banana	5.1	5.0	0.46	5.2	5.3	0.89	5.1	4.7	0.52
Broccoli	4.3	3.7	0.21	4.3	3.7	0.41	4.2	3.6	0.32
Carrot	4.6	3.5	**0.009**	4.4	3.5	0.13	4.8	3.5	**0.02**
Cauliflower	4.1	3.6	0.29	3.7	3.6	0.92	4.5	3.5	0.11
Coffee	3.2	2.9	0.58	3.6	3.1	0.44	2.8	2.7	0.75
Custard	5.8	4.6	**0.001**	6.1	4.9	**0.02**	5.5	4.3	**0.02**
Lemon	2.9	3.2	0.37	2.7	3.1	0.47	3.1	3.3	0.76
Strawberry	4.6	4.1	0.22	4.4	4.2	0.74	4.7	4.0	0.13
Sweet potato	4.6	3.4	**0.009**	4.7	3.0	**0.008**	4.4	4.1	0.49

1 super yucky; 2 really yucky; 3 yucky; 4 just okay; 5 yummy; 6 really yummy; 7 super yummy.
Bold figures represent statistically significant results.
*Kruskall Wallis. PKU, phenylketonuria.

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Table 2 Mean values for ranking of foods by age group

	All ages			4 8 years			9 13 years		
	PKU	Control	*P* value*	PKU	Control	*P* value*	PKU	Control	*P* value*
Apple	5.0	3.5	**0.02**	5.4	3.6	**0.03**	4.5	3.3	0.19
Banana	3.9	3.3	0.22	3.8	3.4	0.62	4.1	3.1	0.22
Broccoli	5.9	5.9	0.93	5.7	5.8	0.93	6.1	6.1	0.93
Carrot	5.3	6.3	0.08	5.8	6.1	0.73	4.7	6.6	**0.03**
Cauliflower	6.0	6.5	0.41	6.0	6.4	0.66	5.9	6.6	0.48
Coffee	7.6	7.7	0.80	6.6	7.3	0.47	8.6	8.4	0.68
Custard	3.4	3.8	0.54	3.1	3.3	0.80	3.8	4.5	0.46
Lemon	7.4	7.2	0.77	7.4	7.5	0.95	7.3	6.7	0.63
Strawberry	4.8	4.4	0.54	4.9	4.5	0.67	4.7	4.3	0.64
Sweet potato	5.3	6.5	**0.03**	5.4	7.4	**0.01**	5.2	5.5	0.71

1 most liked; 10 least liked.
Bold figures represent statistically significant results.
*Kruskall Wallis. PKU, phenylketonuria.

Table 3 Mean (range) number of times/week [1] certain food/food groups consumed

	PKU Mean (range)	Control Mean (range)	*P* value*
Fruit	22.9 (0 89)	20.5 (3 124)	0.55
Vegetables	22.4 (2 56)	22.1 (1 102)	0.47
Potato fries	3.2 (0 14)	1.5 (0 5)	**0.002**
Bread (all types)	13.1 (1 35)	11.0 (0 24)	0.26
Pasta (all types)	5.2 (0 25)	2.4 (0 5)	**0.006**
Rice (all types)	2.1 (0 9)	2.0 (1 3)	0.40
Butter/margarine	11.3 (0 35)	8.7 (0 35)	0.12
Sweet biscuits (including LP)	6.0 (0 27)	3.4 (0 9)	**0.01**
Cake (including LP)	2.4 (0 9)	2.0 (0 7)	0.92
Sweets	6.3 (0 28)	2.8 (0 10)	**0.001**
Ice cream (all types)	1.7 (0 12)	1.7 (0 8)	0.13
Crisps	4.1 (0 7)	2.6 (0 14)	**0.01**
Chocolate (including LP)	1.5 (0 7)	3.9 (0 14)	**<0.0001**
Sugary drinks	9.4 (0 35)	2.9 (0 28)	**<0.0001**
Natural fruit juice	5.5 (0 28)	5.5 (0 21)	0.61
Water	18.1 (0 35)	17.1 (0 35)	0.87
Sugar free drinks	3.7 (0 25)	4.4 (0 21)	0.09
Study foods			
Apple	3.4 (0.1 7)	3.5 (0.3 7)	0.84
Banana	3.6 (0 7)	3.4 (0.1 7)	0.73
Broccoli	1.8 (0 7)	1.8 (0 4)	0.95
Carrot	3.3 (0.3 7)	3.3 (1 7)	0.95
Cauliflower	1.1 (0 4)	0.7 (0 4)	0.24
Coffee	0.2 (0 4)	0.07 (0 1)	0.26
Custard	0.5 (0 4)	0.9 (0.1 4)	0.18
Lemon	0.4 (0 4)	0.7 (0 4)	0.28
Strawberry	4.2 (0 7)	2.8 (0.1 7)	**0.01**
Sweet potato	1.5 (0 4)	0.5 (0 4)	**0.004**

*Kruskall Wallis. LP, low protein; PKU, phenylketonuria.
Bold figures represent statistically significant results.

Children with PKU consumed, on average, more than one sugary drink/day^{-1}, whereas control children only had them 3 times/week^{-1}. Similarly, sweets and sweet biscuits were consumed by PKU children six out of 7 days/week^{-1} compared to 3 days/week^{-1} for control children.

Intakes of fruit and vegetables and most other foods were similar across PKU and control groups (Table 3). Both groups consumed approximately three servings of fruit and three servings/day^{-1} of vegetables; milk/milk replacement 1 2 servings/day^{-1}; bread/low protein bread two servings/day^{-1}; water 2 3 servings/day^{-1}; sugar free drinks 3 4 times/week^{-1}; and natural fruit juice 5 6 times/week^{-1}.

The most frequently eaten (more than once/week^{-1}) study foods were: strawberry, banana, carrot and apple for PKU children, and apple, banana, carrot and strawberry for the control group children (Table 3). Strawberry and sweet potato were eaten significantly more commonly by children with PKU compared to controls, although all other foods were eaten with similar frequency.

Neophobia scale

Children with PKU were consistently reported by caregivers to be more neophobic and, in particular, more food neophobic than control children (Table 4). Caregivers of children with PKU reported that their children were significantly more likely than controls to be particular about what foods they eat and were less trusting and more fearful of new foods. However, although children with PKU were more uncomfortable in new and different situations, their neophobia did not make them less social because both groups were equally comfortable talking with or sitting next to a stranger.

Flavour of phe free L amino acid supplements and influence on preferences

There was no significant difference between groups taking different flavoured phe free L amino acid supplements

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Table 4 Comparison of mean response for general and food neophobia questions

	PKU	Control	*P* value*
Food neophobia			
Frequently tries new and different foods (*r*)	5.0	5.4	0.54
Does not trust new foods	3.4	4.5	**0.002**
Likes foods from different countries (*r*)	4.4	5.3	**0.03**
Thinks ethnic food looks too weird to eat	4.2	5.0	**0.05**
Will try new foods at parties (*r*)	3.9	5.2	**0.002**
Is afraid to eat things they have never had before	3.4	4.6	**0.003**
Is very particular about the foods they will eat	2.7	4.2	**0.001**
Will eat almost anything (*r*)	3.6	5.0	**0.009**
Likes to try new foods when eating out (*r*)	4.0	5.2	**0.007**
Mean total score (range 9 63)	**34.5**	**46.2**	**0.0003**
General neophobia			
Is uncomfortable in new or different situations	3.5	4.4	**0.03**
Prefers to be at home among familiar surroundings	3.0	3.7	**0.05**
Avoids speaking to people they do not know	4.5	4.6	0.91
Feels uneasy in unfamiliar surroundings	3.9	4.7	**0.04**
Does not like sitting next to someone they do not know	4.1	4.7	0.24
Mean total score (range 5 35)	**18.8**	**22.1**	**0.05**

Always 1; mostly 2; sometimes 3; unsure 4; rarely 5; mostly never 6; never 7.
Bold figures represent statistically significant results.
(*r*) scoring reversed for analyses.
*Kruskall Wallis. PKU, phenylketonuria.

and categorisation, ranking or frequency of eating the study foods. Twenty six percent ($n = 9$) of children were taking a strawberry flavoured L amino acid supplement, and 26% ($n = 9$) were taking berry, 23% ($n = 8$) orange, 17% ($n = 6$) unflavoured and 8% ($n = 3$) citrus/lemon flavoured supplements.

Discussion

This is the first study in PKU to demonstrate that children with PKU aged ≥4 years did not prefer bitter tasting foods (coffee, broccoli, cauliflower) associated with the taste of L amino acids. This suggests that, if taste imprinting does occur in PKU[15], it may be limited to infancy and pre school age, decreasing with continuing exposure to a wider range of foods. However, it was clear that children with PKU are apprehensive about trying new foods and eat only a limited range of foods within their dietary restrictions. Food neophobia may have a stronger influence on food acceptance than food taste preferences.

Although the bitter tasting phe free L amino acid formulas prescribed from early infancy in PKU are initially unflavoured (as with standard infant formula), flavoured products are commonly introduced at 12 months of age, and all products for children aged ≥3 years are available in flavoured varieties. Phe free L amino acid supplements are usually administered 3 times/day^{-1} and have added sugar, sweeteners and flavours that are designed to mask the bitter flavour of the L amino acids. This may interfere with the taste imprinting reported in other infants and young children on protein hydrolysate formula. With increasing age in PKU, the contribution of L amino acid supplements to total energy intake declines, whereas the intake of other high energy foods increases and this may further explain the dilution of taste imprinting.

There was some evidence to suggest that children with PKU prefer savoury (vegetables) foods more than control children, although there was also evidence that children with PKU prefer some sweet foods (custard) significantly more than control children. The intake of sweets, sweet drinks and biscuits was certainly greater in PKU children. However, no results demonstrated a preference for (or a liking for) bitter tasting foods more so than control children.

Studies have shown that repeated exposure to a new food dramatically increases infants' intake of other foods that are similar after only one exposure[16], in contrast to pre school children who may take up to six to 10 exposures before accepting a new food[17,18]. This suggests that food neophobia is age related. From an evolutionary point of view, food neophobia is a protective trait designed to prevent humans from ingesting potentially harmful foods[19]. Therefore, it is not surprising that children with PKU are particularly food neophobic because they are educated from an early age that high protein foods are potentially harmful. There is also evidence that children who demonstrate food neophobia have less variety in their diets[20,21], although, in PKU, this is clouded by the necessary dietary restrictions. Although children with PKU may generally be more reluctant to try new foods, in the present study, they appeared to be less fussy, consistently rating most foods higher than control children. Therefore, although neophobia may inhibit children with PKU from trying a wider variety of foods, it is not necessarily because they are averse to the flavour.

There are some limitations to the present study. The texture and seasonal taste of the foods may have altered perception for some children. For example, the strawberries used in the present study were less sweet as a result of the study being conducted in late winter/early spring,

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which may explain the low ratings in children who might normally have liked strawberries. However, all children were exposed to the same tastes and textures for comparison purposes and two different measures were used to determine food preference (ranking and hedonic scale) to check the validity of responses[2]. Because both methods produced similar results, we can assume that responses were relatively reliable. Previous studies have demonstrated that a 'taste and ranking' method of testing food preferences produces reliable results with pre school children[2,22–24]. Furthermore, studies have shown that children from the age of 5 years can reliably and consistently categorise food preferences using a seven point hedonic scale[10,12,13]. Although we did include a few 4 year old children, they were assessed as competent in the tasks required prior to the study. Demographic data such as parent age, education or socio economic status were not collected and this may have influenced the results.

In conclusion, although there is some evidence to suggest that children aged ≥4 years with PKU prefer savoury flavours (vegetables) more than control children, bitter taste imprinting does not occur. Consistent and frequent intake of sweetened phe free L amino acid supplements from the age of 3 years may well mask the bitter flavours of some L amino acids. The increase in the proportion of energy from food and the reduction in energy from L amino acid supplements with increasing age may also alter taste preferences and food acceptance. However, children with PKU are significantly more fearful of new and unfamiliar foods and attention to caregiver influences and food neophobia requires further study.

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Conflicts of interests, source of funding and authorship

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All authors were involved in the initial conception and design, data collection, interpretation of the data, and critical revision of the paper for important intellectual content, as well as final approval of the version submitted for publication. AMcD and SE were additionally involved in the collation of data and drafting of the initial article.

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CHAPTER 3C: THE INFLUENCE OF PARENTAL FOOD PREFERENCE AND NEOPHOBIA ON CHILDREN WITH PHENYLKETONURIA

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The influence of parental food preference and neophobia on children with phenylketonuria (PKU)

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ABSTRACT

Background: In a previous case-control study, we demonstrated that children with PKU and non-PKU controls preferred sweet foods. Additionally, children with PKU exhibited food neophobia, with no preference for bitter tasting foods associated with the taste of phenylalanine (Phe)-free L-amino acid supplements.

Objective: In an observational extension study, we evaluated the influence of parental food choice and neophobia on their children's taste preferences and food neophobia.

Methods: Male and female parents/caregivers of 35 children with PKU and 35 control parents, completed a neophobia and food frequency questionnaire for comparison using the same questionnaires that they completed for their children.

Results: Both groups of children (PKU and non PKU control) were rated as more food neophobic and exhibited more neophobic behaviour than parents, although children with PKU more so than non-PKU controls (PKU food neophobia $p < 0.0001$vs control 0.001; PKU general neophobia $p = 0.003$ vs control $p = 0.04$). Both groups of children ate significantly more sweets, sweetened drinks and potato fries than their parents but differences were greater for children with PKU who also consumed more high carbohydrate (low protein) staple foods such as bread and pasta, and more sweet snacks such as biscuits than their parents. Non-PKU control children's food choices were closer to their parent's choices.

Conclusions: In PKU, parental food choices and their food neophobia have limited influence on their children's eating habits. Food neophobia in children with PKU may be associated with fear of eating unfamiliar foods potentially containing a source of protein or aspartame. Their preference for sweet foods may be influenced by limited food choices and habitual consumption of artificially sweetened L-amino acid supplements.

1. Introduction

In phenylketonuria (PKU), an essential component of management is a low phenylalanine (Phe) diet, and in newborn screened infants is usually commenced within the first 2 weeks of life. Dietary management involves a severe restriction of high protein foods such as meat, fish, eggs, cheese, nuts, seeds and pulses; in addition to supplementation with bitter-tasting Phe-free L-amino acids. Due to severe restriction of high protein foods, the diet by necessity is high in carbohydrate, particularly plant and cereal starches to meet energy requirements. In addition, the artificial sweetener aspartame, that contains phenylalanine, must be avoided thereby limiting intake of some sugar-free products. It is well established that low Phe diets are high in carbohydrate (59–67% of energy) but lower in fat (23–26%) [1–5] than the general populations diet (50–52% carbohydrate and 33% fat) [6]. Eating behaviours evolve in the early years of development, and in PKU, a high carbohydrate diet may influence taste preferences and eating behaviour, potentially influencing the development of obesity in later life [1,7–9]. Parents help mould children's early experiences with food, providing both the genes and the environment for children [10]. It is important to understand the influence of parents on food preferences in PKU.

Although children with PKU might be expected to have a preference for bitter tasting foods associated with frequent exposure to bitter tasting Phe-free L-amino acid supplements from early infancy, a recent study in children with PKU aged 4–13 years [11], concluded that food neophobia (avoidance of unfamiliar foods) played a more significant role in food refusal than taste preferences. Children with and without PKU were given 10-blinded puree foods to taste and rank in order of preference; parents also completed questionnaires about their children's

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neophobia. Both groups demonstrated a strong preference for sweet foods, although children with PKU did show a stronger preference for savoury foods (i.e. vegetables) than control children. Fruits and vegetables were consumed in similar quantities in both groups of children.

Studies have identified that food neophobia (avoidance of unfamiliar food) in children is associated with less healthy food choices, [12] reduced variety [13] and fewer new foods being served in the family home thereby influencing everyday food choices [14,15]. Neophobia and food choice particularly in mothers, has been shown to influence the development of food neophobia in children [15–20]. In non-PKU studies, it is observed that food neophobia is familial i.e. children whose caregivers or siblings are food neophobic, are more likely to exhibit food neophobia [17,18,20,21]. The impact of parental food choice and general neophobia (fear of anything new or unfamiliar) is unreported in PKU.

The aim of this study was to evaluate the influence of parental food choice on food preferences and neophobia in children with PKU and control children.

2. Methods

2.1. Subjects

Male and female parents/caregivers of thirty-five children with PKU aged 4–13 years, and thirty-five age and gender matched control children previously studied and described [11] were recruited. PKU children were all following a low phenylalanine diet providing ≤ 10 g/day of natural protein allocated from 50 mg phenylalanine exchanges (equivalent to 1 g natural protein) from diagnosis (< 14 days of life).

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and the local research ethics committee gave a favourable opinion. Written informed consent was obtained from all parents/caregivers.

2.2. Study design

This was a single-centre, observational, prospective, follow-on, case-control study. Male and female parents/caregivers from PKU and control groups completed a food and general neophobia questionnaire using a 7-item scale (ranging from “always” to “never”). This was modified from an existing questionnaire [22] and was the same questionnaire used in the previous study for the children [11]. This included 9 questions specifically related to food and 5 questions on general neophobia. A lower score indicated more neophobia. Parents/caregivers from the PKU and control groups also completed a food frequency questionnaire to assess the number of times 60 commonly eaten foods from all the major food groups (milk and dairy products; fruit and vegetables; cereals, pasta and bread; meat and meat alternatives; ready meals; snack foods; fats and oils; and drinks) were consumed each day/week.

2.3. Statistical methods for data analysis

Neophobia and food frequency were compared between parent (male and female) and child. Descriptive statistics were used for quantitative outcome measures and more detailed analysis of all parameters was assessed using Kruskal Wallis and Mann Whitney non-parametric tests.

3. Results

3.1. Subjects (Table 1)

The control parents of one child were lost to follow-up and only 31 of 35 fathers were available in both groups. Therefore, 35 mothers and 31 fathers of children with PKU and 34 mothers and 31 control fathers were studied. Median ‘age range’ for mothers of children with PKU was 36–40 years, and for fathers of children with PKU and both control parents median age was 41–45 years. Eighty two percent of parents of children with PKU and 89% of parents of control children were white Caucasian, 8% in both groups were of Asian origin and the remainder were of other descent. Parents in the control group generally achieved a higher level of education ($p < 0.0001$).

Table 1
Subject demographics – parental age, ethnicity and highest educational level achieved.

	PKU		Control	
	Mother n = 35	Father n = 31	Mother n = 34	Father n = 31
Median age range (years)	36–40	41–45	41–45	41–45
Ethnicity	80% white 9% Asian 11% Mixed/ other	84% white 6% Asian 10% Mixed/ other	85% white 9% Asian 6% Mixed/ other	94% white 6% Asian 0% Mixed/ other
Highest educational level	% (n)	% (n)	% (n)	% (n)
None	14% (5)	10% (3)	6% (2)	3% (1)
GCSE	46% (16)	26% (8)	9% (3)	10% (3)
A-level	9% (3)	13% (4)	3% (1)	6% (2)
NVQ	3% (1)	16% (5)	21% (7)	0
Diploma	17% (6)	26% (8)	6% (2)	19% (6)
Degree	9% (3)	6% (2)	6% (2)	35% (11)
Post Grad	3% (1)	3% (1)	21% (7)	0
Masters	0	0	18% (6)	13% (4)
PhD	0	0	9% (3)	13% (4)
Unknown	0	0	3% (1)	0

3.2. Neophobia

3.2.1. Food neophobia (Fig. 1)

When total scores were calculated, children with PKU were significantly more food neophobic than both their mother ($p = 0.004$) and father ($p < 0.0001$). Control children were also more food neophobic than their parents (mother $p < 0.0001$; father $p = 0.0003$) but overall, less significantly than children with PKU. Children with PKU were more food neophobic than control children ($p = 0.0001$). There was no difference between mothers and fathers of children with PKU or between control mothers and fathers, but the parents of children with PKU were more food neophobic than control parents (mothers

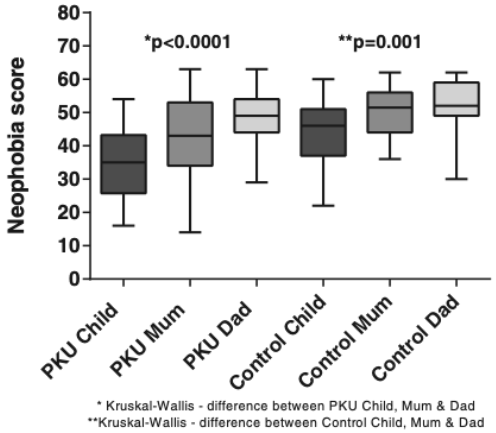

Fig. 1. Median total scores for food neophobia for children with PKU and Control children and their parents (lower score = more neophobic).

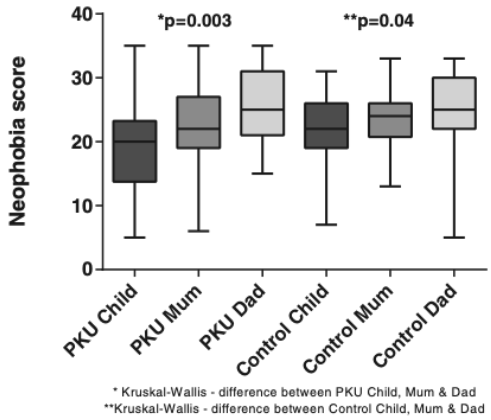

Fig. 2. Median total scores for general neophobia for children with PKU and Control children and their parents (lower score = more neophobic).

p = 0.004; fathers p = 0.05).

3.2.2. General neophobia (Fig. 2)

Children with PKU were significantly more neophobic than both their parents (mothers = 0.05; fathers p = 0.0006). Control children exhibited similar neophobia to their mothers but were more neophobic than their fathers (p = 0.01). There was no significant difference between children with PKU and control children or between mothers and fathers from either group with respect to neophobia.

3.3. Food frequency questionnaire (Table 2)

3.3.1. Parent vs child

Unsurprisingly, the control children's food intake was more similar to parental food intake than children with PKU. Children with PKU ate significantly more high carbohydrate staple foods such as bread, pasta and snack foods such as sweet biscuits and cake (low protein varieties) and there was a non-significant trend for children with PKU to consume more fruit and water and less chocolate than their parents. Both groups of children ate significantly more sweets, sweetened drinks, ice-cream and potato fries than parents. However, except for ice-cream, children with PKU ate significantly more of these foods (although less than daily) compared with control children. The differences between parent and child were greater for children with PKU.

3.3.2. Mothers vs fathers

Control group fathers ate fewer vegetables (p = 0.04) and more potato fries (p = 0.02) and sweets (p = 0.03) than control mothers but there were no significant differences between parents of children with PKU.

3.3.3. Parents of children with PKU vs control parents

Parents of children with PKU ate significantly less chocolate (p = 0.04) than control parents (possibly due to avoidance of eating this in front of their child), and control mothers had more vegetables (p = 0.04) and biscuits (p = 0.008) than mothers of children with PKU. Fathers of children with PKU had significantly more sweetened drinks (p = 0.02) than control fathers and mothers of PKU children (along with their children) consumed more water than control parents (although not statistically significant).

3.3.4. Number of foods consumed per week

The variety of foods consumed by children with PKU was significantly less than control children (p < 0.0001) and both their parents (mothers p < 0.0001; father p = 0.0002) (Table 2). Control children were not significantly different from either parent. Both groups of parents were not significantly different from each other but mothers of PKU children had significantly less variety than control mothers (p = 0.001).

3.4. Influence of mother's age and educational level on food neophobia and food variety

There was a significant difference (p < 0.0001) between PKU and control groups for mother's highest level of education. Mother's (PKU and control groups) with a higher level of education (Diploma or higher), had less food neophobia (median score 55 vs 45; p = 0.006) and ate a wider variety of foods (median number of different foods per week 51 vs 40; p = 0.001) than those with a lower educational level, as did their children (47 vs 37; p = 0.005). However, when divided into PKU and control groups, there was no significant difference between PKU group mothers, or their children, irrespective of educational level

Table 2
Median frequency (per week) of consumption of certain food/food groups consumed by parents and children from both PKU and Control groups.

	PKU				Control			
	Child	Mum	Dad	P value*	Child	Mum	Dad	P value*
Fruit	18	16	11	0.06	17	21	15	0.26
Vegetables	21	19	18	0.79	20	26	21	0.04
Potato fries	3	1	2	0.007	1	1	1	0.02
Bread (including LP)	12	7	8	0.0007	9	9	9	0.31
Pasta (including LP)	3	2	2	< 0.0001	2	2	2	0.31
Rice (including LP)	1	1	2	0.61	2	2	2	0.34
Butter/Margarine	10	7	9	0.45	7	7	5	0.55
Sweet biscuits (including LP)	4	1	2	0.0001	3	3	3	0.49
Cake (including LP)	2	0	1	0.03	2	1	2	0.41
Sweets	5	0	1	< 0.0001	2	0	2	< 0.0001
Ice-cream (including LP)	1	0	0	0.006	1	1	1	0.001
Crisps	5	3	2	0.12	2	2	3	0.35
Chocolate (including LP)	1	2	2	0.29	3	3	3	0.93
Sugary drinks	5	0	1	< 0.0001	1	0	0	0.02
Natural fruit juice	5	3	2	0.61	4	4	5	0.69
Water	21	21	15	0.45	14	14	15	0.54
Sugar-free drinks	0	0	0	0.34	2	0	0	0.24
Median number of different foods/week (range)	33 (12–45)	40 (19–61)	44 (14–62)	< 0.0001	54 (25–80)	51 (28–69)	52 (26–74)	0.09

* Kruskall Wallis LP = low protein.

Table 3
Comparison of food neophobia and food variety with mother's educational level.

	PKU mothers			Control mothers		
	Lower education	Higher education	P value*	Lower education	Higher education	P value*
Mother's median food neophobia score	43	44	0.51	46	55	0.03
Child's median food neophobia score	31	36	0.52	39	48	0.003
Mother's median food variety no./week	39	41	0.73	40	58	0.0009
Child's median food variety no./week	33	33	0.78	47	63	< 0.0001

* Kruskal Wallis.

(Table 3). Conversely, control mothers with a lower level of education (below Diploma level), and their children, were significantly more food neophobic and consumed less variety in their diet. The age of the mother did not significantly influence the level of neophobia or food variety in either the mother or child.

4. Discussion

This is the first study in PKU to compare parental and child neophobia, food preferences and food intake in comparison with age matched control children and their parents. Both groups of children had more general and food neophobia than their parents, although children with PKU were more food neophobic than control children. In addition, both parents of children with PKU (particularly mothers) were significantly more food neophobic than control parents and this in turn may influence the type and variety of foods offered. Children with PKU ate more staple foods such as bread and pasta than their parents or than control children; these are low in phenylalanine, satiating, and more accessible than some other low protein foods. Furthermore, both groups of children, but particularly children with PKU consumed more sweet foods than their parents. The apparent preference for sweet containing food may be influenced by restricted food choices, lack of 'quick' alternative snack foods, avoidance of sugar-free drinks due to their aspartame content or by the 'sweetened' Phe-free L-amino acid supplements which are prescribed and consumed at least three times daily.

Although neophobia may be influenced by genetics [21], several studies show that neophobia declines with age associated with increasing exposure to new and different foods [15,23–25], peaking at around 2–6 years of age [23]. Our children with PKU had a minimal age of 4 years but still had significant neophobia. It is likely many other factors may have influenced their neophobia including innate fear of eating 'unsafe' food, limited food choice, food palatability, and poor appetite associated with regular daytime intake of Phe-free L-amino acids [26]. The mealtime environment is undoubtedly important, including the strategies employed by parents to persuade their children to eat, frequency of exposure to foods, and the effects of parental modelling [23]. When children eat in the presence of others, their eating behaviour is socially facilitated [24] generally leading to an increase in the amount of food consumed relative to the number of people present [23]. Previous studies have demonstrated that children with PKU may eat separately to the rest of the family and receive limited positive encouragement during mealtimes [26,27], exacerbating food neophobia. There is also evidence that parental style and controlling behaviour increases neophobia in children [28]. This type of behaviour may well be reflective of the pressure experienced by parents of PKU children to ensure their children choose appropriate foods in their diet.

The influence of caregivers in food choice and taste preferences cannot be underestimated. Overall, parents of children with PKU lacked variety in their diet compared with control parents who ate fruit and vegetables more frequently. In addition, mothers of children with PKU were more food neophobic than control mothers and this may in turn have influenced the foods offered to children. Food neophobia and food preferences in children are affected by socio-economic status and parents educational level [23] and the quality of children's dietary intake has been shown to be associated with maternal educational level, namely in the form of less variety and less healthy choices [29–31]. In our study cohort, mothers of children with PKU generally achieved a lower educational level than control mothers. However, no difference was found in children with PKU or their mothers with respect to food neophobia and variety of foods consumed irrespective of the mother's education level. Conversely, educational level did influence food neophobia and food variety in control children and their mothers. This suggests that there are other more significant factors influencing neophobia and food variety in PKU. Parents of children with PKU may adjust some of their own eating patterns because of their child with PKU. In addition, food choices may be limited in PKU associated with the complexities of low protein cooking, with parents opting for premeasured manufactured foods for dietary phenylalanine allocation instead of preparing foods from fresh ingredients.

There were some study limitations. The study population had been previously selected for another study with different objectives; therefore, parents were not matched for age and educational level. Moreover, statistical comparisons of mothers' educational level were limited due to the small subject numbers studied. Parents of PKU children generally had lower education levels than control parents and mothers of PKU children were also on average younger than control mothers and these are factors known to influence food choice. However, when controlled for, these factors did not appear to influence results in the PKU group. In addition, the questionnaires for the children in the study were completed predominantly by the children's mothers rather than children themselves. It may be that they perceived their children to be more neophobic than they were and it is possible that their own neophobia or food rejections may have limited exposure to different foods in their children. Furthermore, the BMI of parents was not recorded and it is acknowledged that this may have influenced eating habits. However, previous study in this group of PKU children has indicated that the incidence of obesity is no different than in the general population [8].

In conclusion, this study suggests that food neophobia in the parents/caregivers of children with PKU, has limited influence on their children's food neophobia. However, parental food choice may constrain the exposure to food variety. In early childhood years, although practical guidance is offered on improving children's exposure to different low phenylalanine foods (principles of 'food modelling', eating together as a family, and maintaining positive messages about food choices), this advice adds to the pressure surrounding the central message that blood phenylalanine control should always be maintained within target range. The convenience of offering children low phenylalanine foods that are pre-measured, easy to prepare and readily accepted by their children in a diet that is difficult to maintain is probably an important factor but it remains unstudied. Overall, in PKU, further longitudinal cohort studies are required about dietary patterning, food portion sizes and food choice availability to see how these influence longer-term eating patterns and nutritional status.

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Conflict of interest

Anne Daly – research funding from Vitaflo; financial support from Nutricia and Vitaflo to attend study days and conferences.
Sharon Evans - research funding from Nutricia; financial support from Nutricia and Vitaflo to attend study days and conferences.
Anita MacDonald - research funding and honoraria from Nutricia, Vitaflo International and Merck Serono, Member of European Nutrition Expert Panel (Biomarin), Member of Sapropterin Advisory Board (Biomarin), Member of the Advisory Board for Element (Danone-Nutricia) and Arla.

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CHAPTER 4: BURDEN OF CARE AND EDUCATION

CHAPTER 4A: DEVELOPMENT OF NATIONAL CONSENSUS STATEMENTS ON FOOD LABELLING INTERPRETATION ANDPROTEIN ALLOCATION IN A LOW PHENYLALANINE DIET FOR PKU

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Development of national consensus statements on food labelling interpretation and protein allocation in a low phenylalanine diet for PKU

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Abstract

Background: In the treatment of phenylketonuria (PKU), there was disparity between UK dietitians regarding interpretation of how different foods should be allocated in a low phenylalanine diet (allowed without measurement, not allowed, or allowed as part of phenylalanine exchanges). This led to variable advice being given to patients.

Methodology: In 2015, British Inherited Metabolic Disease Group (BIMDG) dietitians ($n = 70$) were sent a multiple-choice questionnaire on the interpretation of protein from food-labels and the allocation of different foods. Based on majority responses, 16 statements were developed. Over 18-months, using Delphi methodology, these statements were systematically reviewed and refined with a facilitator recording discussion until a clear majority was attained for each statement. In Phase 2 and 3 a further 7 statements were added.

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Results: The statements incorporated controversial dietary topics including: a practical 'scale' for guiding calculation of protein from food-labels; a general definition for exchange-free foods; and guidance for specific foods. Responses were divided into paediatric and adult groups. Initially, there was majority consensus (≥86%) by paediatric dietitians ($n = 29$) for 14 of 16 statements; a further 2 structured discussions were required for 2 statements, with a final majority consensus of 72% ($n = 26/36$) and 64% ($n = 16/25$). In adult practice, 75% of dietitians agreed with all initial statements for adult patients and 40% advocated separate maternal-PKU guidelines. In Phase 2, 5 of 6 statements were agreed by ≥76% of respondents with one statement requiring a further round of discussion resulting in 2 agreed statements with a consensus of ≥71% by dietitians in both paediatric and adult practice. In Phase 3 one statement was added to elaborate further on an initial statement, and this received 94% acceptance by respondents. Statements were endorsed by the UK National Society for PKU.

Conclusions: The BIMDG dietitians group have developed consensus dietetic statements that aim to harmonise dietary advice given to patients with PKU across the UK, but monitoring of statement adherence by health professionals and patients is required.

Keywords: Phenylketonuria (PKU), Delphi method, Food labelling, Consensus, Phenylalanine exchanges

Introduction

Phenylketonuria (PKU) is an inborn error of amino acid metabolism, due to deficiency or absence of the enzyme phenylalanine hydroxylase, leading to accumulation of blood and brain phenylalanine (Phe). Untreated, it will cause severe, irreversible neurological damage [1]. Strict dietary management is the only available treatment option in the UK. The aim is to correct abnormal biochemistry by decreasing the Phe load on the affected pathway and supplementation with Phe -free L-amino acids or low Phe glycomacropeptide protein substitutes [2]. The diet involves avoiding high protein foods (e.g. meat, fish, eggs, cheese, seeds, flour, bread and nuts), with strict control of moderate containing protein foods (e.g. cereals, potato, milk and some vegetables) to maintain blood Phe levels within target range [1, 3]. Several fruits and vegetables are low in Phe and are incorporated in the diet without limit [4]. From weaning when solids are first introduced [5] and later with the ever growing convenience food market, patients with PKU and their caregivers need simple, consistent, easy-to-understand rules for calculating protein intake. A national dietetic re-appraisal of some of the practical dietary advice given to UK patients and families with PKU was necessary because of: the recent publication of European PKU guidelines identifying different criteria for the allocation of fruit and vegetables in a low Phe diet [3]; introduction of new species of fruits and vegetables; new European protein labelling legislation [6]; contradictory information available via social media; and increasing patient usage of manufactured foods.

The British Inherited Metabolic Diseases (BIMDG) Dietitians Group, using the Delphi method set out to agree a set of practical statements about the classifications of foods in a low Phe diet. The Delphi process is a tool used to gain a majority decision in a structured or systematic manor. It helps secure a collective view from a panel of experts about complex issues or problems where there is little or no definitive evidence [7, 8]. Experts respond to questionnaires over several rounds with a facilitator coordinating and summarising responses for feedback. Responses and feedback from each expert, guide the questions for further rounds. With each successive round the number of questions declines as the group moves toward consensus.

Aims

To develop consensus statements about the practical allocation of foods and interpretation of protein food labelling in a low Phe diet for the management of PKU in the UK using the Delphi method to generate consensus.

Methods

In November 2015, BIMDG dietitians convened to discuss the allocation of foods and interpretation of food labeling with the aim of developing consensus statements for PKU. A multiple-choice questionnaire, comprising 14 questions about dietary advice to UK patients or caregivers regarding the allocation of different foods (allowed without measurement, not allowed, or allowed as part of Phe exchanges) and interpretation of protein from food labels, was distributed to 70 BIMDG dietitians from 30 centres treating individuals with PKU. The results of this questionnaire identified 7 specific areas where dietetic practice was variable, leading to contradictory advice being issued to patients and caregivers. These 7 areas are identified in Table 1.

Following a round table discussion of these results at a BIMDG dietitians meeting, 16 consensus statements for

Table 1 Variation in dietary advice given to patients by BIMDG dietitians

Areas of variation in dietary advice given to patients by BIMDG dietitians:
Use of different terminology to describe low protein foods that could be incorporated into the diet without measurement.
Inconsistent advice to parents about the calculation of 1 g protein exchanges (equivalent to 50 mg phenylalanine) from protein labelling analysis of individual food portions i.e. dietitians were either rounding protein values > or < 1 g to the nearest 0.5 exchange.
Use of different upper protein 'cut off' points for foods that could be given in the diet without measurement (exchange-free). There was inconsistent allocation of the following foods: herbs and spices; fats/oils; soya sauce; gravy; cooking sauces; vegetable crisps; sweets; and processed vegetables/vegetable sauces with protein containing ingredients (e.g. milk/wheat).
Inconsistent allocation of manufactured foods that contained low protein (exchange-free) ingredients.
Inconsistent allocation of special low protein foods that contained low protein (exchange-free) ingredients.
Inconsistent allocation of fruits and vegetables (containing phenylalanine from 50 to 100 mg/100 g weight) in the diet. Some dietitians were allowing certain fruits and vegetables in the diet without measurement; others were permitting in restricted amounts only.
Inconsistent interpretation of manufactured food labelling: e.g. some foods are labelled as containing 0 g protein even though some of the ingredients are protein sources. This includes foods containing protein ≤0.5 g/100 g (permissible by European law: Regulation (EU) No 1169/2011) [4] or protein < 1 g/100 g (permissible by USA law).

the practical dietary management of PKU were put forward and agreed in Phase 1. In Phase 2 a further 6 statements, and in Phase 3 one final statement was proposed. The Delphi method was then used to gain consensus from the BIMDG dietitians about each of the statements. The lead author acted as facilitator.

The 23 consensus statements on practical dietary management were circulated to all BIMDG dietitians by email. A written response of agreement or disagreement with each statement was then returned to the facilitator for analysis. After each round of circulation, a telephone conference was held with the BIMDG dietitians to provide collective feedback and the draft consensus statements were further adapted. Modified statements were then recirculated to the BIMDG dietitians with additional questions until a majority decision (> 60%) was obtained on each statement. Dietitians were given 8 weeks to respond in each Delphi round.

Approval of the final consensus statements was sought and received by the UK National Society for PKU (NSPKU).

Results

Phase 1: Round 1 of Delphi method

The 16 draft consensus statements on practical dietary management were prepared with accompanying notes explaining their rationale. They were then distributed for approval or non-approval of each statement to all BIMDG dietitians (73 dietitians: 43 paediatric practice, 20 adult practice and 10 caring for both adult and paediatric patients; from 30 inherited metabolic disorder [IMD] centres).

Replies were received from 40 dietitians (55% of BIMDG dietitians, from 23 centres, 77%) and results were distributed to all dietitians within 5 months of initial circulation. After the first round, there was majority consensus by paediatric dietitians (≥86%; $n = 29$) and dietitians working in adult practice (≥65%; $n = 17$) for all 16 statements. At a BIMDG dietitians group teleconference, minor modifications were made to a small number of the consensus statements and 14 of the 16 statements were formerly agreed by paediatric dietitians (Tables 2 and 3).

However, two of the statements generated considerable debate, so it was decided to further explore individual opinions on these 2 statements following additional investigation into the Phe content of the products. In addition, there was some disparity among dietitians working in adult practice in relation to maternal patients with PKU and Phe consumption from fruits and vegetables permitted without measurement. Consequently, it was decided that for round 2 of the Delphi process, paediatric dietitians and dietitians working in adult practice would separate to examine issues independently.

Phase 1: Round 2 of Delphi method

The remaining 2 'non-agreed' draft consensus statements (statement 1: the upper protein content of soya sauces allowed as an exchange-free food; and statement 2: the allocation of fruits and vegetables containing Phe content from 76 to 99 mg per 100 g weight) were modified following examination of barriers to acceptance (Table 2). An in-depth investigation was conducted about the protein content of all commercial soya sauces available to purchase as well as issuing analysis about the Phe content of fruits and vegetables. These 2 consensus statements were then recirculated to the BIMDG dietitians; with 3 possible options for each statement.

Soya sauce was considered differently to other table top sauces. Its protein content is highly variable from < 0.5 g per 100 ml up to 15 g per 100 ml. Soya sauce option 1: *protein ≤ 1 g per 100 ml is exchange-free,* was consistent with the statement for wet cooking sauces but allowed very little brand choice; option 2: *protein ≤ 1.5 g per 100 ml is exchange-free*, allowed more choice but was inconsistent with statements for other foods; and option 3: *up to 2 tablespoons per day of any soya sauce,* allowed a wide choice of brands but could lead to a higher protein intake.

Table 2 Summary of Consensus Statements

PHASE 1

Consensus statements	Delphi Process – **Round 1** % agreement (n)		Delphi process – **Round 2** % agreement (n)		Delphi process – **Round 3** % agreement (n)	
	Paediatric Dietitians $n = 29$ 18 centres	Dietitians in adult practice $n = 17$ 11 centres	Paediatric Dietitians $n = 36$ 18 centres	Dietitians in adult practice $n = 19$ 12 centres	Paediatric Dietitians $n = 25$ 10 centres	Dietitians in adult practice $n = 20$ 13 centres
In PKU, low protein, free, unmeasured or non-exchange foods are referred to as 'exchange-free' foods.	93 (27)	100 (17)	Agreed		Agreed	Agreed
Foods are 'exchange-free' if protein content is ≤ 0.5 g/100 g of food:						
• e.g. sweets	90 (26)	88 (15)	Agreed		Agreed	Agreed
• e.g. gravy	97 (28)	76 (13)	Agreed		Agreed	Agreed
Exceptions that remain 'exchange-free' if > 0.5 g per 100 g or per 100 ml of food:						
• all herbs, spices and seasonings (irrespective of the protein content on the food ingredient label, due to the small quantities consumed);	100 (29)	100 (29)	Agreed		Agreed	Agreed
• fats (oils, butter, margarine) (as only small amounts are used)	100 (29)	88 (15)	Agreed		Agreed	Agreed
• 'wet cooking sauce' if the protein content is ≤1.0 g/100 g	100 (29)	65 (11)	Agreed		Agreed	Agreed
Soya sauce:						
ROUND 1: soya sauce that contains Phe ≤ 1.0 g/100 ml (based on the quantities commonly consumed)	97 (28)	88 (15)	N/A		N/A	N/A
ROUND 2:						
• Option 1: Any soya sauce that contains protein ≤1.0 g/100 ml is considered 'exchange-free'	N/A	N/A	25 (9)*		N/A	N/A
• Option 2: Any soya sauce that contains protein ≤1.5 g/100 ml is considered 'exchange-free'	N/A	N/A	67 (24)*		Agreed	Agreed
• Option 3: 2 tablespoons per day of any soya sauce is 'exchange-free' (existing guideline)	N/A	N/A	14 (5)*		N/A	N/A
For 'wet cooking sauces' with a protein content > 1.0 g /100 g:						
• If they contain exchange ingredients (e.g. cream, egg, coconut) they are counted as an '**exchange**' food;	100 (29)	65 (11)	Agreed		Agreed	Agreed
• If they contain 'exchange free' ingredients only (e.g. fruit/ vegetables) they are considered an 'exchange-free' food.	100 (29)	65 (11)	Agreed		Agreed	Agreed
Allocation of fruits and vegetables (except fresh/frozen potatoes)						
ROUND 1:						
• Fruits & vegetables (except potatoes) containing a Phe content ≤75 mg/100 g weight will be consisdered 'exchange-free' foods.	100 (29)	100 (17)	Agreed		Agreed	Agreed
• Fruits & vegetables with a Phe content of ≥100 mg/100 g, use the actual Phe content to calculate **exchange** amounts.	100 (29)	100 (17)	Agreed		Agreed	Agreed

Table 2 Summary of Consensus Statements *(Continued)*

• Phe content 76-99 mg/100 g weight of fruit & vegetable: allow 1 portion/day 'exchange-free'	86 (25)	82 (14)	N/A	N/A	N/A
ROUND 2/3:					
• Option 1: Fruit & vegetables containing a Phe content 76–99 mg/100 g weight of fruit & vegetable, count as **'exchange'** foods	N/A	N/A	35 (13)*	64 (16)	Agreed
• Option 2: Allow 1 exchange portion 'exchange-free' per day with additional portions to be counted as an **exchange**	N/A	N/A	42 (15)*	24 (6)	
• Option 3: Only 1 portion of these fruits/ vegetables are allowed 'exchange-free' per day (existing guideline)	N/A	N/A	33 (12)*		
Additional statement: Fruits & vegetables containing a Phe content of 76–99 mg/100 g, use a standard **'exchange'** amount of 60 g weight to provide approximately 50 mg/Phe	N/A	N/A	97 (35)	Agreed	Agreed
• ***Fresh/frozen potatoes*** are counted as **'exchange'** foods. If the Phe analysis is available, this will be used to determine exchange amount; if Phe analysis is unavailable (e.g. potato waffles, hash browns), the protein content will be used to calculate exchange amounts.	97 (28)	94 (16)	Agreed	Agreed	Agreed
• ***Manufactured fruit/vegetable*** products containing only fruits or vegetables that are designated as 'exchange-free' in their fresh form, should still be considered 'exchange-free' foods. However, if they contain added ingredients that are protein containing (e.g. milk or wheat), they are counted as **'exchange'** foods with their protein content used to determine the amount of food allocated for one Phe exchange.	100 (29)	88 (15)	Agreed	Agreed	Agreed
• ***Vegetable crisps*** containing exchange free vegetables are counted as **'exchange'** foods due to the concentration of protein associated with cooking methods; their protein content should be used to determine the exchange amount.	88 (21)	88 (15)	Agreed	Agreed	Agreed
Low protein special foods:					
• Low protein special products (e.g. bread, flour) should be allowed without measurement if all ingredients are 'exchange-free', irrespective of the phenylalanine analysis per 100 g on the label.	93 (27)	76 (13)	Agreed	Agreed	Agreed
alculating food protein exchanges from protein analysis on the food label:					
• Food **'exchange'** amounts for food portions should be rounded up or down based on the 'rule of maths' (Table 3 for guidance).	97 (28)	94 (16)	Agreed	Agreed	Agreed
• Patients/caregivers are advised to read ingredient lists as well as the protein content on food labels. The following was concluded as guidance:	100 (29)	88 (15)	Agreed	Agreed	Agreed
- If ingredients contain protein but the protein label content appears low, establish a more accurate protein analysis before consuming.	100 (29)	88 (15)	Agreed	Agreed	Agreed
- If ingredients are 'clearly' exchange-free but the protein	100 (29)	100 (17)	Agreed	Agreed	Agreed

Table 2 Summary of Consensus Statements *(Continued)*

label content is not available then it is suitable to give as an exchange-free food.						
- If there is no protein content on the product label, but it contains **exchange** ingredients, an accurate protein analysis should be obtained before consuming.	100 (29)	100 (29)	Agreed		Agreed	Agreed
- If a label states its protein content is 0 g but ≥1 ingredient contains a protein source (e.g. gelatine), avoid until an accurate protein analysis is known.	97 (28)	65 (11)	Agreed		Agreed	Agreed
Agree with all consensus statements (as above)	N/A	N/A	N/A	N/A	N/A	75 (15)
Separate maternal PKU statements preferred	N/A	N/A	N/A	N/A	N/A	40 (8)
PHASE 2						
Consensus statements	Delphi Process – Round 1 % agreement (n)		Delphi process – Round 2 % agreement (n)		Final Decision	
	Paediatric Dietitians *n* = 19 13 centres	Dietitians in adult practice *n* = 9 8 centres	Paediatric Dietitians *n = 19* 11 centres	Dietitians in adult practice *n* = 14 8 centres		
Low protein milk						
ROUND 1:						
• Option 1: A daily volume of 250 ml is exchange free if it provides ≤0.5 g protein (25 mg Phe)/daily	16 (3)	56 (5)	N/A	N/A		
• Option 2: A daily volume of 500 ml/daily volume is exchange free if it provides ≤0.5 g protein (25 mg Phe) /daily	42 (8)	11 (1)	N/A	N/A		
• Option 3: A daily volume of 1000 ml/daily volume is exchange free if it provides ≤0.5 g protein (25 mg Phe) /daily	21 (4)	22 (2)	N/A	N/A		
• Option 4: A daily volume of 1000 ml/daily volume is exchange free if it provides ≤0.5 g protein (25 mg Phe) /daily	11 (2)	11 (1)	N/A	N/A		
• Unsure	11 (2)	–	N/A	N/A		
ROUND 2:						
• Any plant milk (e.g. coconut, rice or almond) that provides a total protein intake of > 0.5 g over 24 h when consumed should be counted as an exchange food. If the total protein intake provides ≤0.5 g over 24 h, it should be considered exchange-free.	N/A	N/A	84 (16)	79 (11)	Agreed	
• Any low protein special milk (e.g. Sno Pro, Taranis, Prozero) that provides a total phenylalanine intake of > 25 mg (half an exchange) over 24 h when consumed should be counted as an exchange food. If the total phenylalanine intake provides ≤25 mg over 24 h, it should be considered exchange-free.	N/A	N/A	79 (15)	71 (10)	Agreed	
Soups that contain exchange-free ingredients are exchange-free.	79 (15)	78 (7)	N/A	N/A	Agreed	
Any ***coconut yoghurt/dessert*** with a protein content ≤0.5 g/100 g	79 (15)	56 (5)	N/A	N/A	Agreed	

Table 2 Summary of Consensus Statements *(Continued)*

is exchange-free.					
Any ***dried coconut product*** with a protein content > 0.5 g/100 g should be counted as an **exchange** food.	95 (18)	89 (8)	N/A	N/A	Agreed
Any food containing ≤0.5 g/100 g protein (but contains **gelatine**) can be allocated as exchange-free as it is likely to contain ≤10 mg phenylalanine from this source.	95 (18)	89 (8)	N/A	N/A	Agreed
The weight rather than the volume of ***ice-cream*** should be used to calculate the protein exchange amount.	100 (19)	100 (9)	N/A	N/A	Agreed
PHASE 3					
Consensus statements	Delphi Process – Round 1 % agreement (n)				Final Decision
	n = 17 13 centres				
Low protein special foods					
If the special low protein food contains exchange ingredients but contains up to 25 mg Phe/100 g, it is exchange-free. If the special low protein food contains exchange ingredients but contains ≥26 mg Phe/100 g, it is an exchange food.	94 (16)				Agreed

NB: Dietitians covering both paediatrics & adults – responses are included in both groups. *some dietitians were undecided and chose 2 options

Table 3 Calculating food protein exchanges from protein analysis on the food label

Protein content per item when calculated from label	Calculated exchange
0 g protein per food portion	Exchange free
0.1 g protein per food portion	Exchange free (if total volume consumed is ≤0.5 g protein)
0.2 g protein per food portion	Exchange free (if total volume consumed is ≤0.5 g protein)
0.3 g protein per food portion	Exchange free (if total volume consumed ≤0.5 g protein). Suggest 1 portion is exchange-free, 2 portions is ½ exchange[a]
0.4 to 0.7 g protein per food portion	½ exchange protein
0.8 to 1.2 g protein per food portion	1 exchange protein
1.3 to 1.7 g per food portion	1.5 exchange protein
1.8 to 2.2 g per food portion	2 exchange protein
2.3 to 2.7 g per food portion	2.5 exchange protein
2.8 to 3.2 per food portion	3 exchange protein

[a]This may apply to ice cream lollies, gluten-free cakes, very small packets crisps, sweets

For fruit and vegetables with a Phe content between 76 to 99 mg per 100 g: option 1: *count as part of the 50 mg Phe exchange system*, considered that their uncontrolled consumption may increase dietary Phe intake considerably, but may be difficult to enforce in patients already established on dietary management who do not currently restrict their intake. Option 2: *permit only one serving daily of any fruits and vegetables in this category, but calculate Phe intake from any additional servings of fruits and vegetables from this category,* overcame some of the issues associated with option 1, but was complex for both dietitians and patients or caregivers. Option 3: *permit 1 portion daily of any one of the fruits and vegetable in this category*, was the existing guidance and whilst aiming to control overall intake, it did not consider increased opportunities for consumption of some of the more novel forms of these fruits and vegetables e.g. vegetable rice, vegetable pasta. An additional question was asked in this round (Table 2) about the acceptance of a standard exchange weight i.e. 60 g for fruits and vegetables with a Phe content between 76 and 100 mg per 100 g.

For BIMDG paediatric dietitians, option 2 for the soya sauce consensus statement received a majority response (67% of respondents; $n = 24/36$) and was agreed; but opinion was divided between the 3 options for the final consensus statement on fruits and vegetables containing Phe content from 76 to 99 mg per 100 g weight. However, there was consensus on the need for a standard exchange weight for these fruits and vegetables (Table 4). The dietitians working in adult practice continued to debate both questions but did not reach consensus at this round.

Table 4 New exchanges (previously exchange free)

Fruit and vegetables containing Phe ≥ 76 mg/100 g	Amount for 1 exchange
Figs	60 g
Asparagus	60 g
Beansprouts	60 g
Broccoli	60 g
Brussel sprouts	60 g
Cauliflower	60 g
Yam	60 g
Sugar snap peas	60 g
Mange tout	60 g
Whole hearts of palm	60 g

Phase 1: Round 3 of Delphi method

The remaining 'non-agreed' consensus statement concerning the allocation of fruits and vegetables was reissued with the 2-options scoring highest in the previous round. Option 1 statement was finally agreed by 64% ($n = 25$) of paediatric dietitians and endorsed at the dietitians BIMDG teleconference in February 2017.

In the final Delphi round, 75 % of dietitians working in adult practice ($n = 15$) agreed with the 16 consensus statements for adult patients following diet, concluding that consistency in dietary care in the transition from paediatric to adult services was important. However, 40% ($n = 8$) of dietitians working in adult practice proposed that separate maternal PKU consensus statements were required.

NSPKU endorsement

The initial consensus statements were endorsed by the UK National Society for PKU (NSPKU) in April 2017.

Phase 2: Round 1of Delphi method

Additional food items not covered by the initial statements were identified in the 6 months following completion of Phase 1, so 6 new statements on: low protein milks, soups, coconut desserts, coconut products, ice-cream and gelatine containing products, were distributed with accompanying notes. Replies were received from 25 dietitians (from 18 centres) and results were discussed at a BIMDG dietitians group teleconference in January 2018. There was majority consensus by dietitians (≥76%) for all but the low protein milks statement which it was agreed needed redefining (Table 2).

Phase 2: Round 2 of Delphi method

Two amended statements on plant milks and low protein milk replacements were sent out in February 2018 and results discussed and agreed at a teleconference in May

2018. There were 30 replies and 77% ($n = 23$) agreed with the plant milks statement and 80% (n = 24) with the low protein milk replacements statement (Table 2). Other low protein special foods were also discussed in detail and it was agreed that the initial statement in Phase 1 required elaboration due to significant protein containing ingredients in some products.

Phase 3: Round 1 of Delphi method

A statement regarding low protein special foods was distributed along with information on their content in May 2018. Results were conclusive and this along with Phase 2 statements were agreed at a BIMDG dietitians meeting in June 2018. A summary of all agreed guidelines was issued in July 2018 and endorsed by the NSPKU (Table 5).

Discussion

National consensus statements on the practical interpretation of dietary management in PKU are central to ensuring consistent advice is given to all families and patients with PKU. We have systematic agreement on many basic dietary rules and definitions which have received united support from BIMDG dietitians. Using the Delphi method allowed all BIMDG dietitians to have the opportunity to give their opinion and influence several areas of dietary management in PKU where scientific evidence was unavailable to dictate practice. For most of the statements, clear consensus was reached in the first round of Delphi discussions, leaving only 4 controversial statements requiring more extensive discussion and negotiation. The process we

Table 5 Summary of Final Consensus Statements for PKU

1. Any food given without measurement is referred to as an exchange-free food.

2. Foods are 'exchange-free' if they contain protein ≤0.5 g/100 g. e.g. sweets, coconut products, foods containing gelatine.
Exceptions:
- herbs, spices, seasonings, fats (oil, butter, margarine) – as the quantity used is very small.
- Any soya sauce containing protein ≤1.5 g/100 ml is exchange-free.
- Any 'wet cooking sauce' containing protein ≤1.0 g/100 g is exchange-free. If it contains > 1 g protein/100 g and contains exchange ingredients (e.g. cream, egg, coconut) it should be counted as an ***exchange food***. *If it contains > 1 g protein/100 g and contains 'exchange free' ingredients only (*e.g. vegetables such as tomatoes) it is an exchange-free food.

3. 'European PKU guideline 2017' [3] is used for fruit and vegetable allocation:
- Phe content ≤75 mg/100 g weight of fruit and vegetables: exchange-free.
- Phe content ≥76 mg/100 g weight of fruit and vegetables: count as **exchange foods**.

Exception:
Potatoes – use Phe analysis to determine exchange amounts. If potato products contain additional exchange ingredients (e.g. wheat flour, or milk), use protein analysis on the packet to determine its exchange amount.

4. A standard exchange amount of 60 g for any fruit/vegetables containing Phe between 76 and 99 mg/100 g will be used. For any fruit/vegetables containing Phe ≥100 mg/100 g (e.g. peas, sweetcorn), the actual Phe content will be used to calculate exchange amounts.

5. If any frozen/canned product is designated 'exchange-free' in their fresh form, they are considered exchange-free foods e.g. carrots, mushrooms, tomatoes.
Exception:
Vegetable crisps - although derived from exchange-free food, are concentrated in protein due to cooking methods so should be counted as **exchange foods**. Use protein content per 100 g to determine the amount that can be given for one exchange.

6. Low protein special products (e.g. bread, flour) are exchange-free if all ingredients are exchange-free. If they contain exchange ingredients but contains ≤25 mg Phe/100 g, they are exchange-free. If they contain exchange ingredients but contains ≥26 mg Phe/100 g, they are an **exchange food**.

7. Any low protein special milk that provides a total Phe intake of > 25 mg (1/2 exchange) over 24 h in the volumes consumed, should be counted as an **exchange food**. If the total Phe intake provides ≤25 mg over 24 h, it should be considered exchange-free.

8. Any plant milk (e.g. coconut, rice, almond) that provides a total protein intake of > 0.5 g over 24 h in the volumes consumed, should be counted as an **exchange food**. If the total protein intake provides ≤0.5 g/100 g over 24 h, it should be considered exchange-free.

9. Soups that contain exchange-free ingredients are exchange-free. If soups contain exchange ingredients and their protein content is > 0.5 g/100 g, then they should be counted as an **exchange food**.

10. Weight rather than the volume of ice-cream should be used to calculate the protein exchange amount.

11. Food 'exchange' amounts for food portions should be rounded up or down based on the 'rule of maths'. See Table 3

12. All patients/caregivers are advised to read ingredient lists as well the protein content/100 g on food labels.
If ingredients are protein containing (but the protein analysis appears very low), it is important to establish more accurate protein analysis before consuming.
If ingredients are 'clearly' exchange-free, then it is appropriate to give as an exchange-free food even if the protein analysis is unavailable on the label.
If there is no nutritional analysis on the product but it contains ingredients that are **exchange foods**, then further food protein analysis must be obtained before consuming.
If a label states protein content is 0 g but one or more ingredients is a protein source (e.g. gelatine), then an accurate food protein analysis must be obtained before consuming.

have undertaken should lead to harmonization and consistency of dietetic practice in PKU with less confusion for professionals, patients and their families. Adoption of these consensus statements by the national patient's society, NSPKU, will enable uniform written dietary information to be available for all patients. Further adaptation of these statements is necessary for maternal PKU, where dietary management practices are particularly rigorous. This exercise will be taken forward by the BIMDG dietitians working in adult practice.

The advantage of the Delphi method is that it involves the collective knowledge of a group of experts which is likely to be better than that of each individual; comparing, contrasting, challenging and complementing each other [8]. The process is directed, impartial, helps engender group ownership, encourages consensus among individuals with diverse views and is an alternative to conventional meetings where strong personalities, status and group pressures can influence individual responses [7]. It was clear that every opinion was valued and would be important in influencing the final decisions. Because this method is structured and focused, it can avoid much of the counterproductive digressions identifiable in face-to-face group discussions [7]. Due to the combined contribution of all members, varying ideas and viewpoints were generated and these further directed later responses.

The Delphi Method did have some limitations. Being a qualitative method, it is considered subjective. However, in our consensus statements, only best practice opinion was available to support any of the specific decisions reached; when new scientific evidence is available these statements will be challenged. Also, 70 dietitians participated, which could be considered a limitation. However, the number of experts chosen was designed to be inclusive of all centres and dietitians in the UK who were members of the BIMDG dietitians group. Another drawback of the process was the tendency for participants to maintain the status quo rather than voting for change. For example, for two of the consensus statements, despite good initial agreement in round 1, further discussion identified the impracticality of the statements which prompted the generation of 3 possible options for round 2. A further limitation was the lack of response from some members. Unfortunately, this was unavoidable due to maternity leave, job changes and retirements occurring during the 18-month period of the project. Some dietetic non-responders were new to the IMD specialty or were from centres with fewer patients and or less clinical experience and may have been reluctant to respond due to uncertainty or a lack of definitive opinion. Despite some non-responding dietitians, most UK IMD centres were represented in each round.

Conclusion

In conclusion, this process of agreement between BIMDG dietitians across the UK will enable the introduction of consistent, easy-to-understand rules for calculating protein intake for professionals and patients with PKU. Both dietitians working in paediatric and adult services have endorsed these consensus statements which should contribute to a smooth transition between services, maintaining uniformity of information across all ages. Modification of the consensus statements may be warranted for maternal PKU patients requiring more rigorous dietary restriction. It will be important to perform an evaluation of the interpretation of these statements by dietitians and patients in clinical practice. Overall these consensus statements contribute to harmonising dietary advice offered to British PKU patients. Longitudinal monitoring of their application, acceptance and adherence by health professionals and patients or caregivers is essential.

Abbreviations

BIMDG: British Inherited Metabolic Diseases Group; IMD: Inherited Metabolic Diseases; NSPKU: National Society for Phenylketonuria; Phe: Phenylalanine; PKU: Phenylketonuria

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Availability of data and materials

The supporting datasets analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

All authors were involved in the initial conception and design (through BIMDG dietitians group meetings, conferences and teleconferences), data collection (using the Delphi Method), interpretation of the data and critical revision of the paper for important intellectual content and final approval of the version to be published. SE and AMac were additionally involved in the collation of data and drafting of the initial article and SE acted as facilitator for the Delphi process.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

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CHAPTER 4B: UNIFORMITY OF FOOD PROTEIN INTERPRETATION AMONGST DIETITIANS FOR PATIENTS WITH PHENYLKETONURIA: 2020 NATIONAL CONSENSUS STATEMENTS

nutrients

MDPI

Article

Uniformity of Food Protein Interpretation Amongst Dietitians for Patients with Phenylketonuria (PKU): 2020 UK National Consensus Statements

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Abstract: In phenylketonuria (PKU), variable dietary advice provided by health professionals and social media leads to uncertainty for patients/caregivers reliant on accurate, evidence based dietary information. Over four years, 112 consensus statements concerning the allocation of foods in a low phenylalanine diet for PKU were developed by the British Inherited Metabolic Disease Dietitians Group (BIMDG-DG) from 34 PKU treatment centres, utilising 10 rounds of Delphi consultation to gain a majority (≥75%) decision. A mean of 29 UK dietitians (range: 18–40) and 18 treatment centres (range: 13–23) contributed in each round. Statements encompassed all foods/food groups divided into four categories based on defined protein/phenylalanine content: (1) foods high in protein/phenylalanine (best avoided); (2) foods allowed without restriction including fruit/vegetables containing phenylalanine ≤75 mg/100 g and most foods containing protein ≤0.5 g/100 g; (3) foods that should be calculated/weighed as an exchange food if they contain protein exchange ingredients (categorized into foods with a protein content of: >0.1 g/100 g (milk/plant milks only), >0.5 g/100 g (bread/pasta/cereal/flours), >1 g/100 g (cook-in/table-top sauces/dressings), >1.5 g/100 g (soya sauces)); and (4) fruit/vegetables containing phenylalanine >75 mg/100 g allocated as part of the protein/phenylalanine exchange system. These statements have been endorsed and translated into practical dietary management advice by the medical advisory dietitians for the National Society for PKU (NSPKU).

Keywords: phenylketonuria (PKU); consensus; Delphi method; food labelling; phenylalanine; Phe; protein; exchanges

1. Introduction

Phenylketonuria (PKU) is a rare, inherited metabolic disorder (IMD) caused by phenylalanine hydroxylase deficiency, leading to an abnormal accumulation of blood phenylalanine. Without treatment, it causes severe and irreversible intellectual disability. However, national newborn screening programmes detect PKU, which enables treatment to commence in early infancy with outcomes associated with a broad range of normal general ability. In the UK, the only available treatment is a rigorous, life-long dietary restriction of natural protein (i.e., meat, eggs, fish, cheese, nuts, bread, flour, pasta) in order to control blood phenylalanine levels [1,2] within the target range, as pharmacological treatments are not reimbursed by the National Health Service. Individuals with PKU tolerate only a limited amount of natural protein, with the amount individually determined. It is estimated that 80% of those with classical PKU are prescribed less than 10 g/day of protein [3].

Whilst the goal of dietetic management is essentially the same, the method for allocating phenylalanine/protein intake varies between and sometimes within countries, with insufficient evidence to recommend any one method [4–9]. Broadly there are two different methods, each with its merits and drawbacks: (1) patients are allocated a daily amount of phenylalanine/protein from all food, which is calculated to provide the prescribed phenylalanine intake; and (2) a phenylalanine/protein exchange system whereby amounts of food are calculated for a defined amount of phenylalanine (ranging from

10–50 mg of phenylalanine for each exchange) or protein (e.g., 1 g protein = 1 exchange). One food exchange can be replaced with an alternative exchange of an equivalent phenylalanine/protein amount.

In the UK, natural protein is apportioned using a protein exchange system whereby one exchange is equivalent to the amount of food that is calculated/measured to provide 1 g protein or 50 mg of phenylalanine. Individual tolerance based on blood phenylalanine levels determines the number of exchanges allocated, but is typically between 3–10 g exchanges/day (or 150–500 mg/day phenylalanine). The diet is then supplemented with a phenylalanine-free/low-phenylalanine protein substitute, foods naturally low in phenylalanine/protein, and special low protein foods (SLPF) (e.g., bread, flour, pasta) approved by the Advisory Board for Borderline Substances (ACBS), and prescribed by the General Practitioner (GP). Overall dietary management is complex, and a high degree of patient/caregiver knowledge and application is required to effectively implement dietary care. Successful dietary treatment is hampered by inconsistent dietary advice associated with unclear and historical recommendations, lack of comprehensive food phenylalanine analysis, similar plant species containing a variable phenylalanine content per 100 g of food, and an overwhelming range of manufactured foods with unclear declarations of protein content. In the UK, national practical guidance about the implementation of dietary treatment in PKU has not been reviewed since 1993 [10].

Patients with PKU are cared for by multidisciplinary teams of health professionals working across many care settings within the UK. Almost every professional involved has an opinion about the practical implementation of dietary management. This causes wide variability in dietary advice given by practitioners, leading to uncertainty for patients and caregivers and inexperienced health professionals seeking advice from peers. In addition, patients/caregivers readily turn to social media for information that may be based on the erroneous interpretations of others. Multiple sources of discordant information are also available through apps and specialist manufacturers of dietary products. International dietary practices also vary, adding another tier of misunderstanding and ambiguity [4]. It is important that patients with PKU and their caregivers receive reliable and uniform dietary information from their health care professionals.

In 2016, the British Inherited Metabolic Disease Dietitians Group (BIMDG-DG) identified several controversial issues concerning the calculation of protein in a low phenylalanine diet [11]. This included EU legislation that foods containing a protein content of ≤0.5 g/100 g do not need to declare their specific protein content on food labels [12]. This has caused considerable confusion about which foods could be permitted within a phenylalanine restricted diet. There was also discrepancy about the allocation of fruits and vegetables according to their phenylalanine/protein content. This led to the development of 23 BIMDG-DG national consensus statements about the interpretation of protein food labelling and allocation of foods in a low phenylalanine diet [11].

Since the publication of the first report [11], the BIMDG-DG have continued to develop new statements about food allocation in a low phenylalanine diet utilising Delphi methodology. This time, all food categories have been systematically examined, and a comprehensive set of consensus guidelines for interpreting their protein content and allocating these foods within a low phenylalanine diet has been developed.

2. Materials and Methods

Over a 4-year period (November 2015 to September 2019), 112 consensus statements from UK dietitians working in PKU were developed regarding the inclusion, exclusion, and allocation of foods within a low phenylalanine diet *specifically for use by dietitians*. These were aimed at restrictions of protein of ≤10 g/day or phenylalanine ≤500 mg/day. These statements considered most commercially available foods as well as special low protein foods. The process included 10 rounds of Delphi consultation to gain a majority decision in a structured and systematic way [13]. It involved an independent facilitator collecting the opinion of clinical dietitians working with PKU. The facilitator issued proposed statements about food allocation, each time presenting any available research evidence along with the protein/phenylalanine content of foods. Questionnaire responses in each round were

gathered and results discussed by telephone conference. Metabolic dietitians from the BIMDG voted on each statement. The process was repeated, with modification of the statements, until there was at least 75% agreement (an arbitrary figure chosen to represent a majority decision). Any unresolved statements at round one of the Delphi process were carried over to the next round of statements for further discussion. A more detailed description of this process has been reported previously [11].

All foods/food groups were systematically divided into nineteen subgroups with a mean of six statements per subgroup (range: 1–14): milk and milk replacements (n = 2); dairy products and alternatives (n = 12); breads and cereals (n = 4); spreads and dips (n = 5); sauces and soups (n = 13); pasta and rice (n = 2); potato and potato products (n = 6); fruit and vegetables (n = 7); meat and alternatives (n = 7); drinks (n = 8); sweet snacks (n = 13); savoury snacks (n = 10); sugars, sweeteners and syrups (n = 9); herbs and spices (n = 1); low protein special foods (n = 2); flours and starch (n = 5); baking ingredients (n = 4); gluten-free products (n = 1); and gelatine containing products (n = 1).

No ethical approval was required for this project as it is not considered research as defined by the UK Policy Framework for Health and Social Care Research [14]. Descriptive analysis was used to present the results.

3. Results

In the UK, there are nine paediatric and nine adult PKU specialist centres for PKU, with around 18 district general hospitals who share PKU care with the specialist centres.

In total, 93 BIMDG dietitians (57% (n = 53) paediatric; 32% (n = 30) adult; 11% (n = 10) working with both adults and children) from all represented UK PKU treatment centres (n = 34) responded to the consensus statements during the four year study period. In each round of the Delphi process, there were approximately 70 active BIMDG dietetic members potentially able to participate. The numbers of dietetic contributors varied in each round due to the movement of dietitians (maternity leave, retirement, illness, secondment, or specialty change). A median of 29 dietitians (range: 18–40) and 18 centres (range: 13–23) contributed to each round. All specialist UK PKU IMD centres were represented in each round except for one adult centre only able to participate in two of 10 rounds. All BIMDG dietitians received copies of the results for each round and the minutes of meetings. Fifty-eight percent (n = 65/112) of statements received 100% agreement, a further 26% (n = 29/112) received ≥90% agreement, and 15% (17/112) were between 79–89%.

Of the 112 statements, most had majority agreement within one round of the Delphi process. Only nine statements received less than 75% agreement on the first round of discussion (fruit/vegetables, low protein milks, coconut desserts, soya sauce, special low protein foods, vegetable crisps, eggs, cheese, and seeds). These statements were then modified and reconsidered in the next round. New statements for other foods continued to be added at each round. Consensus was agreed in seven of nine statements on the second round of discussion. Two statements (fruit/vegetables and low protein milks) required discussion, modification, and voting over three consecutive rounds to achieve consensus. No statements required deletion, only modification, as all foods/food groups were required to have a statement.

Each food subgroup was systematically discussed via the Delphi process and allocated into one of four categories (with further subcategories), based on their protein or phenylalanine content.

Category 1: Foods high in protein or phenylalanine that are best avoided (Table 1). This included two subcategories:

(a) Foods high in protein (generally containing protein >15 g/100 g).

(b) Foods containing aspartame and therefore phenylalanine.

Category 2: Foods allocated without restriction or measurement (defined as exchange-free foods) (Table 2). Generally, any foods with an upper protein content cut-off point ≤0.5 g/100 g or containing exchange-free ingredients were considered exchange-free. Exceptions included spices (with a higher protein content) that are used for flavouring purposes only and consumed in small quantities. Overall, this category consisted of four subcategories:

Table 1. Category 1: Foods high in protein or phenylalanine.

1. Foods High in Protein or Phenylalanine that Are Best Avoided	
(a) Foods high in protein (approximately >15 g/100 g)	• Meat, fish, eggs, nuts, cheeses, seeds, soya products, Quorn, goji berries, peanut butter, tofu, spreadable yeast extracts. • Exceptions: soft cheeses, soya cheese, baked products containing seeds or eggs as an ingredient, baked goods with eggs as an ingredient - these fall into category 3b and are used as part of the exchange system. • Eggs contain protein <15 g/100 g. Although they are used as part of the protein exchange system in baked goods, one hen's egg is high in protein and best avoided.
(b) Foods containing aspartame	• Aspartame containing food and drinks (e.g., fizzy drinks, fruit juice, fruit tea, milkshake powders/syrup, smoothies, squash, chewing/bubble gum, desserts, jelly, sweets, tabletop sweeteners).

Table 2. Category 2: Foods allocated without restriction.

2. Foods Allocated without Restriction or Measurement (Defined as Exchange-Free Foods)	
(a) Fruits and Vegetables containing phenylalanine ≤75 mg/100 g (except potatoes and vegetable crisps)	• Apples, apricots, avocado, bananas, banana chips, bilberries, blackberries, blueberries, candied angelica, candied peel, cherries, clementines, cranberries, currants, custard apples, damsons, dates, dragon fruit, fruit crisps (e.g., apple, pineapple), fruit pie filling, fruit mincemeat, fruit salad, glacé cherries, gooseberries, grapes, grapefruit, greengages, guavas, jackfruit, kiwi fruit, kumquats, lemons, limes, loganberries, lychees, mandarins, mango, medlars, melon, nectarines, olives, oranges, papaya (paw paw), peaches, pears, physalis, pineapple, plums, pomegranate, prickly pear, prunes, quince, raisins, raspberries, rhubarb, satsumas, Sharon fruit, star fruit, strawberries, sultanas, tamarillo, tangerines, watermelon. • Artichoke, aubergine, baby corn, beetroot, cabbage, capers, caperberries, carrots, cassava, celeriac, celery, chayote, chicory, courgette, cucumber, dudhi, eddoes, endive, fennel, garlic, gherkin, ginger, green beans (dwarf, French, runner), karela, kohlrabi, leeks, lettuce, marrow, mooli, mushrooms, okra, onion, pak choi, parsnips, peppers, pickled vegetables (e.g., onion, gherkins, red cabbage), plantain, pumpkin, radish, salad cress, samphire, squash (butternut, acorn, spaghetti), swede, sweet potato, tomato, turnip, watercress, water chestnuts. • Fruit and vegetable-based foods containing exchange-free fruits/vegetables and other exchange-free ingredients (e.g., frozen or canned fruit/vegetables, tomato puree/passata).
(b) Manufactured foods containing protein ≤0.5 g/100 g or exchange-free ingredients	• Sugar (brown, cane, caster, demerara, fruit, glucose, granulated, icing, molasses, muscovado, white). • Jam, honey, marmalade, syrup (agave, fruit, golden, maple, treacle). • Fats (oils, oil sprays, ghee, lard). • Baking ingredients (arrowroot, baking powder, bicarbonate of soda, cassava/tapioca flour, cornflour/maize starch, cream of tartar, sago). • Aspartame-free drinks (squash, fruit drinks, soft drinks, black/herbal tea and coffee). • Artificial sweeteners (except aspartame). • Condiments (mint jelly, mint sauce, salt, vinegar). • Fibres/gums (e.g., psyllium fibre/husks, xanthan gum). • Aspartame-free milkshake powders/syrups and custard powder containing exchange-free ingredients. • Plants & cereals (konnyaku, sago, tapioca, cassava crisps).
(c) Manufactured food containing protein >0.5 g/100 g but used in small amounts	• Fats (butter, margarine). • Herbs, spices, condiments (e.g., pepper). • Food colouring and flavourings/essences.
(d) Special low protein foods containing exchange-free ingredients or a phenylalanine content <25 mg/100 g	• Low protein: bread (sliced, rolls, baguettes), biscuits, breakfast cereals, cereal bars, cakes, chocolate, chocolate spread, cheese sauce, crackers, cake mix, dessert/custard mixes, egg replacer, fish substitute mixes, flour, pizza bases, pasta, rice, sausage/burger mixes. • Low protein milk replacement Prozero (Vitaflo). • Includes most UK ACBS prescribed low protein products.

(a) Fruits and vegetables containing phenylalanine ≤75 mg/100 g.

(b) Manufactured foods containing protein ≤0.5 g/100 g or exchange-free ingredients.

(c) Manufactured food containing protein >0.5 g/100 g, but used in small amounts so provide minimal contribution to protein intake.

(d) SLPF containing exchange-free ingredients or a phenylalanine content <25 mg/100 g that have been ACBS approved and are available on prescription for low protein diets.

Category 3: Manufactured foods/SLPF allocated as part of the protein/phenylalanine exchange system according to their protein/phenylalanine content per 100 g (Table 3). One protein/phenylalanine exchange is the amount of food that is calculated/measured to provide either 1 g of protein or 50 mg of phenylalanine from its food analysis. This category included any SLPF and manufactured foods with a phenylalanine/protein content above the upper cut-off point (>0.5 g/100 g) and containing protein exchange ingredients (e.g., milk, wheat, flour, rice, egg, and soya). This section was divided into four subcategories with different protein/phenylalanine upper cut-off points based on the food portion size that would typically be consumed or if they contained a high proportion of exchange-free fruit or vegetables (e.g., commercial cooking sauces):

Table 3. Category 3: Manufactured foods allocated as part of an exchange system based on protein or phenylalanine content.

3. Manufactured Foods Allocated as Part of the Protein Exchange System According to Their Protein/Phenylalanine Content per 100 g	
(a) Liquid plant and animal milks with protein >0.1 g/100 g or 0.1 g/100 mL or specialist low protein milks with phenylalanine >5 mg/100 mL	• Animal milks (e.g., cow, goat, sheep), full fat, semi-skimmed, skimmed, condensed. • Plant milks (e.g., coconut, oat, almond, soya). Includes coffee with these added (e.g., lattes, cappuccino, frappuccino, macchiato, coffee pods/sachets). • Low protein milk replacements–Dalia 6.4 mg Phe/100 mL (Taranis), Lattis 12 mg Phe/100 mL (Mevalia), Loprofin 10 mg Phe/100 mL and SnoPro 8.7 mg Phe/100 mL (Nutricia).
(b) Foods containing protein >0.5 g/100 g or specialist low protein foods containing phenylalanine >25 mg/100 g and containing exchange ingredients	• Bread and bread products, biscuits and cakes made from regular flour, butter, cheese spread, cream, cream cheese, chocolate spreads, cocoa powder, breakfast cereals, cereal grains, cereal bars, cereal products (pancakes, waffles, stuffing, Yorkshire pudding), chocolate, coconut based desserts and products, corn/rice based snacks, crackers, cream, dairy desserts (custard, instant, fromage frais, mousse), dips (sweet & savoury), drinking chocolate, flour and flour products, free-from and vegan/plant cheeses, fondant icing, fruit bars, fudge, gelatine containing foods, gluten-free foods, gravy, herb/spice rubs and coatings, hummus, ice cream (dairy/non-dairy), ice lollies, icing/frosting, jelly, legumes/pulses (baked beans, lentils), lemon curd, liquorice, marshmallows/mallows, marzipan, milk based sauces, milkshake powders/syrups, mustard, nut spread, pasta/noodles, pesto, plant/vegetable spreads, popcorn, potato crisps, pretzels, pot noodles, puddings/desserts, rice, rice/oat cakes, soft cheese, sorbets, soups, stock cubes, sweets, tapenade, toffee, tofu, vegan meat/fish or egg alternatives, vegetable crisps, vegetable soups, yoghurt (dairy/non-dairy). • Note: yogurts, dairy desserts and coconut-based puddings with a protein content ≤0.5 g/100 g should be limited to 1 per day. • Low protein ACBS prescription products: Promin potato pots, Promin potato cakes, Taranis fish substitute.
(c) Commercial sauces and tabletop sauces containing protein >1 g/100 g and containing exchange ingredients	• Cook-in, pour-over or liquid sauces (curry, sweet & sour, tomato, vegetable), oil-based dressings (mayonnaise, salad cream, vinaigrette), table top sauces (brown, chilli, chutney, horseradish, mint, pickles, tartare, tomato ketchup). • Cake decorations/sprinkles.
(d) Soya sauces containing protein >1.5 g/100 g	• Most soya sauces have protein >1.5 g/100 g.

(a) Liquid plant milks and animal milks that contain protein >0.1 g/100 mL or specialist low protein milks with a phenylalanine content >5 mg/100 mL that have been approved by the UK ACBS and available on prescription for low protein diets. The upper protein/phenylalanine cut-off is set at a low amount due to potential high daily volumes that may be consumed.

(b) Manufactured foods containing protein >0.5 g/100 g or SLPF containing phenylalanine >25 mg/100 g and containing exchange ingredients. This group contained most of the manufactured foods. It also included some SLPF approved by the UK ACBS and available on prescription for low protein diets.

(c) Commercial sauces and tabletop sauces containing protein >1 g/100 g and exchange ingredients. This subcategory mainly consisted of commercial sauces containing vegetables, which have a lower phenylalanine content per 1 g of protein than cereals or animal foods [15]. This group also included cake decorations, as the amounts consumed are small.

(d) Soya sauces containing protein >1.5 g/100 g. This subcategory allowed soya sauce with a higher amount of protein because the amount consumed is small and only a few brands contained protein less than this amount.

Category 4: Fruit/vegetables containing phenylalanine >75 mg/100 g allocated as part of the protein/phenylalanine exchange system (Table 4). This included vegetable crisps prepared from exchange-free vegetables that had a higher phenylalanine content and were discussed in Evans et al. [11]. Potatoes have a lower phenylalanine content than 75 mg/100 g but are calculated/measured as exchange foods due to the amount consumed daily in the UK diet.

Table 4. Category 4: Exchange fruit and vegetables (phenylalanine >75 mg/100 g).

4. Exchange Fruit/Vegetables Containing Phenylalanine >75 mg/100 g Allocated as Part of the Protein Exchange System According to Their Phenylalanine/Protein Content per 100 g	
Fruit & Vegetables with phenylalanine content 75–99 mg/100 g	• Figs • Asparagus, bamboo shoots, beansprouts, broccoli, brussels sprouts, cauliflower, mange tout, sugar snap peas, whole hearts of palm. • A standard portion size of 60 g is used for 1 phenylalanine exchange.
Fruit & Vegetables with phenylalanine content >100 mg/100 g	• Passionfruit • Broad beans, chestnuts, choi sum, corn on the cob, kale, mixed vegetables, peas and petit pois, romanesco, rocket, spinach, spring greens, sweetcorn kernels, sweet potato fries with coating, vine leaves, yams. • Phenylalanine content is used to determine amount for 1 phenylalanine exchange.
Potatoes	• All potatoes and potato products.
Vegetable crisps	• All vegetable crisps (except cassava).

All statements were accepted and endorsed by the NSPKU. The statements were then translated into easier guidance for patients and carers [16]. A detailed list of all statements is provided in Supplementary Materials.

4. Discussion

This paper reports the results of four years of in-depth national discussions amongst experienced UK metabolic dietitians using Delphi methodology to gain consensus on the suitability and allocation of foods in a low phenylalanine diet, for patients tolerating a natural protein intake of ≤10 g/day. Having uniform national recommendations across all UK centres treating PKU should enable health professionals and support groups to provide consistent information to patients with PKU. Securing consensus amongst health professionals was challenging, but essential, as there were many differing opinions leading to disparate patient information and unfounded dietary practices. The Delphi methodology was systematic, impartial, and consistent, involving representation from all major UK PKU centres. All terms of reference were agreed in advance and an impartial facilitator ensured the process was conducted transparently and without bias [13]. Eighty-four percent (94/112) of statements received at least 90% agreement.

Historically in the UK, exceptionally low protein foods such as sugar, jam, honey, and vegetable oils have been permitted as exchange-free and there is no evidence to suggest this advice should change.

There was much discussion about the allocation of protein cut off points for different food groups and categorisation of foods/drinks within each group considering the impact on blood phenylalanine control if foods were not calculated/measured as part of the phenylalanine exchange system. It was clear that not all food subgroups could be considered in the same way, dependent on the weight of food that would be consumed and the role of each food within the diet. Most manufactured foods were defined as exchange-free only if they had a protein content ≤0.5 g/100 g of food. However, even within this subcategory, some foods required additional rules to prevent over consumption when protein content was close to the upper cut off point, and the amount consumed would exceed 100 g in one portion. This applied to dairy-free yoghurts and desserts based on plant milks that contained a protein content of around 0.5 g/100 g.

Since the 1960s, UK health professionals have given most fruits and vegetables (except potatoes) with a phenylalanine content ≤75 mg/100 g as exchange-free in a low phenylalanine diet. This guidance was reconsidered when developing the consensus statements, as evidence from a series of studies indicated that this maximum cut off did not adversely impact blood phenylalanine control in PKU [17–20]. This was also consistent with the 2017 PKU European Guidelines [21] and in turn, this influenced other consensus statements. For example, many cook-in/pour over sauces are primarily made from exchange-free vegetables and other exchange-free ingredients such as starches and seasonings only. It was therefore decided to calculate/measure vegetable sauces as part of the phenylalanine exchange system only if the sauce contained exchange ingredients (such as cream, flour) and a protein content >1 g/100 g. Soya sauce was considered exchange-free if the protein content was ≤1.5 g/100 g. A higher cut off point was given for soya sauce, as the amount used in recipes is generally small.

Plant milks and special milk replacements were a group that also required more specific definition of protein/phenylalanine cut off points due to potentially high daily volumes being consumed, thereby contributing a significant amount of protein/phenylalanine. A stringent exchange-free upper limit of ≤0.1 g protein/100 mL was set for regular and plant milks, and an upper limit of phenylalanine of ≤5 mg/100mL for low protein special milks available on ACBS prescription. This was because the volumes consumed may be high when taking this as a regular hot or cold drink, 'milk' shake or 'latte'. Only a small number of plant milks (some coconut milks) and low protein special milks (protein-free only) can therefore be given without calculation/measurement in the diet.

Currently, around 10% of SLPF contain more phenylalanine than the upper cut off point (≤25 mg/100 g) due to added ingredients such as milk, seeds, and rice flour [22]. It is hoped that these consensus statements will encourage manufacturers of SLPF to develop new low protein special foods with a phenylalanine content of ≤25 mg/100 g, so that they can be eaten without measurement or restriction. There seems to be little value in having SLPF that must still be limited and controlled within the diet. All SLPF should have clear labelling identifying their phenylalanine content (per 100 g of raw ingredients and per 100 g after preparation) with the full list of ingredients given, and any protein containing ingredients identified in bold on the ingredients list [23].

There were study strengths and limitations. Although dietitians represented both paediatric and adult care, they had differing opinions on the stringency of dietary guidance needed. Whilst this added to discussions, it also increased the challenge of producing statements that would meet the needs of the majority of the PKU population. Although all BIMDG dietitians had the opportunity to comment on each statement and vote in each round of the Delphi process, due to career changes or other circumstances such as maternity leave, the dietitians responding in each round were not necessarily the same each time. Even so, a representative from almost all major IMD specialty treatment centres (in both paediatric and adult care) was represented in each round. Overall, 100% consensus was reached for 64 statements. It was considered impractical to aim for 100% agreement for all statements, but a consensus cut off of 75% was agreed upon as it represented the majority of opinion. It may have been valuable to seek patient/carer opinion on each statement. Pragmatically, it was considered

important as a first step to gain dietetic consensus as this was considered a substantial barrier to consistency of care, before translating into practical guidance for patients.

It is acknowledged that internationally, different systems are used to calculate/measure protein/phenylalanine in the PKU diet, each with its own inherent weaknesses [4–9]. Using upper protein/phenylalanine cut-off points has the disadvantage of having to measure/calculate foods as part of an exchange system if they contain protein marginally over the cut-off, whilst eating foods as exchange-free if they are just under the cut-off point, but it does give direct guidance and allows many foods to be eaten without measurement or calculation. Overall, these statements are designed to provide broad guidance for dietitians with limited experience in treating PKU on how to safely manage individuals with PKU. It is also important that they understand the foundation for the statements. The proposed guidance should be used in conjunction with individual tailored advice considering patient food likes, aversions, and level of understanding.

The statements were designed to be used by dietitians and heath care professionals. However, since completion of the statements, the NSPKU medical advisory dietitians have developed a dietary information guide using the statements as a basis for practical advice [16]. The next step will be to evaluate the adherence to these statements by both health professionals and patients with PKU and their caregivers.

5. Conclusions

The development and publication of UK consensus statements for food labelling and protein allocation in the PKU diet is an important step in harmonising dietary advice and effecting consistency of care for patients with PKU. Developing these statements in partnership with BIMDG dietitians using Delphi methodology has ensured that all dietitians have had the opportunity to participate in the development in an impartial, transparent, and consistent process. In the UK, this is the first time that such extensive agreement has been reached amongst specialist dietitians, with the results being simplified and implemented into patient care. It is also hoped that these guidelines will be respected and adopted by manufacturers of SLPF to ensure patients with PKU can gain full advantage from consuming low protein foods in a low phenylalanine diet.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/8/2205/s1, Low protein labelling consensus statements for people with PKU on ≤10 g/day of natural protein.

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CHAPTER 4C: EFFICACY OF A NEW LOW PROTEIN MULTIMEDIA APP FOR PKU

nutrients

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Article

Efficacy of a New Low-Protein Multimedia Diet App for PKU

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Abstract: Patients with phenylketonuria (PKU) require a phenylalanine/protein-restricted diet, with limited food choice. Interpreting food labels, calculating protein intake, and determining food suitability are complex and confusing tasks. A mobile multi-media low-protein diet app was developed to guide food choice, label interpretation, and protein calculation. '*PKU Bite*'® includes >1100 specialist and regular low-protein foods, is colour-coded for suitability, and features a protein calculator. A 12-week randomised controlled trial assessed app efficacy, compared with written/pictorial material, in 60 parents/caregivers of children with PKU, aged 1–16 years, and 21 adolescents with PKU. Questionnaires examined self-efficacy and label-reading knowledge; food records evaluated natural-protein intake, compared with prescriptions. There was no difference between groups in label-reading knowledge or self-efficacy, but there was a trend for improved accuracy of dietary protein calculation, when using the app (baseline/12-weeks: app 35%/48%; control 39%/35%). Parents of children <10 years of age (median 5.5 years), were most likely to use the app to check the phenylalanine/protein content of a food or to verify suitability of foods. Whilst the app was popular (43%), so too was contacting the dietitian (43%), using written/pictorial information (24%), or using social media (18%). This is the first dietary app for PKU to be studied in a systematic way as well as validated by healthcare professionals. It is a useful adjunct to existing resources and will be a valuable tool for educating parents of younger children.

Keywords: phenylketonuria (PKU); app; multimedia; low-protein diet

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1. Introduction

Stringent dietary phenylalanine (Phe) restriction is an essential treatment strategy, to prevent severe neurological sequelae in children with phenylketonuria (PKU). This inborn error of metabolism is characterised by a deficiency of the enzyme phenylalanine hydroxylase, essential for the metabolism of the amino acid phenylalanine, leading to accumulation of Phe in the blood and brain. Phe is found in protein-containing foods, and protein intake is commonly reduced to <10 g/day (<500 mg Phe) in patients with classical PKU, to maintain metabolic control [1].

The amount of natural protein tolerated in PKU is dependent on the PKU phenotype. In the UK, protein is allocated in the form of an 'exchange' system (one exchange is the amount of food that provides one gram of protein, or 50 mg Phe for fruit and vegetables). This provides some flexibility in food choice. High-protein foods (e.g., meat, fish, eggs, cheese) and the artificial sweetener aspartame (high in Phe) are avoided, and plant-protein food sources are calculated accurately, to maintain satisfactory blood Phe control. Remaining protein requirements are provided by Phe-free L-amino acids or glycomacropeptide

(a low Phe peptide), which is supplemented with added amino acids. Energy is supplied by specially manufactured low-protein foods such as bread, pasta, and cereals [1], as well as regular foods that are naturally low in protein.

A Phe-restricted diet is complex, confusing, and, even, overwhelming. For example, whilst most foods contain 50 mg of Phe for each gram of protein, fruits and vegetables vary in their Phe content, and no Phe analysis is available for regular manufactured foods, although protein content is reported on food labels. European legislation states that manufacturers can declare foods as protein free, if they contain a threshold of $\leq$0.5 g/100 g [2]. Thereby, a food product may have a protein-containing ingredient listed, but the nutrition label states 0 g/100 g protein [3,4]. Ingredient lists on manufactured products can, also, include an extensive range of unusual additives or food ingredients. Novel and exotic foods and ingredients are continually introduced. Recently spirulina, a type of algae, has been used as an additive in sweets and smoothies to give a green colour, but it is high in protein. Powdered custards, dessert mixes, drinking chocolate, and milkshake powders regularly describe nutrient values, assuming they have been reconstituted with milk or egg, which are not permitted in a Phe-restricted diet; so, it is challenging to identify the protein content of the powder only [3]. Initiatives to lower the sugar content of manufactured foods have led to a higher use of aspartame [5]. In addition, some ingredients (e.g., spice mixes) used in small quantities may have high protein content, so uncertainty exists about their suitability. A recent survey of individuals with PKU or their caregivers reported that 31% found it difficult to calculate food protein exchanges from food labels, and 90% had experienced problems with food labelling in the previous six months [3].

There is substantial patient/caregiver demand for quick access to information about the suitability of foods for PKU. On social media (Facebook/Instagram), caregivers/patients discuss interpretation of protein on food labels and suitability for inclusion in a low-protein diet. Whilst this is a useful forum, with input from health professionals, sometimes inaccurate information may be shared and poor dietary practice perpetuated. Furthermore, professional written/pictorial information may be misplaced or inaccessible when required, prompting phone calls to dietitians for quick advice on individual food products and the suitability of a food.

Mobile media are commonly used to access dietary information, but current apps for PKU are often commercially developed, country specific, under researched, inattentive to food suitability, or not compatible with all devices [6]. No existing UK apps provide information on food suitability for PKU, and none have been validated by a healthcare organisation or certified by a recognised body. However, studies in other health and medical conditions have demonstrated that apps can improve adherence compared with traditional interventions, such as written educational information [7–17].

We have developed a UK mobile app, specifically written for PKU, accessible on iOS, Android, and web-based smart phones and tablets. The app contains a databank of 1100 specialist and regular low-protein foods/ingredients that might be consumed on a Phe-restricted diet. It was designed to inform patient/caregivers about food labelling interpretation, give information about food exchanges (protein/Phe), aid calculation of food protein exchanges, and delineate the suitability of each food item. The principles of the dietary information within the app are based on professional consensus, by UK Inherited Metabolic Disorder (IMD) dietitians utilising Delphi methodology [18,19].

The aim of this study was to investigate if the use of a mobile app in PKU was able to increase self-efficacy in dietary management, improve knowledge and interpretation of food labels, and improve the accuracy of patient/caregiver daily food protein calculations, when selecting fresh, manufactured, and specialised dietary products in a low-protein diet, compared with use of 'traditional' written dietary resources.

2. Materials and Methods

This was a 12-week randomised, controlled, parallel, intervention study. It aimed to determine if the use of a mobile application (available via any smart phone or tablet) by

caregivers of, and patients with PKU, who are following a strict low-protein diet, improved food label interpretation, knowledge of food suitability for inclusion in the diet, protein calculations, dietary adherence, and self-care, compared with control subjects who used written/pictorial information only. All subjects from both the app and control groups were then encouraged to download and use the app, and were followed up for a further six months. Figure 1 shows the app study design and procedures.

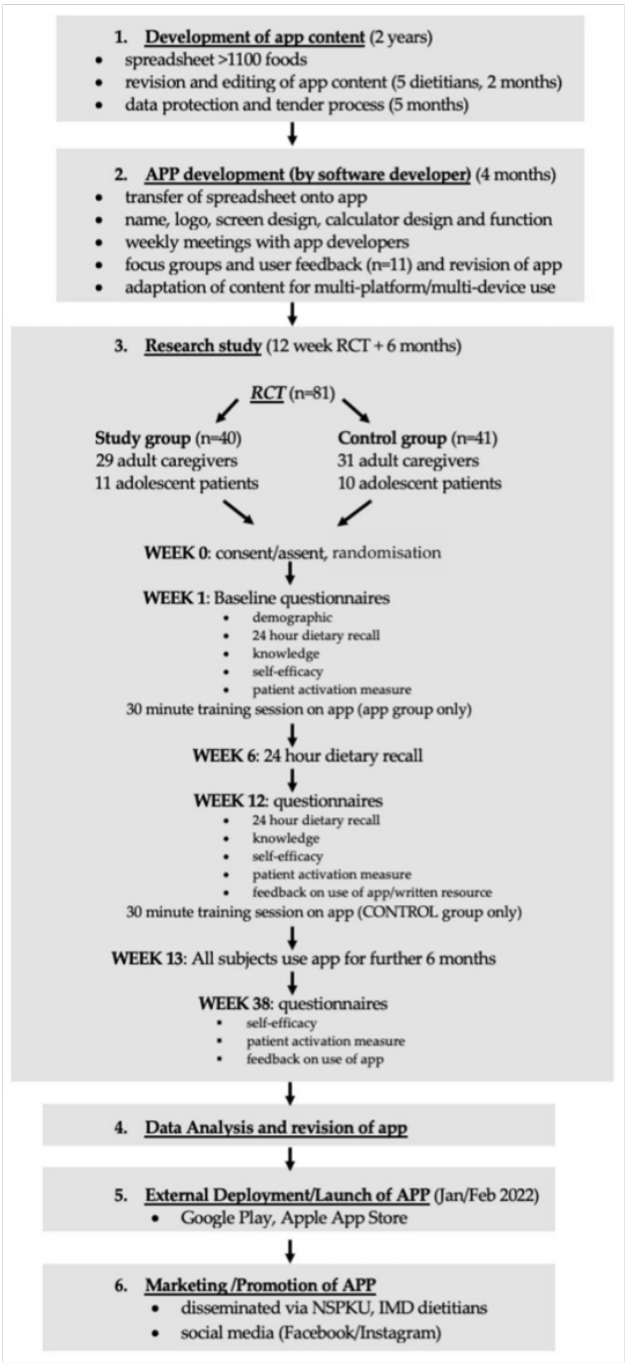

Figure 1. App study design and development procedures. (Abbreviations: RCT = Randomised Controlled Trial; NSPKU = National Society for Phenylketonuria; IMD = Inherited Metabolic Disorder).

2.1. App Development

2.1.1. App Content

A multimedia app, specifically for PKU, was developed by a team of 5 specialist IMD dietitians, in consultation with the software development company Imobisoft. App content for over 1100 specialist and regular low-protein foods/ingredients was developed over a 2-year period.

This included for every food:

- a general description of the food such as appearance and origins;
- suitability for inclusion in a low-protein diet (based on British Inherited Metabolic Disease Group (BIMDG) UK dietetic consensus statements [18,19], including colour coding (**green:** low-protein exchange-free foods; **orange:** exchange foods that contain protein/Phe and so should be calculated as part of the daily allowance; **red:** high-protein foods that are best avoided);
- menu ideas and food serving suggestions;
- exchange-free (low-protein) recipes;
- food preparation;
- food storage recommendations;
- food availability information, including details of home delivery companies that supply low-protein prescription items.

Foods were selected for their suitability for a low-protein diet, including specialist low-protein foods, foods naturally low in protein, and foods that are calculated as part of the daily protein allowance. Some high-protein foods were included with a warning that they were best avoided. All app data were cross-checked individually, by 3 IMD dietitians at least twice, and by 2 dietitians at least 5 times. Users could search by food name or food category. Food categories (n = 12) included:

- all special low-protein prescription items;
- plant foods (e.g., fruits and vegetables, whether fresh, frozen, canned, or dried);
- cheese, yogurt, cream, and alternatives (e.g., regular, low-protein, or vegan cheeses, yoghurts, cream);
- savoury foods/meat alternatives (e.g., low-protein or plant burgers, sausages, soups, legumes, plant-based meat alternatives);
- flours, pasta, and cereals (e.g., breads, bread products, and pastries, including gluten-free varieties);
- fats, oils, sauces, dips, and spices (e.g., butter, margarine, oils, gravy, ketchup, dressings, marinades);
- cakes, biscuits, and desserts (e.g., plant and milk-based ice-cream, custards, jellies, puddings);
- drinks (e.g., fizzy drinks, squash, juices, smoothies, milk, plant milks, hot chocolate, milkshake powders);
- baking ingredients (e.g., sugar, baking powder, cake decorations, colourings, flavourings);
- sweet spreads, sweets, and syrups (e.g., chocolate, sweets, chewing gum, jams, honey, chocolate spread);
- snack foods (e.g., crisps, crackers, ice lollies);
- food label ingredients (e.g., food additives).

The app also included a protein exchange calculator, for calculating the protein content of a food per 100 g or per portion, using information from food labels.

Any foods, food groups, or ingredients that may contain aspartame were identified with a red warning triangle with an exclamation mark and the words “aspartame warning”. In addition, there was a filter function to enable all possible aspartame-containing foods/products in the app to be listed.

The app was written in the English language only.

2.1.2. App Design

The app was designed and developed over 4 months, which included choice of name ('*PKU Bite*'®), logo design (Figure 2), background, and page designs. During this development phase, the app design was discussed with user focus groups, including parents of children with PKU (n = 7) and children with PKU (n = 4), to collect feedback on usability and to direct development.

Figure 2. '*PKU Bite*'® logo.

2.2. Randomised Controlled Trial

2.2.1. Subjects

Eighty-one patients aged 10–16 years, or caregivers of children aged 1–16 years, on low-protein diets for PKU were recruited (40 study and 41 control subjects). Subjects were recruited from one specialist IMD centre (Birmingham Women's and Children's NHS Foundation Trust). Subjects were excluded, if the child with PKU had co-morbidities (e.g., cancer, inflammatory bowel disease, diabetes), or was following a special diet for any other medical reason. All subjects were well established on their Phe-restricted diet.

2.2.2. Randomisation

Individual subjects or families were randomised, by computer-generated random number block sequences (blocks of 2 and 4), to use the app, called '*PKU Bite*'® (study group), or to use written/pictorial resources (control group) only.

2.2.3. App Group

Study group subjects were sent a trial download of the '*PKU Bite*'® app for their phone/tablet and attended a hands-on 30 min training session, to explain how to download the trial app and use the app functions. A copy of the teaching slides was given for reference. Subjects were asked to use the app for 12 weeks, as a source of information for food suitability, and to use the exchange calculator to determine the appropriate portion size for any protein-containing foods searched. They could use the recipes, menu ideas, preparation, storage, and availability information, as required. Other non-app sources of dietary information available to this group included: direct contact with an IMD dietitian, any previously distributed written/pictorial information, social media (e.g., Facebook/Instagram), and the NSPKU (National Society for PKU) webpage.

2.2.4. Control Group

Control group subjects were issued with an NSPKU exchange calculator card, dietary information book, and written pictorial product information booklets, produced by the IMD dietetic team. This included pictorial guides on: the basic PKU diet; food exchanges; low-protein fruits and vegetables; ice lollies, ice-creams, and sorbets; low-protein cheese; low-protein baking and desserts; plant milks, creams, yoghurts, and desserts; low-protein sweets; and savoury low-protein alternatives. This information was comparable to the data on the app. They, also, had direct access to an IMD dietitian and information from social media. They used these resources as a reference for 12 weeks for information on food suitability, protein exchange amounts, and menu ideas.

2.2.5. Demographic Questionnaire

At baseline, all subjects completed a demographic questionnaire, describing parent/child age, gender, ethnicity, parent educational level, frequency of use of social media, any apps currently used, and other dietary resources.

2.2.6. Knowledge Questionnaire

A 22-item multiple choice non-validated questionnaire on low-protein labelling knowledge and interpretation (see Supplementary File S1) was completed, by all subjects at baseline and after 12 weeks of using the app (app group) or written educational material (control group). The questionnaire was pilot tested with a user group of adolescent patients with PKU or their parents and IMD dietitians, prior to the study. It included pictures of 22 manufactured foods, information on their protein content, and questions about whether these products could be included in a Phe-restricted diet. For example, how many 1 g protein exchanges would a specified weight of a food product contain, or how much of a food product could be eaten for 1 exchange, when the protein content per 100 g was given. Information given on the app or in the control written information supported subjects in answering these questions.

2.2.7. Self-Efficacy Questionnaire

This validated 8-item questionnaire measured how confident subjects were with managing different aspects of their own, or their child's PKU health care, on a 10-point Likert scale (not confident = 1, to totally confident = 10) [20]. This included questions such as "I can choose appropriate foods [for my child] to eat when hungry" and "I know what to do when [my/my child's] blood Phe level goes higher or lower than it should be". This was completed by all subjects at baseline and at 12 weeks.

2.2.8. Patient Activation Measure

This validated 13-item questionnaire, with a 4-point Likert scale (strongly disagree to strongly agree) [21], was completed by all subjects at baseline and at 12 weeks. Similar to the self-efficacy questionnaire, this also measured confidence with PKU self/child-care management. Examples of questions included: "I am confident I can take action to minimise symptoms/problems associated with [my/my child's] PKU", "I am confident I can maintain lifestyle changes like diet even during times of stress", and "I understand the nature and cause of PKU".

2.2.9. Feedback Questionnaire

A non-validated feedback questionnaire on subject frequency and reasons for use of the app, written information, and other resources used during the study period was completed at 12 weeks.

2.2.10. Natural Protein Intake

A 24 h dietary recall was completed at baseline, 6 weeks, and 12 weeks. Subjects (caregivers and patients) were asked to record the foods they calculated/measured, as part of their protein prescription, and the number of food exchanges (1 g protein/50 mg Phe) allocated for each item. An IMD dietitian then checked the number of food exchanges actually eaten, compared with prescribed amounts, and documented any calculation errors.

2.2.11. Metabolic Control

All routine weekly blood Phe results were recorded, for 6 weeks prior to study commencement and throughout the study period. Mean blood Phe results were then calculated for each child at baseline, 12 weeks, and 6 months.

2.3. Six Month Follow Up

At the end of the 12-week randomised controlled trial (RCT), control subjects were sent a download of the '*PKU Bite*'® app for their phone/tablet, and they attended a 30 min training session on the app. Both groups (app and control), then, used the app for a further 6 months, completing the self-efficacy, patient activation measure, and feedback questionnaires on completion. Routine weekly blood Phe results were, also, recorded.

2.4. Statistics

From a hospital patient population of approximately 120 children with PKU, a sample size of 80 (40 study, 40 controls) had 80% power to detect a 30% improvement in test scores for protein calculation in the study group (using the app), compared with the control group (not using the app), with a significance level of 0.05 (two-tailed). Continuous data are summarised as median (IQR), and categorical data are summarised as frequencies of counts, with associated percentages. Comparisons of outcome data between treatment groups or timepoints were performed using Wilcox tests.

2.5. Ethical Approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and a favourable ethical opinion was obtained from the West Midlands–Edgbaston National Research Ethics Service (NRES) Committee (REC reference: 20/WM/0010 and IRAS ID: 260370). Written informed consent was obtained from the parent/carer of all children, as was assent from the children where appropriate, according to level of understanding.

3. Results

3.1. Subjects

Table 1 describes participant characteristics. There was no significant difference between the demographic characteristics of the two groups, except for control group mothers, who had a lower median level of education compared with the app group mothers (2.0 vs. 3.0; $p = 0.04$ Mann–Whitney). Six of the control group parents/carers (all mothers) and three of the children (two female) declined to do the app training, so did not complete the final six months using the app. Reasons for withdrawal included: disengagement with technology +/− adolescent fixed-eating habits ($n = 3$); patient transfer to an adult hospital ($n = 2$); extended holiday ($n = 1$); family social issues ($n = 1$); and patient psychological/psychiatric issues ($n = 2$). Sensitivity analyses, which restrict the patient population only to those patients who remained in the study, were performed. These had no meaningful impact on any of the study's interpretations (data not shown).

Table 1. Characteristics of subjects.

	App Group	Control Group
Number of child subjects (gender)	11 (6 F, 5 M)	10 (8 F, 2 M)
Median age of child subjects (years) (IQR)	12.0 (10.5–13.0)	12.7 (12.0–14.0)
Number of parent/carer subjects (gender)	29 (18 mothers, 10 fathers, 1 grandmother)	31 (24 mothers, 5 fathers, 2 grandmothers)
Median age of mothers (years) (IQR)	36.5 (31.8–39.3)	35.5 (31.3–41.0)
Median age of fathers (years) (IQR)	39.0 (35.5–44.5)	38.0 (30.0–44.0)
Mother's highest educational qualification # median (IQR)	3.0 (2.0–6.5)	2.0 (2.0–3.0)
Father's highest educational qualification # median (IQR)	5.0 (2.0–6.0)	4.0 (2.0–5.0)
Total number of children (including children of parent/carer participants) (gender)	21 (10 F, 11 M)	25 (15 F, 10 M)

Table 1. *Cont.*

	App Group	Control Group
Median age of all children (including children of parent/carer participants) (years) (IQR)	10.0 (5.5–12.5)	9.0 (2.5–12.0)
Ethnicity of all children	19 White/European 1 Asian 1 mixed race	20 White/European 4 Asian 1 mixed race

IQR interquartile range; M male, F female; # Educational levels were recorded as qualification levels 0–8, as described on gov.uk (accessed on 19 April 2018) [22] (0 = no qualifications, 8 = Ph.D.).

3.2. Baseline Use of Apps and Sources of Dietary Information

The top five apps, used by more than 50% of the children, were YouTube, TikTok, Snapchat, WhatsApp, and Instagram, whilst for parents/carers they were WhatsApp, Facebook, Twitter, YouTube, and Messenger. Parents used more apps than children. There was no significant difference between the groups for the total number of apps used, overall, weekly, or daily (Table 2). Parents/carers used a median of 36 different apps, 10 weekly and 5 daily; whilst children used a median of 30 apps, 7 weekly and 4 daily. However, there was wide variation in app use within the groups.

For parents/carers at baseline, written/pictorial dietary information, contacting the dietitian by phone or email, and social media (Twitter/Facebook) were the most common (>50% of subjects) sources of low-protein diet information (Table 2). For children, the most common source was the dietitian, followed by written/pictorial information, and nearly one quarter reported that they relied on their parent(s). There was no significant difference between parent/carer or child app and control groups, for the source(s) of low-protein dietary information used at baseline.

Table 2. Baseline use of apps and dietary information.

	Parent/Carer			Child		
Number of Apps Used	**App Group *n* = 29 Median (IQR)**	**Control Group *n* = 31 Median (IQR)**	**Total *n* = 60 Median (IQR)**	**App Group *n* = 11 Median (IQR)**	**Control Group *n* = 10 Median (IQR)**	**Total *n* = 21 Median (IQR)**
Total no. apps used per participant	40 (25–100)	30 (13–61)	36 (18–74)	30 (7–79)	30 (19–47)	30 (10–60)
No. apps used/day per participant	5.5 (3–15)	4 (3–7)	5 (3–10)	4 (1–6)	4.5 (4–6)	4 (3–6)
No. apps used/week per participant	11 (6–25)	8 (6–14)	10 (6–19)	7 (3–12)	7 (4–10)	7 (4–10)
Sources of dietary information % of subjects (*n*)						
Written info	86 (25)	81 (25)	83 (50)	36 (4)	60 (6)	48 (10)
Phone/email dietitian	83 (24)	71 (22)	77 (46)	55 (6)	80 (8)	67 (14)
Social media	69 (20)	61 (19)	65 (39)	18 (2)	40 (4)	29 (6)
Clinic/home visit dietitian	55 (16)	39 (12)	47 (28)	45 (5)	30 (3)	38 (8)
Ask others with PKU	21 (6)	23 (7)	22 (13)	9 (1)	10 (1)	10 (2)
Child's parent(s)	N/A	N/A	N/A	18 (2)	30 (3)	24 (5)

3.3. Frequency of Using the 'PKU Bite'® App at Twelve Weeks and Six Months Follow Up

The app was used at least once a week, by 58% (n = 11) of female and 40% (n = 4) of male parent/carers in the app group at 12 weeks, and 53% (n = 10) of females and 60% (n = 6) of males at 6 months (Table 3). In the control group, at 6 months (after using the app) 46% (n = 12) of females and 60% (n = 3) of males used the app at least weekly. Amongst children, only a few used the app regularly: at 12 weeks, in the app group, 60% (n = 3) of

male and 17% (n = 1) of female children used the app at least weekly, but by six months only one male and one female used it weekly, whilst two female control children used the app weekly.

When the parents/carers were divided into app users (used the app at least once a week at six months; n = 31) and app non-users (rarely/never used the app at six months; n = 29), there was a significant difference in the age of their children (median age (IQR): users 5.5 years (1.3–10) vs. non users 10.0 years (6–13); p = 0.002 Wilcoxon signed rank), suggesting that the app was more likely to be used by parents/carers with children under the age of 10 years.

Table 3. Frequency of using app.

	App Group 12 Weeks		**App Group 6 Months**		**Control Group 6 Months**	
	Parent (n = 29)	**Child (n = 11)**	**Parent (n = 29)**	**Child (n = 11)**	**Parent (n = 25)**	**Child (n = 7)**
Never	5 (2 M, 3 F)	3 (1 M, 2 F)	6 (3 M, 3 F)	3 (1 M, 2 F)	5 (1 M, 4 F)	4 (1 M, 3 F)
Rarely	8 (4 M, 4 F)	4 (1 M, 3 F)	7 (1 M, 6 F)	6 (3 M, 3 F)	5 (1 M, 4 F)	1 (1 F)
Once a week	8 (2 M, 6 F)	2 (1 M, 1 F)	9 (5 M, 4 F)	0	3 (2 M, 1 F)	2 (2 F)
Two–six times per week	4 (2 M, 2 F)	1 (1 M)	7 (1 M, 6 F)	2 (1 M, 1 F)	9 (1 M, 8 F)	0
Once a day	3 (3 F)	1 (1 M)	0	0	1 (1 F)	0
>Once a day	0	0	0	0	2 (2 F)	0
No response	1 (1 F)	0	0	0	0	0

M male, F female.

3.4. Frequency of Seeking Dietary Assistance at Baseline, Twelve Weeks, and Six Months

3.4.1. Between Group Differences

At baseline, more control group parents/carers reported using written dietary information at least weekly than the app group (carers: 71% vs. 34%), but this was not statistically significant at 12 weeks or 6 months (Table 3). More control children reported contacting the dietitian at least weekly than the app group at baseline (50% vs. 9%) and at 12 weeks (30% vs. 0%).

3.4.2. Within Group Changes

During the study, there were some reported changes in the frequency of dietary resources used within groups (Table 4). Fewer app group parent/carers used social media at least weekly at 12 weeks and 6 months, compared to baseline (41% vs. 72%; p = 0.0003; 52% vs. 72%; p = 0.01). Fewer control group parent/carers reported contacting the dietitian and using social media and written information at least weekly at six months than at baseline (dietitian: 28% vs. 42%; p = 0.06) (social media: 40% vs. 65%; p = 0.001) (written information: 52% vs. 71%; p = 0.0009).

Table 4. Percentage of respondents accessing different sources of dietary information at least weekly at baseline, 12 weeks, and 6 months.

	Parent/Carer Baseline		**Parent/Carer 12 Weeks**		**Parent/Carer 6 Months**		**Child Baseline**		**Child 12 Weeks**		**Child 6 Months**	
	App Group n = 29	**Control Group n = 31**	**App Group n = 29**	**Control Group n = 31**	**App Group n = 29**	**Control Group n = 25**	**App Group n = 11**	**Control Group n = 10**	**App Group n = 11**	**Control Group n = 10**	**App Group n = 11**	**Control Group n = 7**
Phone/text/ email dietitian % (n)	45 (13)	42 ** (13)	38 (11)	45 (14)	45 (13)	28 ** (8)	9 (1)	50 (5)	0 (0)	30 (3)	18 (2)	43 (3)
p-value	0.90		0.41		0.10		0.05		0.01		0.11	

Table 4. *Cont.*

	Parent/Carer Baseline		Parent/Carer 12 Weeks		Parent/Carer 6 Months		Child Baseline		Child 12 Weeks		Child 6 Months	
	App Group *n* = 29	Control Group *n* = 31	App Group *n* = 29	Control Group *n* = 31	App Group *n* = 29	Control Group *n* = 25	App Group *n* = 11	Control Group *n* = 10	App Group *n* = 11	Control Group *n* = 10	App Group *n* = 11	Control Group *n* = 7
Social media e.g., Facebook/ Twitter % (*n*)	72 $^{*,\#}$ (21)	65 $^{++}$ (20)	41 * (12)	55 (17)	52 $^{\#}$ (15)	40 $^{++}$ (10)	18 (2)	60 (6)	27 (3)	10 (1)	18 (2)	14 (1)
p-value	0.54		0.30		0.22		0.45		0.48		0.99	
Written information % (*n*)	34 (10)	71 § (22)	38 (11)	52 (16)	28 (8)	52 § (13)	0 (0)	50 (5)	9 (1)	0 (0)	9 (1)	14 (1)
p-value	0.004		0.22		0.09		0.06		0.03		0.23	

p = Wilcoxon signed rank; * $p = 0.0003$; $^{\#}$ $p = 0.01$; ** $p = 0.06$; $^{++}$ $p = 0.001$; § $p = 0.0009$.

There were no statistically significant changes for either app or control group children during the study, in the reported frequency of using any dietary resources, however, subject numbers were small.

3.5. Self Confidence in Managing PKU

Throughout the study, there were only small changes in self-confidence with managing PKU for parents/carers or children in either group, when measured by self-efficacy or the patient-activation measure (Table 5).

Self-efficacy: children in the app group had more self-efficacy than controls after six months (median score 8.20 vs. 6.65; $p = 0.01$), but this was, also, the case at baseline (7.95 vs. 6.35; $p = 0.02$), suggesting there was no real change (Table 5). App group children were less self-confident at 12 weeks compared to baseline, but improved after 6 months on the app, although results were not significant. Similarly, app group carers had no significant change in self-confidence. However, whilst app group carers were more confident at baseline than controls (median 8.85 vs. 8.20; $p = 0.02$), at six months control carers improved significantly compared with baseline (8.20 vs. 9.20; $p = 0.02$), suggesting control carers had improved self-efficacy. Whilst the six carers who did not complete the six-month questionnaires may have skewed the baseline data (based on qualification levels of 0–3 [22]), when their data was omitted, results did not differ. When all parents/carers (from both app and control groups) were divided into users (used the app at least once a week at six months; $n = 31$) and non-users (rarely/never used the app at six months; $n = 29$), there was no significant difference in self-efficacy scores at baseline and six months, either within or between groups.

Patient activation measure: control group carers were more self-confident after six months of using the app, compared with baseline (carers: mean score 3.60 vs. 3.70; $p = 0.009$) (Table 5). Similarly, compared with baseline, carers in the app group showed improved confidence after 12 weeks of using the app and after 6 months (3.70 vs. 3.60; $p = 0.02$), but there was no significant change for children. However, as with the self-efficacy questionnaire, app group children were significantly more confident at six months compared with controls (3.20 vs. 2.90; $p < 0.0001$). Control children were also less confident at six months than at baseline (3.10 vs. 2.90; $p = 0.01$). Patient-activation scores for app users versus non-users showed no significant difference between groups at baseline and six months, but app users did improve from baseline to six months (3.5 vs. 3.8; $p = 0.03$).

Table 5. Self-efficacy and patient activation measure mean scores for app and control groups at baseline, 12 weeks, and 6 months.

	Baseline Median (IQR)	12 Weeks Median (IQR)	6 Months Median (IQR)	*p*-Value * (Baseline vs. 6 Months)
	Self-efficacy questionnaire (1 = not confident, 10 = confident)			
App group parent	8.85 (8.6–9.1)	8.70 (8.4–9.1)	9.00 (8.7–9.2)	0.38
Control group parent	8.20 (7.9–8.5)	8.65 (8.2–8.9)	9.20 (9.1–9.3)	0.02
***p*-value ***	**0.02**	**0.70**	**0.11**	
App group child	7.95 (7.7–8.5)	7.60 (6.8–8.4)	8.20 (7.9–8.5)	0.16
Control group child	6.35 (5.6–7.8)	7.05 (6.4–7.5)	6.65 (5.6–7.8)	0.37
***p*-value ***	**0.02**	**0.17**	**0.01**	
App users (parents/carers from app and control groups, who used app ≥weekly)	9.00 (8.3–9.5)	NA	9.10 (8.3–9.8)	0.08
App nonusers (parents/carers from app and control groups, who rarely/never used app)	8.60 (7.7–9.8)	NA	9.45 (8.4–10.0)	0.10
***p*-value ***	**0.75**		**0.51**	
	Patient activation measure (1 = strongly disagree, 4= strongly agree)			
App group parent	3.60 (3.4–3.8)	3.60 (3.5–3.8)	3.70 (3.5–3.8)	0.02
Control group parent	3.60 (3.4–3.7)	3.60 (3.4–3.7)	3.70 (3.6–3.8)	0.009
***p*-value ***	**0.93**	**0.39**	**0.42**	
App group child	3.30 (3.1–3.3)	3.10 (3.0–3.2)	3.20 (3.2–3.4)	0.35
Control group child	3.10 (3.0–3.3)	3.20 (2.9–3.3)	2.90 (2.7–3.1)	0.01
***p*-value ***	**0.11**	**0.87**	**<0.0001**	
App users (parents/carers from app and control groups, who used app ≥weekly at 6 months)	3.50 (3.3–3.8)	NA	3.80 (3.5–4.0)	0.03
App nonusers (parents/carers from app and control groups, who rarely/never used app at 6 months)	3.70 (3.2–3.9)	NA	3.50 (3.2–4.0)	0.48
***p*-value ***	**0.76**		**0.24**	

* Wilcoxon signed rank; NA not applicable.

3.6. Knowledge and Interpretation of Low-Protein Labelling

There was no significant difference between or within groups for knowledge and interpretation of low-protein labelling, at baseline or 12 weeks, for children or carers (Table 6). When the app group parents/carers were divided into those who used the app regularly (≥weekly) at 12 weeks (n = 16) and those who rarely or never used the app (n = 13) at 12 weeks, there was a non-significant trend toward improved knowledge between baseline and 12 weeks for users, with higher scores than nonusers.

Table 6. Mean percentage of correct answers on protein-labelling knowledge/interpretation questionnaire, by all respondents.

	Baseline Mean% (SD)	12 Weeks Mean% (SD)
App group parent	65.4 (18.0)	66.7 (15.9)
Control group parent	61.4 (14.8)	60.2 (17.3)
App group child	52.1 (22.1)	49.3 (15.3)
Control group child	48.1 (13.7)	47.7 (14.3)
App users (parents/carers from app group, who used app ≥weekly at 12 weeks)	64.9 (18.3)	69.0 (14.1)
App nonusers (parents/carers from app group, who rarely/never used app at 12 weeks)	66.0 (18.4)	63.9 (18.0)

3.7. Accuracy of Calculating Daily Natural Protein Intake

There was a trend toward improvement in ability to calculate protein intake, for those subjects using the app. Both parents/carers and children using the app made fewer calculation errors at 6 and 12 weeks compared to baseline, and at 12 weeks there were more subjects making no errors (Table 7). In the control group, there was some improvement at 12 weeks, but not at 6 weeks for the parents/carers, and there was no change in errors throughout the 12-week study period for the control children. However, no differences reached statistical significance. When the app group was divided into users (used the app at least once a week at 12 weeks; $n = 16$) and nonusers (rarely/never used the app at 12 weeks; $n = 13$), there were no significant differences in the percentage of incorrectly calculated items, but both groups improved from baseline to 12 weeks, and both groups had more subjects correctly calculating all of their protein exchanges.

Table 7. Accuracy of calculating protein intake from diet diaries.

		Baseline Mean (SD)	6 Weeks Mean (SD)	12 Weeks Mean (SD)	*p* Value (Baseline vs. 12 Weeks)
App group Parent/carer $n = 29$	% of incorrectly calculated food items *	20.6 (20.9)	15.5 (18.0)	15.5 (18.9)	0.18
	No. of subjects correctly calculating all protein intake	11	9	19	
Control group Parent/carer $n = 31$	% of incorrectly calculated food items *	21.4 (27.4)	24.4 (22.5)	11.5 (19.5)	0.32
	No. of subjects correctly calculating all protein intake	14	7	10	
***p* value (% incorrect app group vs. control group)**		**0.73**	**0.12**	**0.72**	
App group Child $n = 11$	% of incorrectly calculated food items *	28.2 (32.5)	15.9 (20.5)	11.5 (18.2)	0.09
	No. of subjects correctly calculating all protein intake	3	3	4	
Control group Child $n = 10$	% of incorrectly calculated food items *	31.6 (32.8)	40.8 (36.6)	34.3 (39.4)	0.88
	No. of subjects correctly calculating all protein intake	2	0	1	
***p* value (% incorrect app group vs. control group)**		**0.61**	**0.09**	**0.18**	
App users (parents/carers from app group, who used app ≥weekly at 12 weeks) $n = 16$	% of incorrectly calculated food items *	18.2 (21.3)	14.2 (19.2)	16.6 (21.5)	0.97
	No. of subjects correctly calculating all protein intake	6	5	12	
App non-users (parents/carers from app group, who rarely/never used app at 12 weeks) $n = 13$	% of incorrectly calculated food items *	23.7 (29.9)	17.0 (17.0)	14.3 (16.3)	0.13
	No. of subjects correctly calculating all protein intake	5	4	7	
***p* value (% incorrect app users vs. non users)**		**0.54**	**0.61**	**0.89**	

* foods where protein content has not been calculated or has been incorrectly calculated—either over or under the true amount.

3.8. Metabolic Control

There was no significant difference between or within the app and control groups, for mean blood Phe levels at baseline, 12 weeks, or 6 months (Table 8), and all median levels were within the recommended target range of 120–360 µmol/L (<12 years) or 120–600 µmol/L (>12 years), indicating that the children had acceptable blood Phe control. However, there was a trend toward lower mean blood Phe levels at 12 weeks compared to baseline, in both groups, possibly associated with increased attention to diet in the initial weeks of the study.

Table 8. Median blood Phe levels at baseline, 12 weeks, and 6 months.

	Baseline Median (IQR)	**12 Weeks Median (IQR)**	**6 Months Median (IQR)**	***p* Value (Baseline vs. 12 Weeks)**	***p* Value (Baseline vs. 6 Months)**
App group children *	288 (222–376)	260 (207–397)	315 (232–428)	0.58	0.25
Control group children *	287 (174–338)	221 (198–403)	246 (199–347)	0.59	0.10
***p* value**	**0.50**	**0.52**	**0.22**		

* includes child subjects and children of parent/carer subjects.

3.9. Participant Feedback

Responses about the most useful features of the app are given in Table 9. Seeking advice on food suitability was a common use for the app, as well as looking up the protein/Phe content of a food, or the number of exchanges in a portion or per 100 g of a food. It was also noted to be helpful for accessing diet information quickly.

Table 9. Most useful features about the app.

	App Group 12 Weeks		**App Group 6 Months**		**Control Group 6 Months**	
	Parent % (*n* = 29)	**Child % (*n* = 11)**	**Parent % (*n* = 29)**	**Child % (*n* = 11)**	**Parent % (*n* = 25)**	**Child % (*n* = 7)**
Able to look up the number of exchanges in a food	52 (15)	55 (6)	48 (14)	55 (6)	44 (11)	29 (2)
Able to look up protein/Phe content of a food	48 (14)	27 (3)	62 (18)	36 (4)	52 (13)	43 (3)
Able to get advice on suitability of foods	41 (12)	0	52 (15)	18 (2)	52 (13)	0
Quick and easy to access diet information immediately when needed	28 (8)	27 (3)	41 (12)	27 (3)	44 (11)	0
Able to calculate daily exchanges	17 (5)	9 (1)	28 (8)	27 (3)	28 (7)	0
Able to look up guidelines/rules about certain foods	24 (7)	0	28 (8)	18 (2)	20 (5)	0
Able to get advice on how to use foods/ingredients in a low-protein diet	17 (5)	9 (1)	31 (9)	18 (2)	8 (2)	14 (1)
Able to get advice on how to cook/prepare a food	14 (4)	18 (2)	21 (6)	18 (2)	8 (2)	14 (1)
Do not need to contact the dietitian as often	14 (4)	0	14 (4)	18 (2)	16 (4)	0

When subjects were asked for their preferred method of obtaining diet information, whilst the app was popular (43%) across all subjects, so too was contacting the dietitian (43%), followed by written information (24%) and social media (18%). Children were less likely than carers to use any of the methods, but contacting the dietitian was rated the highest (Table 10). Some respondents used a combination of different resources.

At the end of the study, subjects were asked how satisfied they were with the app. Overall, 51% percent (n = 37) were very or extremely satisfied, 21% (n = 15) were satisfied, and 8% (n = 6) were unsatisfied or very unsatisfied (19%, n = 14, did not respond).

There was a lot of positive feedback about the app and the functions that people found particularly helpful, such as the calculator and the mobile nature of the app (Table 11). Comments on what did not work well mostly related to being unable to find a specific food, a desire for more branded and individual food items, or difficulties with the calculator or technology in general. Some teenagers or their carers reported being 'set in' their dietary habits, so did not vary their diet much, meaning the app was not required.

Table 10. Preferred method of obtaining diet information.

	App Group 6 Months		Control Group 6 Months		Total 6 Months	Direct Quotes
	Parent % (*n* = 29)	**Child % (*n* = 11)**	**Parent % (*n* = 25)**	**Child % (*n* = 7)**	**Total % (*n* = 72)**	
App	45 (13)	27 (3)	48 (12)	43 (3)	43 (31)	*App preferred because it's mobile and always with me (much easier than the sheets of paper). App is more discrete* [mother of teenager] *I think it will be really useful when she's older* [mother of toddler]
Dietitian (phone/email)	38 (11)	18 (2)	52 (13)	71 (5)	43 (31)	*I don't think the app will ever be able to replace contacting our dietitian who has so much knowledge and experience but it's definitely very handy and convenient when dietitian is unavailable or when you just want to double check things to be safe* [mother] *I would rather speak to someone re diet* [mother]
Written/pictorial information	28 (8)	9 (1)	28 (7)	14 (1)	24 (17)	*I like the books when they arrive; they make me want to look at them and try new foods. It gives me ideas and my family always try to get me to eat different foods. I tend to stick to the same foods that I like. I don't like change!* [teenage girl] *I just kept going back to using a calculator and looking in my written paper work for clarity on things, but probably would use the app more if away from home or on holiday* [father of teenager]
Social media	21 (6)	9 (1)	24 (6)	0	18 (13)	*Find that because I am used to using social media that is where I still tend to go back to first* [mother of teenager] *Currently the app does not list branded food items (like on Twitter/written information) so cannot replace those yet* [mother]
Reading food labels	3 (1)	18 (2)	0	0	4 (3)	*It has helped but I have still used other ways of getting info* [mother] *Use a mixture of all* [mother]
Others with PKU	10 (3)	0	8 (2)	0	7 (5)	
Mum	0	18 (2)	4 (1) *	14 (1)	6 (4)	
NSPKU	0	0	4 (1)	0	4 (3)	

Note: some respondents gave more than one choice; * grandparent.

When subjects were asked about additional app functions for the future, common themes were: an exchange tracker, a barcode/label scanner, more recipes and menu ideas, and a bigger range of branded foods (Table 11).

Table 11. Feedback about '*PKU Bite*'® app.

App Feedback	Responses	Examples of Direct Quotes
Positive feedback on existing functions: • mobile nature of app/useful for shopping • like calculator • other	5% (n = 4) 4% (n = 3) 2% (n = 2)	• *Liked green/red/orange colours-found this very helpful* [mother] • *I loved the portion size option on the protein exchange calculator. I used this the most* [mother] • *Very easy to use; calculator easier than the card* [mother] • *Easier to use app when shopping* [mother] • *It was useful out and about not carrying the food booklets* [teenage boy] • *Mobile and quick* [grandmother]
Difficulties with app: • finding foods • want more branded items • using calculator	9% (n = 7) 11% (n = 9) 4% (n = 3)	• *Sometimes I would search up a food and it wouldn't come up, but when I went through the categories I found it* [teenage girl] • *More information on everyday foods e.g., crisps, more supermarket foods* [mother] • *Would like to see foods included like 'McDonalds' and other restaurants* [mother] • *Calculator was confusing at first but got used to it* [mother of teenager]
Reasons for not using the app: • not into technology • set in ways/unchanging diet	2% (n = 2) 9% (n = 7)	• *I am very stuck in my ways and not great with technology* [father of teenager] • *I am used to calculating diet and my son does not change much what he eats* [mother of teenager] • *I know what to eat and I don't change what I eat* [teenage boy] • *I can imagine it would be better for new families; I can definitely see it would help people* [teenage boy]
Ideas for additional functions: • Exchange tracker • Label scanner • More recipes • Other ideas	11% (n = 9) 9% (n = 7) 7% (n = 6) 10% (n = 8)	• *Meal tracker function. Multiple users log in to the same account-sharing information across family members* [mother] • *Food diary-to be able to record how many exchanges he had and from what food* [mother] • *Section to enter your exchanges and medicine so you can have everything in one place. Also access for the dietitian to see* [father] • *Ability to scan the barcode of a food packet and it tells you exchanges/100 g and exchanges/portion* [teenage boy] • *More meal ideas for toddlers; easy quick meals* [mother] • *Recommendations and ideas for meals and recipes that take into account exchanges* [father of teenager] • *Digital versions of the picture books-I usually take pictures on my phone of the picture books* [mother] • *Would like index of recipes so know what recipes are on the app* [father of teenager]

4. Discussion

This is the first dietary app designed specifically for PKU, which has been formally evaluated to assess efficacy. Feedback from the RCT and patient focus groups has facilitated development of a user-friendly app, with information explained in a clear effective way, and its value analysed objectively. Previous dietary apps for inherited metabolic disorders, both within and outside the UK, have not included the full range of criteria represented in this app, in terms of content, availability on all devices, non-commercial bias, and validation by health professionals or credible organisations [6].

The results of this RCT provide valuable insight into how this app is used by both adolescents with PKU and parents/carers of children with PKU, the key features used, and suggestions on how the app could be improved. It was apparent that whilst the app was popular, it could not replace other commonly used resources for obtaining dietary advice, such as contact with the dietitian, written/pictorial information booklets, and social media. There is substantial variation in individual preference, for methods of seeking

dietary information; the app offers an additional choice to existing resources, for those who commonly use this form of technology.

During the time subjects used this app, there were trends toward improvement in the accuracy of protein calculation, self-confidence in dietary management, and reduced reliance on other dietary information resources. However, it is difficult to isolate this to solely the influence of the app, as subjects had access, concurrently, to other sources of dietary information, such as the dietitian, written/pictorial information, and social media. Both app users and nonusers improved in their protein calculations from baseline to 12 weeks, suggesting that the extra attention associated with diet during the study led to subjects being more vigilant with food calculations, irrespective of their source of dietary information. There was no evidence of improved labelling interpretation or metabolic control, during the study period. However, this study included subjects who were well established with and knowledgeable about the Phe-restricted diet, were already self-confident in management of their PKU care, and had good metabolic control. In particular, adolescents and their parents/carers had been accustomed to dealing with the Phe-restricted diet in excess of 10 years, and commonly self-reported that they were 'set in their ways' and they varied their diet very little. Therefore, they were not regularly seeking dietary information, and, when they did, they often preferred to use the method they were accustomed to, such as contacting the dietitian or using written/pictorial educational material. Initial interest in the app may have faded, when adolescents realised that it did not provide any further information than they already knew or could access using existing resources. As children with PKU move into adolescence, they typically exhibit decreasing dietary adherence, and, consequently, reduced metabolic control [23], which is associated with the adolescent perception of invincibility and a lack of acknowledgment that their health may be affected by their actions [24]. Engaging adolescents in education about their dietary treatment is challenging, and PKU is no exception. Teenagers are high-level users of smartphones and social media, so they would, generally, be expected to be more receptive to these platforms [25]. However, there is also evidence that apps that enable content creation, sharing, and networking are more effective in this age group [25]. This is supported by the top five apps used by adolescents in this study being largely social media apps. Hence, an information-giving app such as '*PKU Bite*'® is less likely to engage adolescents. Interactive features, such as a diet or exchange tracker or an ability to share recipes, might generate higher engagement.

At the end of the study, 50% of subjects were using the app regularly (at least weekly), whilst a quarter had rarely used it, and a quarter not at all. Uptake by adolescents was particularly poor, with only 4 out of 21 using the app regularly. In addition, it tended to be the parents of younger children that used the app frequently, rather than those with teenage children. For those not using, or rarely using the app, no change in knowledge, accuracy of protein calculation, or self-efficacy would have been expected. This lack of uptake is similar to other studies looking at educational dietary resources and reinforces the concept that provision of the resource alone does not guarantee motivation to utilise [26]. Furthermore, there is much evidence to suggest that a combination of teaching methods incorporating different learning styles is more effective than one resource or intervention alone, so expecting a solitary resource to engage all people with PKU is an unrealistic target [24,26–28].

Even with patient/caregiver education and provision of written/pictorial material, patients/caregivers find reading and interpretation of food labels confusing, leading to errors in protein calculation [3]. In addition, patients/caregivers commonly interpret suitability of foods differently to professionals, and, even within the same family, caregivers can vary in the interpretation of food-protein values on food labels. A study of 45 patients/caregivers of children with PKU and 49 dietitians demonstrated that dietitians are generally more relaxed about protein labelling; patients/caregivers were more likely to use exact protein analysis than dietitians (38%vs. 6%) and calculate the Phe content of special low-protein foods (64% vs. 30%) [29]. Uncertainty about interpreting food labels may, therefore, cause

over-restriction in a diet that is already very limited. The app provides concordance in label interpretation, and targets an unmet national need to deliver more consistent care for patients with PKU who are on strict low-protein diets. Used in conjunction with other forms of dietary information, it provides another tool with the potential to impact and improve longer-term clinical outcomes.

The '*PKU Bite*'® app provides caregivers/patients with accurate, reliable, and readily available information about the protein content of foods, with the aim of improving self-care skills in the interpretation of food-protein suitability. It is free to download from the App Store and Google Play and includes a brief set of instructions on how to use the app, so it is accessible for all patients with PKU in the UK. It is estimated that approximately 2360 patients are currently monitored by NHS hospitals in England [30]. It can also be used by parents/carers, extended family, friends, occasional carers, manufacturers of dietary products, catering companies, and school and nursery workers. It may also be useful for adult patients with PKU returning to a diet and for maternal PKU patients as a tool to re-educate. It is hoped that widespread availability will assist with UK standardisation of dietary instruction and minimise conflicting information.

Use of dietary resources, such as '*PKU Bite*'®, has the potential to reduce reliance on dietitians for basic dietary queries, thereby enabling more time for complex clinical care. It was originally intended that any contact made by subjects with IMD dietitians with dietary queries during the initial 12-week study period, and the 6-month follow-up period, would be recorded, to see if the app helped with reducing dietetic contacts. However, this proved to be too complex, due to the multi-faceted nature of phone calls. Parents/carers frequently call for other reasons, but will commonly use the opportunity to ask about suitability of different foods. Similarly, during dietetic calls to report blood Phe results, questions about food suitability commonly arise. In addition, the app often identified new foods they could eat, prompting clarification with the dietitian. Thereby, '*PKU Bite*'® may have initially increased dietetic contacts, but for appropriate reasons.

There was some evidence that use of social media and written information had decreased during the six-month study period. However, it is difficult to isolate this as being due solely to the introduction of the app. Choice of dietary resources is influenced by many factors, including individual preference and learning styles. For PKU, educational material is usually issued on diagnosis and new materials are issued at regular intervals. Therefore, these documents were already available to all subjects, however, they may have been misplaced, lost, or forgotten. In addition, older children, and parents of teenagers, are more familiar with the dietary restrictions and may refer less often to informational resources, but the release of new booklets may generate interest in new products, thereby altering their normal eating behaviour. Issuing written/pictorial educational material and using the app, may also have drawn additional attention to the diet, potentially leading to changes in dietary patterns and behaviours, such as protein calculation.

Our intention is to further develop the '*PKU Bite*'® app and add functions specifically requested by users, including a label scanner to calculate protein content of manufactured foods, a diet and formula tracker for users to record and monitor daily protein intake, and a pre-clinic assessment questionnaire to assist with collecting data for clinic appointments. This will include a validated food-frequency questionnaire that can also be used for research purposes [31]. In addition, a DTAC application (Digital Technology Assessment Criteria) is being submitted to NHSX—a UK government organisation responsible for setting national policy and developing best practice for National Health Service technology, digital information, and data, including data-sharing transparency [32]. This assessment is the national baseline criteria for digital health technologies entering into the NHS and social care [33]. It gives users confidence that the app meets clinical safety, data protection, technical security, interoperability, usability, and accessibility standards [33]. In the future, the app could be adapted to suit the dietary requirements of other inborn errors of amino acid metabolism.

There were some study limitations. Six mothers and three children in the control group chose not to complete the final six months of the study, so they did not download the app or attend the training session. Consequently, there was no six-month questionnaire data for nine control subjects. This included subjects with lower education, which may have influenced their engagement. However, when sensitivity analyses, which restrict the patient population only to those patients who remained in the study, were performed, these had no meaningful impact on any of the study's interpretations. These were individuals who, whilst able to commit to the completion of questionnaires as control subjects, were less confident about committing to using the app. Seven of the nine subjects who withdrew from the study were adolescents, or parents of adolescent children, who were more likely to be set in their ways and resistant to change. The two subjects with younger children had family commitments and social circumstances that made it difficult to commit. It is likely that there were similar subjects in the app group who, whilst remaining on the study, reported not using the app. Randomisation of subjects in the study was complicated by families where more than one member participated (e.g., parent and child). In order to mimimise confounding variables, it was necessary to ensure that all members of the same family were randomised to the same study group. This may have had a bearing on the lack of homogeneity in the education level of mothers across study groups and the number of withdrawals from the control group, due to disengagement and social issues. Follow-up multicentre studies on the longer-term use of '*PKU Bite*'® and its potential impact on dietary adherence, metabolic control, and reliance on dietitians for diet queries would be beneficial. It is, particularly, important to examine its efficacy alongside differing teaching styles and care protocols. Moreover, focusing on a cohort of parents of younger children, who are still learning about PKU, may be helpful.

5. Conclusions

'*PKU Bite*'® is a dietary app, specifically designed for PKU, which has been developed in close partnership with patients and caregivers, independent of industry involvement, and has been extensively studied to examine its efficacy. The app has the potential to improve protein calculation, knowledge, and self-efficacy with PKU management, and is one of a multitude of tools that can be used to educate and inform patients and parents/carers of children with PKU. Validation by a UK national approval process, supported by the NHS, will further support its use as an adjunct to existing resources for PKU.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14112182/s1, File S1: protein-labelling knowledge questionnaire.

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CHAPTER 5: DISCUSSION

5.1 Overview of Results

This research examines the development of food preferences, dietary patterns, food acceptance and food neophobia in children with PKU, commencing during the weaning period when a second stage weaning protein substitute is introduced. It describes how dietary adherence to a phenylalanine restricted diet and the administration of a phenylalanine-free protein substitute causes stress and anxiety for parents/caregivers of children with PKU. It also explores how the child environment and management of feeding behaviour can influence successful dietary treatment. It further shows the complexity of the dietary knowledge required to interpret food suitability for inclusion in a phenylalanine restricted diet and how this can be lessened through a low protein food app. National dietetic professional agreement on allocation of protein and interpretation of food labels in PKU was essential before national resources were introduced, guiding content and aiding effective application of these agreed principles in clinical practice.

5.2 Weaning protein substitute

Weaning is an important formative period in developing and instilling longer term healthy food patterns, preferences and behaviours in young children. This has rarely been studied in PKU. Prior to weaning, protein and energy requirements for infants with PKU are met by a phenylalanine-free liquid formula (protein substitute) together with a controlled amount of breast milk or standard infant formula. These protein substitutes provide a percentage of energy from fat and carbohydrate (Table 5.1) similar to human milk and regular infant formula and in line with dietary reference values (DRVs) [1]. By weaning age (6 months), it is difficult to achieve the higher protein equivalent

requirements required for infants with PKU from phenylalanine-free infant formula alone as the volume required is high [2]. Instead, protein requirements are partially met by introducing a phenylalanine-free weaning protein substitute. These are low volume, amino acid based powders with added carbohydrate (starch), vitamins and minerals that are mixed with a small amount of water to make a spoonable age-appropriate textured paste. They contain negligible amounts of fat (mostly omega 3 fatty acids) and are lower in energy and carbohydrate (Table 5.1). Third stage protein substitutes (usually introduced from the age of 3-5 years) are even lower in energy than weaning protein substitutes containing less carbohydrate, and minimal fat (as essential fatty acids) but include high amounts of protein equivalent. The carbohydrate content of infant protein substitutes is mainly starch based, with less than 15% contribution from sugars. Weaning and third stage protein substitutes are more concentrated, and have a larger percentage contribution from sugar, as well as artificial sweeteners.

Prior to the development of second stage weaning protein substitutes in 2001, infants were either given concentrated liquid phenylalanine-free infant formula, infant formula supplemented with preparations designed for older children, or protein substitutes mixed into a paste with added water [2-4]. Despite 15 years since the introduction of weaning protein substitutes, their use had not been systematically investigated, particularly examining their tolerance and method of introduction. A review in 2012 clearly identified the need for observational and intervention studies examining the weaning process in PKU, focusing on biological, maternal, infant, social and environmental factors associated with weaning to guide evidence-based guidelines [2].

Table 5.1 Energy and macronutrient composition of infant, weaning and third stage protein substitutes for PKU

	Volume providing 10g protein equivalent	Energy Kcal/10g protein equivalent	Protein equivalent g (% energy)	Carbohydrate g/10g protein equivalent (% energy)	Sugars g/10g protein equivalent (% energy)	Fat g/10g protein equivalent (% energy)
Infant protein substitute 1 (PKU Start)	500ml prepared	340	10 (12%)	36 (42%)	3.5 (4%)	17.5 (46%)
Infant protein substitute 2 (Anamix Infant)	500ml prepared	350	10 (12%)	37.5 (43%)	5.5 (6%)	17.5 (45%)
Weaning protein substitute 1 (PKU Gel)	24g powder + 30ml water	76	10 (49%)	8.9 (51%)	4.8 (25%)	0.02 (<1%)
Weaning protein substitute 2 (Anamix First Spoon)	25g powder + 20ml water	82	10 (49%)	9.6 (47%)	6.4 (31%)	0.3 (3%)
3rd stage protein substitute 1 (PKU Cooler)	87ml liquid	65	10 (62%)	4.4 (27%)	3.0 (18%)	0.8 (11%)
3rd stage protein substitute 2 (Lophlex LQ)	62.5ml liquid	58	10 (69%)	4.4 (31%)	3.7 (26%)	0 (0%)

In a retrospective study, the medical and dietetic records of 31 children with PKU were examined to investigate the introduction and administration of a second stage

weaning protein substitute and solid foods from commencement until 2 years of age. They were all given the same second stage phenylalanine-free weaning protein substitute administered as a semi-solid gel or paste consistency. The age at which parents chose to commence solid foods varied between 12-25 weeks of age, and the weaning protein substitute was introduced between 13-37 weeks under the direction of a dietitian. Weaning protein substitute was well accepted when introduced in a stepwise manner and administered from a spoon before solid foods or any liquid infant formula. This coincided with a stepwise decrease in liquid infant protein substitute formula as well as the gradual introduction of phenylalanine-containing exchange foods to replace breast feeds or standard infant formula. Despite the lower energy content of the weaning protein substitute compared to the infant protein substitute it replaced, growth and metabolic control were within expected ranges. The main obstacles with administering weaning protein substitute were its refusal associated with teething, illness and general food rejection, with problems peaking at 12-14 months of age. Routine and consistency with protein substitute administration were important during challenging periods. Early (<17 weeks) or late (>26 weeks) weaning, delayed introduction of coarser textured foods, and first-born children were more likely to experience refusal of protein substitute or food particularly during teething or illness.

For dietitians managing PKU, discouraging very early or late weaning, ensuring a consistent approach by caregivers and providing intensive support for parents particularly during the most challenging ages (12-14 months) was paramount. Educating and enlisting the support of grandparents and nurseries may also assist parents at this time, as well as parent social support groups that enable discussion with others who have already been through a similar experience.

The limitations of retrospective data, the lack of dietary pattern data and the inability to assess parental anxiety and dietary management strategies in this study led to the design of a longitudinal prospective case-control study. Twenty infants with PKU commencing a weaning protein substitute, and 20 healthy control infants were recruited. Subjects were followed prospectively from weaning until 2 years of age. The study reported growth, energy and macronutrient intakes; weaning progression including the introduction of textured food (e.g., thin puree, thick puree, mashed, finger foods), self-feeding motor skills, stepwise reduction of infant formula, the feeding environment (where they eat and with whom), gastrointestinal symptoms and negative feeding behaviour. Mealtime emotions, anxiety and coping strategies were also examined to assess the impact of the additional dietary burdens of weaning protein substitute administration and natural protein prescription adherence in PKU.

5.3 Growth, macronutrient intake and weaning protein substitute

Weight, height, BMI, head circumference and dietary intake were assessed monthly from weaning commencement to 12 months of age, then at 15, 18 and 24 months. The aim was to establish if growth in PKU was comparable to non-PKU infants during this critical period and examine the energy and macronutrient intakes in infants with PKU transitioning from an infant to a lower energy dense weaning protein substitute.

Z-scores for growth did not differ between PKU and control infants and were within normal ranges at all ages. No child's z-scores for any parameters were below -2 (underweight/stunted) at 2 years of age and only one child with PKU had a BMI z-score >2. Length and length-for-age z-scores showed that infants with PKU were statistically

taller at all ages. This was explained by the girls with PKU being taller than their non-PKU controls. This early accelerated growth has previously been reported [5, 6] and may be related to the quantity or quality (natural vs synthetic) of protein intake and a possible effect on bone age [6]. Boys with PKU also had a slower growth rate than controls and girls with PKU, and there were trends indicating that female infants with PKU had a higher weight and BMI than male infants, as previously reported [5]. Studies of older children and adults have demonstrated that girls with PKU are more susceptible to overweight and obesity than boys [7-10]. At what age this susceptibility begins is unknown, but it may well occur as early as weaning.

A detailed 24-hour prospective dietary intake record was collected monthly until 12 months of age and then at 15, 18 and 24 months. Protein intake from protein substitute (both infant and weaning) remained constant at a mean of 2.8g/kg body weight from 7 months of age when infant protein substitute intake started to decline in a linear fashion as weaning protein substitute intake correspondingly increased. Natural protein intake was constant throughout the study at 0.7-0.8g/kg/day. At all ages, total protein intake in both groups exceeded WHO/FAO/UNU safe protein levels (0.97-1.77g/kg/day) [11] plus the additional 40% recommended for PKU to compensate for the reduced utilisation of protein from amino acid based protein substitutes. Whilst it is difficult for children with PKU to meet non-phenylalanine protein requirements in the early stages of weaning due to the volume of infant protein substitute required [2], this study shows that if weaning protein substitute was introduced in a consistent stepwise manner, the protein intake was maintained at recommended levels. What impact this has on the observed growth rates in boys and girls is unknown.

Total energy intakes were comparable across all ages in both study groups. Energy intake from protein substitutes (weaning and infant) remained constant between weaning (4-6 months) and 2 years of age, at a mean of 423kcal/day. Protein substitute (mostly weaning type) contributed 75% of protein intake and 20% of energy intake by the age of 2 years, with food making up the remainder.

Until 12 months of age, the percentage of energy from carbohydrate and fat was the same in both groups. However, between the ages of 1-2 years, infants with PKU had a higher percentage of energy from carbohydrate but lower fat (60% and 25%) compared to controls (48% and 36%). This difference had previously been reported in other studies of older children and adults with PKU [12-14], but not in early childhood, and this was the first time that the age at which these differences occur has been identified. This is likely to be influenced by the low fat, high carbohydrate composition of SLPF needed to meet energy requirements in PKU. This in turn may have an impact on appetite, adherence, and feeding problems [15-17]. Nearly 70% of SLPFs are higher in carbohydrate and 60% are lower in fat than their equivalent regular varieties [18]. The most consumed SLPFs in children include the staple foods: bread, pasta/rice, flour and milk substitutes [13, 19, 20]. Their most significant contribution is carbohydrate (mostly high glycaemic index starches), and therefore energy, and they all contain more added sugar than their protein containing equivalents [18]. This could have implications on the incidence of overweight and obesity, insulin resistance [21, 22] and risk of metabolic syndrome [23] in PKU. However, a recent systematic review and meta-analysis suggested that body mass index (BMI) was similar between patients with PKU and healthy controls, thereby providing no evidence that a phenylalanine-restricted diet is a specific risk factor for overweight [24].

There is also evidence that higher carbohydrate intake reduces ghrelin [21, 25], a gut hormone that affects appetite, adiposity, and regulates energy expenditure and glucose homeostasis by inhibiting insulin secretion [26]. Ghrelin may also have a role in bone formation and metabolism, and there is evidence that bone mineral density in PKU, whilst within the normal range, is below that of non-PKU peers, [27-32] due to a possible imbalance between bone formation and resorption. Furthermore, there is evidence that the high carbohydrate content of phenylalanine-restricted meals containing SLPFs raises postprandial plasma glucose and insulin, lowers fat oxidation and the thermic effect of feeding (a metabolic factor associated with regulation of energy balance) more so than a regular meal, and this may, over time, contribute to insulin resistance, overweight and obesity in susceptible individuals [15].

Whilst the dietary imbalance of carbohydrate and fat might be corrected by adding more fat to weaning protein substitutes, this would impact on texture, volume and satiety and potentially reduce adherence. The main role of protein substitutes is to provide a source of non-phenylalanine protein, vitamins and minerals. The addition of starch, sugar and sweeteners are designed to improve the taste, texture and mouthfeel, so balancing the volume and composition of protein substitutes is challenging. It is also difficult to alter the composition of SLPFs as many are staple foods that are fundamentally high in carbohydrate and are an important source of energy and bulk. Adding more fat and less carbohydrate could also alter their acceptability. Judicious use of low protein milk replacements could help address the dietary balance if their composition was modified by increasing the quality and quantity of their fat content.

Further longitudinal studies are required to determine if the growth trends seen in this study continue into childhood, adolescence and adulthood. This includes longitudinal

data on dietary patterns, food intake, body composition and growth rate at all ages. Identifying the point at which trends begin and why will be important in preventing and controlling lifestyle diseases such as obesity in adulthood. Further detailed studies examining ghrelin concentrations, glycaemic index and glycaemic load are important to assess the impact of dietary composition of a phenylalanine restricted diet.

5.4 Feeding development and progression during weaning

In PKU, there is a strong focus on food, and feeding is directed, controlled, and monitored by health professionals. Knowing more about what happens at mealtimes, children's reactions, and the approaches parents/caregivers use to manage mealtimes is important in developing educational strategies that will guide parents through this challenging feeding stage.

In the longitudinal weaning study, infants with PKU did not differ significantly from their non-PKU peers in most aspects of weaning progression. However, the following was observed in the infants with PKU: prolonged bottle feeding (with phenylalanine-free infant formula), deferred transitioning from a bottle to a cup, protracted parental spoon feeding, and delayed self-finger feeding. Delays in independent feeding were associated with parental anxiety and the perceived need to control mealtimes to ensure that prescribed protein substitute and protein exchanges were consumed to maintain metabolic control. In the healthy population, baby led weaning, which encourages self-feeding, reduces fussiness compared with parent led spoon feeding [33], increases variety and exposure to vegetables and more textured foods at a younger age [34, 35], improves satiety, reduces parental pressure at mealtimes [36], and stimulates interest in family

foods and shared family meals [37]. Previous studies have identified that children with PKU may have distorted feeding patterns and eating behaviours including food aversions, faddiness, neophobia, limited variety, poor appetite, eating in isolation and the need for more parental coercion [38-40]. The daily coercion required to encourage children to take their protein substitute and prescribed natural protein intake, is exhausting and stressful for caregivers [41].

Some children with PKU commenced weaning at a younger age (median 4 months) than controls (median 5 months). First weaning foods were similar to controls, but puree fruit and vegetables were consumed more by children with PKU, whilst for the control group baby rice/porridge was the main first food. Thus, it might be expected that vegetables and fruits may be better accepted by children with PKU in the longer term than in non-PKU children due to this earlier and persistent introduction in infancy.

Parents in this study reported more gastrointestinal symptoms such as flatulence, burping and retching than controls, possible side effects of the high osmolality of the protein substitutes [16, 39, 42]. For this reason, it is recommended that protein substitutes be taken with additional water, particularly if they are made up more concentrated than recommended by the manufacturers. They should also be administered in small frequent doses (3 or 4) that are spread evenly throughout the day [16, 43, 44]. This helps to minimise 24-hour fluctuations in blood phenylalanine levels, improves nitrogen retention, and minimises oxidation [45-49].

Various strategies have been reported for improving adherence with protein substitutes [16, 50]. For example, routine and consistency are important, administering the protein substitute at the same time every day, with the same adult supervision, and ideally before meals whilst the child is hungry. The environment should be calm,

encouraging, but persistent, and all caregivers should follow the same procedure and use the same strategies [16, 50]. Flexibility in timing pre and post meals may be important in finding the right balance between giving protein substitute when the child is hungry (before meals) but preventing the protein substitute from inhibiting appetite and slowing development of feeding skills.

Results from the longitudinal study showed that children with PKU were less likely to sit at the table with the rest of the family at 2 years of age, as previously reported [38, 51]. This may be due to preparation of separate meals to the rest of the family resulting in a negative mealtime atmosphere and a lack of social experiences associated with eating [16]. Eating meals together enables infants to learn and model adult and older sibling behaviour. When mothers of non-PKU children eat shared meals, distractions are minimised (e.g., TV, toys) and children are allowed some choice in food type and quantity, they are observed to refuse fewer foods and are easier to feed [52]. Getting the balance right between structured mealtimes where the family eat together but still allowing children some autonomy in terms of food choice, may be important in minimising food neophobia and fussiness [52].

Further study into the gastrointestinal side effects attributed to protein substitutes and compositional product development aimed at minimising these would be beneficial. In addition, studies looking at the implementation of baby led weaning in PKU could help to identify if this can reduce fussy eating, increase variety and minimise stress during mealtimes. Mothers who have experience of weaning their own children with PKU could act as role models and provide support for first time parents of children with PKU.

5.5 Parental feeding strategies, anxiety and coping behaviour during weaning

It was observed that support from their dietitian and family, confidence in the medical team, and optimism, helped mothers of children with PKU adapt and cope during weaning, minimising anxiety and stress to comparable levels observed in control mothers. However, this study identified a critical period between 12-24 months of child age when additional emotional support was needed. All 4 study questionnaires showed peaks in maternal stress, anxiety and coping behaviour at around 15 months of age. Fears about protein substitute refusal peaked between 12-24 months of age and mealtimes were reported as being less enjoyable for both mother and child in the PKU group particularly between 8 and 24 months of age, coinciding with the age of protein substitute refusal, teething and growing independence.

Some of the feeding difficulties observed in children with PKU reflected unrealistic parental expectations about how much food their child should eat, especially when protein substitutes commonly provided up to 25% of energy requirements [16]. Parental anxiety and fear of the neurological consequences of poor dietary control weigh heavy in the second year of life for parents of children with PKU and can produce parenting practices that restrict a child's emotional and social development. In contrast, the control mothers improved in their confidence and were less anxious with increasing child age, although they experienced a peak in concerns at 24 months when their child became more independent. Force feeding, unpleasant mealtime environments and associated negative behaviour such as crying, gagging and refusal have previously been reported in PKU and were observed in the longitudinal protein substitute study [16]. A recent study suggested that across all ages, parents of children with PKU were more frustrated, angry and anxious when feeding their children [51].

Blood phenylalanine concentrations in this weaning study were higher, although within target reference ranges, and more variable between 13-18 months of age and were correlated with teething and illness, maternal mealtime stress, mood, and fears of protein substitute refusal. Parents of non-PKU fussy eating toddlers, tended to use more controlling prompts to eat rather than autonomy-supportive prompts and this was associated with food refusal [53]. In a recent study looking at non-PKU maternal feeding practices, it was found that unpleasant mealtime emotions (stressful for the mother, or tearful for the child), coaxing the child to eat, and the mother's own neophobia, were all associated with a higher degree of child neophobia [54].

Whilst an anxious, coercive and controlling parenting style around mealtimes may produce satisfactory blood phenylalanine concentrations, higher levels of anxiety and psychiatric disorders have been observed in early and continuously treated adults who had good metabolic control as children [55-58]. Social problems such as withdrawal, anxiety, depression, poor attention and low self-esteem have been reported in children with PKU [41, 58, 59]. It is unknown if these symptoms are caused by poor blood phenylalanine control, the nature of dietary treatment, or having a chronic illness, but parental coping mechanisms may account for some of the variance [41, 59]. The psychological adjustment of parents to PKU can therefore have a longer-term impact on their child. A PKU specific quality of life (QoL) questionnaire identified the emotional impact of PKU, and anxiety about blood phenylalanine levels and protein substitute intake, as having the most impact on QoL irrespective of patient age [60]. These impacts were less for patients with mild or moderate PKU and those on sapropterin treatment [60].

Further study into strategies for dealing with parental anxieties at mealtimes with a particular focus on independent feeding, normalising the feeding environment, and

including children in the social aspects of family mealtimes, are needed. This might include resources to build parental confidence such as parent support groups particularly during the latter stages of the weaning period (1-2 years of age).

5.6 Food frequency questionnaires and dietary patterns

It is important to identify nutrient imbalances and examine differences in dietary patterns that may influence lifestyle disease risk in PKU [43]. Achieving optimal nutrient status is challenging given that a significant proportion (80%) of the nutrient intake is chemically derived with poor bioavailability and little natural protein to aid absorption and utilisation [41, 43]. It is not surprising that in trying to adhere to a highly restrictive diet, feeding patterns and food choices are affected, and may lead to disordered eating including preoccupation with food, anxiety associated with eating, food poverty, feelings of guilt and shame around eating, and a sense of loss of control over their diet [16]. Therefore, examining food patterns and changes in nutritional status are important areas for monitoring [16].

A better understanding of food preferences in PKU, what patients actually eat, how these preferences develop and how patients/carers manage their daily restrictions is essential. To do this, a PKU specific validated tool for assessing dietary patterns is necessary. Only one such tool had been validated but was specific to adults not children with PKU, and based on food types and portion sizes unique to the USA [61].

A FFQ specifically designed for low protein diets was developed and assessed for reproducibility (test/retest reliability), criterion validity (comparison with an existing standard: the 24-hour dietary recall technique) and construct validity (ability to

differentiate between different subject groups) for energy and macronutrient intake and individual foods. It was studied in 50 children with PKU and 50 healthy control children. A portion size photographic booklet was designed to accompany the questionnaire and a database of food item composition was developed to aid analysis.

The low protein FFQ was composed of 89 food items and 11 general dietary pattern questions. There was good reproducibility and criterion validity indicating that the FFQ was a valid tool for assessing habitual intake of energy, macronutrients and commonly eaten foods, providing comparable data to repeated 24-hour dietary recall assessments.

FFQ are useful tools for collecting data on habitual intake. This is the first FFQ developed specifically for children with PKU, and will be invaluable in measuring changing patterns in dietary intake over time. It is a comprehensive tool covering a wide range of foods commonly eaten in PKU, including SLPFs and fruits and vegetables of differing phenylalanine content, and is therefore a useful adjunct to 24-hour dietary records that are designed for collecting data on current rather than habitual food intake. Whilst the FFQ can be self-completed, administration by a dietitian gives valuable additional information that might otherwise be overlooked. For example, dietitians may identify foods patients have not yet tried or have tried in the past but wish to retry, or foods they have difficulty in accessing. In focussing on calculating how many food portions they have, patients/caregivers may concentrate less on what they think the dietitian wants to hear. Hence more accurate intake data may be obtained.

With the introduction of pharmacological treatments such as sapropterin, that can increase dietary phenylalanine tolerance, and reduce reliance on protein substitute, assessment of changing food patterns is essential. There are few studies describing the long-term effect of sapropterin treatment on dietary intake [43]. The 3-year European

SPARK (Safety Paediatric EfficAcy PhaRmacokinetics with Kuvan) study was the first clinical trial of sapropterin to demonstrate safety and efficacy in children before the age of four years in combination with a phenylalanine restricted diet [62]. Commencing sapropterin treatment early is likely to be beneficial in aiding development of a normal healthy dietary pattern but this remains unstudied. Although sapropterin treatment may liberalise protein restriction, it is rare for the diet to be completely liberalised and may even lead to nutritional imbalances [63]. The FFQ will enable assessment of dietary intake before and after sapropterin treatment to identify any nutritional imbalances and help to characterise the dietary patterns associated with treatment change.

Further studies examining accuracy and adherence with self-completion of online versions of the FFQ with parents/carers and patients with PKU are necessary. Longitudinal studies assessing dietary patterns in children, teenagers and adults with PKU are also required. The FFQ will prove a useful tool to examine dietary choices and their impact on the cost of living, burden of care and quality of life.

5.7 Food acceptance and neophobia

At diagnosis, infants with PKU start dietary treatment immediately which includes the introduction of a phenylalanine-free infant formula [16]. Amino acid based infant protein substitutes, introduced in the first weeks of life, are critical for metabolic control but are bitter tasting in contrast to the sweet taste of breast milk or standard infant formula and may impact on taste preferences in children with PKU.

A prospective case-control study examined food preferences, neophobia and dietary patterns in 35 children with PKU and 35 age and gender matched healthy controls. Parents

completed a pre-validated version of the FFQ previously described, to assess their children's habitual dietary intake, and a validated questionnaire on their child's food and general neophobia. Children were asked to taste 10 blinded puree/liquid foods (including sweet, savoury, sour and bitter taste sensations) in random order and to rate them on a 7-point hedonic scale and then rank them in preferential order.

All the children (in PKU and control groups) rated and ranked sweet foods the highest. There was no evidence for a conditioned preference or acceptance of bitter foods in the children with PKU associated with early and consistent exposure to bitter tasting protein substitutes. In this study, children with PKU preferred vegetables more than controls, and controls preferred fruits more than the PKU group. However, there was no difference in their reported intakes; a mean of 3 servings/day of fruit and 3/day of vegetables in both groups. Food neophobia was also significantly higher in the children with PKU and is likely to play a bigger role in dietary patterns and food refusal in PKU than taste preferences.

All healthy children are predisposed to prefer sweet and salty foods and many display significant food neophobia particularly in the pre-school years [64]. Food preferences develop over time and are acquired via experience, and so they have the potential to be modulated. For example, in healthy infants, acceptance of sweet and salty tastes has been shown to be modified by early dietary experience [65] and pre-school children tend to prefer the tastes they are familiar with [66]. So repeated introduction of healthy foods of varied flavours increases hedonic appeal of healthier foods later in life [67]. In PKU, it is likely that the introduction of artificially sweetened protein substitutes after weaning (3-5 years) and the sugar content of many unrestricted foods, modulate any preference there may be for bitter tasting foods associated with early exposure to bitter tasting unflavoured

infant and weaning protein substitutes. In PKU, a study comparing 4 flavours of amino acid with the same 4 flavours of CGMP protein substitutes found a preference for the sweeter flavours of chocolate and strawberry, whilst tomato flavour, described by participants as bitter and salty, was the least liked [68], as might be expected in healthy non-PKU children.

Understanding the taste preferences of children with PKU and how these might differ to those in non-PKU children is important so that the food manufacturers developing protein substitutes and SLPFs produce tailored foods with respect to taste preferences to help improve patient adherence with the diet [43, 69]. Identifying if there is a preference for bitter tastes in infancy, when this preference fades, and if it is related to the introduction of artificially sweetened protein substitutes or sweet foods requires future study.

Food neophobia is a significant barrier to dietary change, is associated with lower dietary variety and is primarily mediated by fear of encountering unacceptable tastes [70]. In PKU food neophobia may also inherently protect patients from eating foods that are high in protein. There is also evidence that food neophobia is associated with the timing of introduction of semi-solid foods [71] suggesting that late weaning or a delay in introduction of more textured foods, may increase its incidence.

One of the study limitations was the subjective nature of parents describing their child's food neophobia. It was postulated that some of the results seen in this study may be attributable to the influence of parental food preferences and neophobia, as they are the main providers of children's food. There is evidence that parents with food neophobia tend to report neophobia in their children despite observations to the contrary [54, 72]. A follow on prospective observational case-control study examined the food preferences

and neophobia of the parents (mothers and fathers) of the same 35 children with PKU and 35 matched controls using the same FFQ and neophobia questionnaire used for their children. Both PKU and control groups had more food neophobia than their parents, and mothers did not differ significantly from fathers in either group. However, both mothers and fathers of children with PKU were more food neophobic than the mothers and fathers of control children and this may affect the foods offered to their children. Mothers and fathers of children with PKU did not differ significantly in their dietary intakes, although mothers' diets were less varied than mothers of control children. It may be that parents of children with PKU adapt the variety of foods they eat to simplify mealtimes and avoid having to prepare two different meals.

Parents of children with PKU frequently report stress associated with food preparation and restrictions on social life [42]. In contrast, control children ate more similarly to their parents than children with PKU who, not surprisingly, consumed a significantly less varied diet than both their parents and the control children.

An understanding of general neophobia and food neophobia in PKU and how these change from early childhood, through adolescence and into adulthood is important. Studies examining the age at which food neophobia is first identified, how it develops over time and how it relates to general neophobia and the incidence of other eating disorders are needed. With sapropterin treatment, and subsequent diet relaxation, it will also be important to identify the impact this has on the incidence of food neophobia.

5.8 Food labelling interpretation and protein calculation

In PKU there is much complex information for parents/caregivers and patients to understand before they can implement the dietary restrictions. This includes understanding and calculating the protein/phenylalanine content of foods and interpreting protein content from nutrition labels. Teaching patients/caregivers how to read and interpret food labels is confusing [16, 73].

In an online survey of adult patients with PKU and parents/caregivers of children with PKU, 90% had difficulty with food labelling in the previous 6 months [73]. There were several issues that complicated reading and interpreting the nutrient content on food labels for PKU, including cut off points for declaring protein content [73-75], lack of data on phenylalanine content, lack of clarity as to whether the nutrition analysis is for the cooked/prepared or uncooked/unprepared version of the food, and analyses based on addition of other protein containing ingredients [16, 73]. This has substantial implications for a diet that is already significantly restricted.

To further add to the uncertainty, phenylalanine is found in varying amounts in natural protein food sources. Animal and cereal protein sources (e.g., meat, fish, eggs, milk, wheat) contain about 5% phenylalanine, so 1g of protein provides approximately 50 mg of phenylalanine, and this can be estimated from the protein content on food labels [16]. Fruits and vegetables contain a lower and more variable amount of phenylalanine (20-40 mg per 1g of protein), so using the protein content of fruits and vegetables on a food label, overestimates phenylalanine content due to a lower phenylalanine:protein ratio [16]. For this reason, many fruits and vegetables have been analysed specifically to identify their phenylalanine content [76-78].

The collaborative study conducted with IMD dietitians aimed to facilitate the development of consensus statements about the interpretation of protein food labelling

and the allocation of protein in a low phenylalanine diet for the management of PKU. Over 4 years, 10 Delphi rounds were used to systematically obtain consensus from 93 British inherited metabolic disorder group (BIMDG) dietitians, representing 34 centres treating patients with PKU. One hundred and twelve practical statements for classifying food for inclusion in a low phenylalanine diet were agreed.

Aimed at patients with PKU tolerating ≤10g/day of natural protein, the statements were divided into 4 categories of food based on their phenylalanine content:

1) foods high in phenylalanine and best avoided (foods containing aspartame or with a protein content >15g/100g);

2) foods allowed without measurement (fruits and vegetables containing ≤75mg phenylalanine/100g, most foods containing ≤0.5g protein/100g, foods containing protein >0.5g/100g but used in small quantities (e.g., fats, oils, herbs, spices), and SLPFs containing phenylalanine <25mg/100g), in line with the EU PKU guidelines [41] and previous studies [79-83];

3) manufactured foods that should be calculated as an exchange food; and

4) fruits and vegetables containing >75mg phenylalanine/100g that are calculated as exchange foods.

In Europe, fruit and vegetables with a phenylalanine content ≤75mg/100g are allowed without restriction except for potatoes which tend to be consumed in larger quantities so are usually calculated as part of the daily phenylalanine allocation [41]. Fruit and vegetables with phenylalanine content 76-100mg/100g were more controversial, and it required 3 rounds of the Delphi process to reach agreement to include these as exchange

foods and simplify calculation by using a 60g portion size as the standard for 1 exchange for all foods in this category.

Statements included guidance for incorporating SLPFs, canned, frozen and dried fruits and vegetables, plant milks, soups, cooking sauces, soya sauce and ice cream in the PKU diet. Patients and caregivers find it confusing when SLPFs, specifically designed to be low in protein, must be calculated as part of their protein exchanges, and for this reason, manufacturers were encouraged to comply with the 25mg/100g cut off point.

These statements have brought about uniform national interpretation of the suitability of foods for inclusion in a phenylalanine restricted diet. This provides consensus ground rules for dietitians when providing education for patients with PKU and their families. However, these statements were aimed at dietitians so it was necessary to translate them into a format that could be easily understood by patients and caregivers. An information booklet was developed by the National Society for PKU (NSPKU) [84] and a low protein diet app was developed. Patient experience, blood phenylalanine control and feedback from patients/parents/caregivers, dietitians and low protein food manufacturers will guide amendments and additions to these statements over time. Follow up studies assessing the impact and application of these statements will be necessary to validate their acceptance in the PKU community and to assess any impact they might have on long-term adherence, metabolic control and food neophobia.

5.9 A low-protein multimedia diet app for PKU

A low protein app ("PKU Bite") was created that provided a user-friendly and readily accessible patient guide to the 2020 UK national consensus statements on food protein

allocation and labelling interpretation for PKU. The app was designed and developed using feedback from dietitians and user focus groups. A 12-week randomised controlled trial (RCT) and a 6-month follow-up study was conducted in 60 caregivers of children, and 21 adolescents, with PKU to test the app's efficacy in improving interpretation of food labels, accuracy in food protein calculation and independence in dietary self-management compared with traditional written dietary resources.

The app contained a database of over 1100 foods. A description of each food outlined its suitability in a low phenylalanine diet, how to calculate/interpret the protein content, ideas on its incorporation in the diet, exchange-free recipes, and information on its preparation, storage and availability. A protein exchange calculator estimated protein exchange amounts. Users could filter the foods to identify all products that might contain the artificial sweetener, aspartame (E951).

This RCT demonstrated that parents of younger children (<10y of age) were more likely to use the app than either adolescents or their parents/caregivers. Among frequent app users, there was a trend toward improved knowledge of food labelling interpretation and those using the app made fewer protein calculation errors than controls. Whilst there was no difference between groups for blood phenylalanine control during the study, there was a trend toward lower blood levels in both groups at 12-weeks, suggesting that increased attention to dietary management during the study period may have improved metabolic control. The app was more popular than written information or social media as a tool for deciding on food suitability and for protein exchange calculation, but contacting the dietitian for information was equally preferred. Three quarters of participants were still using the app after 6-months, half of these at least weekly.

The app received over 1000 downloads in the first 4 months of public release and further additions to the app are planned based on feedback from participants. This includes a protein substitute and exchange tracker, a label scanner that can read and calculate the protein content and an assessment questionnaire for PKU clinics that will include the food frequency questionnaire specifically designed for children with PKU.

Dietary treatment in PKU imposes a significant social burden. The practical application of identifying suitable low protein foods from the vast number of manufactured products on the commercial market is problematic and time consuming. Studies on living with PKU for patients and their caregivers have identified time constraints and stress associated with food preparation, limited food choice and record keeping as the primary obstacles to achieving better dietary adherence [42, 85]. A study examining the time burden of dietary care estimated that caregivers in the UK spend 19 additional hours per week on dietary management [75]. Educating parents, carers and patients requires considerable time and dedication. Although no single intervention can serve as a panacea [85], the app is a useful tool for accessing suitable dietary information on PKU. It is hoped that the app will save caregiver/patient time. Validation by the government agency NHSX, responsible for national health service (NHS) technology, will ensure that it meets clinical safety, data protection and useability standards. Accessibility via app stores and NHS.uk will enable patients/caregivers to easily find and download the app as a source of immediate and trustworthy information when out shopping.

Long-term studies during weaning and early childhood (<5 years of age) are necessary to assess if parent/caregiver knowledge, self-efficacy and accuracy of protein calculation can be improved when using the app. In addition, studies assessing the

usefulness of the app for extended family members, and staff in nurseries and schools, would be valuable.

5.10 CONCLUSION

In PKU, dietary management is the principal treatment. This thesis draws together the results of studies examining feeding development, food acceptance, food neophobia, dietary patterns and education in PKU. Feeding progression was hampered by many factors throughout childhood, including difficulties with protein substitute administration, the weaning process, food acceptance, food preferences, food neophobia, distorted feeding patterns and behaviours, and maternal stress. Patients/caregivers experienced practical difficulties with dietary application, sometimes associated with misunderstandings and misinformation. The development of nationwide consensus statements for the interpretation of food labelling and allocation of protein in the PKU diet has brought about consistency amongst healthcare professionals in the delivery of education in PKU. The dietary assessment tools and educational resources developed as part of this thesis were validated and are now accessible to professionals and patients and are in line with technological trends to support ongoing training, learning and monitoring in patients and their caregivers.

There is a critical time for the introduction of weaning in PKU and studies in this thesis identified a vulnerable stage in the second year of life where protein substitute and food refusal were common. Alongside feeding difficulties, parental stresses and anxieties were common. Parents are understandably fearful of refusal of both protein substitute and the prescribed natural protein. These stresses can lead to controlling and coercive parental

behaviour during mealtimes, causing delays in independent feeding, food neophobia, and a negative impact on the social aspects of eating compared to similar aged non-PKU children. Whilst on the surface parents appeared to cope well, this was commonly at the expense of their own social and psychological wellbeing.

These studies also highlight the differences in dietary patterns observed between children with PKU and their non-PKU peers. There are subtle differences in their growth trajectories with girls being taller than their peers and having higher weight and BMI than boys with PKU. The significance of this in terms of long-term health and incidence of lifestyle diseases is unknown but warrants further study. Children with PKU, have a distinct preference for sweet foods and this is a concern as many low protein foods that can be eaten without restriction are high in sugar. Despite most fruits and vegetables being low in protein and therefore exchange-free, children with PKU do not appear to consume these foods in quantities any greater than non-PKU children. Further studies using the new validated FFQ will enable further longitudinal analysis to identify age-specific dietary patterns in PKU.

Together, these studies provide new knowledge and innovative practical tools that improve dietary management in PKU and will help health professionals to better support patients with PKU and their families.

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APPENDICES

APPENDIX 1: SUPPLEMENTARY DATA FOR CHAPTER 2D

Supplementary

Details of the mixed models used to model the change in anthropological measures over time. The trend over time is analysed to allow for a linear relationship over time with a single term for time. Longitudinal mixed modelling is applied including a random intercept and slope terms.

Table 1. Longitudinal modelling results for Weight z-scores.

	est	Std. dev	t-value	*p*-value
(Intercept)	0.135	0.223	0.608	0.652
Time	0.042	0.015	2.893	0.032
Gender (Male vs Female)	–0.204	0.191	–1.069	0.472
Treatment (PKU vs Control)	–0.101	0.175	–0.574	0.664
Time: Gender Interaction	–0.020	0.015	–1.320	0.375
Time: PKU Interaction	0.004	0.014	0.314	0.752

Table 2. Longitudinal modelling results for Head Circumference z-scores.

	est	Std. dev	t-value	*p*-value
(Intercept)	–0.405	0.203	–1.990	0.163
Time	0.311	0.192	1.614	0.271
Gender (Male vs Female)	–0.173	0.181	–0.952	0.519
Treatment (PKU vs Control)	0.009	0.014	0.643	0.639
Time:Gender Interaction	–0.039	0.012	–3.218	0.016
Time:PKU Interaction	–0.001	0.015	–0.047	0.827

Table 3. Longitudinal modelling results for BMI z-scores.

	est	Std. dev	t-value	*p*-value
(Intercept)	–0.041	0.243	–0.170	0.795
Time	–0.077	0.206	–0.372	0.734
Gender (Male vs Female)	–0.675	0.188	–3.591	0.006
Treatment (PKU vs Control)	0.036	0.015	2.405	0.083
Time:Gender Interaction	0.025	0.014	1.830	0.205
Time:PKU Interaction	0.019	0.015	1.232	0.409

Table 4. Longitudinal modelling results for Length z-scores.

	est	Std. dev	t-value	*p*-value
(Intercept)	0.316	0.214	1.475	0.319
Time	0.019	0.015	1.278	0.391
Gender (Male vs Female)	–0.348	0.200	–1.739	0.232
Treatment (PKU vs Control)	0.733	0.189	3.872	0.003
Time:Gender Interaction	–0.012	0.016	–0.717	0.611
Time:PKU Interaction	–0.018	0.015	–1.165	0.435

Table 5. Longitudinal modelling results for Length-for-age-difference.

	est	Std. dev	t-value	*p*-value
(Intercept)	1.401	0.454	3.088	0.021
Time	0.022	0.049	0.439	0.711
Gender (Male vs Female)	–0.379	0.508	–0.747	0.599
Treatment (PKU vs Control)	1.488	0.486	3.060	0.022
Time:Gender Interaction	–0.084	0.042	–1.995	0.162
Time:PKU Interaction	–0.029	0.039	–0.752	0.597

Table 6. Longitudinal modelling results for total Protein Equivalent g/kg/day.

	est	Std. dev	t-value	*p*-value
(Intercept)	2.410	0.183	13.160	0.000
Time	0.070	0.013	5.426	0.000
Gender (Male vs Female)	0.065	0.177	0.369	0.735
Treatment (PKU vs Control)	0.543	0.162	3.357	0.011
Time:Gender Interaction	–0.001	0.014	–0.077	0.820
Time:PKU Interaction	–0.042	0.013	–3.230	0.015

Table 7. Longitudinal modelling results for Protein Intake g/100kcal.

	est	Std. dev	t-value	*p*-value
(Intercept)	2.320	0.170	13.681	0.000
Time	0.102	0.013	7.615	0.000
Gender (Male vs Female)	0.040	0.176	0.225	0.779
Treatment (PKU vs Control)	0.736	0.161	4.566	0.000
Time:Gender Interaction	–0.018	0.014	–1.248	0.403
Time:PKU Interaction	–0.057	0.013	–4.411	0.001

Table 8. Longitudinal modelling results for Carbohydrates g/kg/day.

	est	Std. dev	t-value	*p*-value
(Intercept)	12.340	0.700	17.619	0.000
Time	–0.074	0.053	–1.397	0.347
Gender (Male vs Female)	1.137	0.699	1.627	0.267
Treatment (PKU vs Control)	–1.490	0.639	–2.331	0.095
Time:Gender Interaction	–0.001	0.056	–0.023	0.833
Time:PKU Interaction	0.232	0.051	4.521	0.000

Table 9. Longitudinal modelling results for Total Energy kcal/day.

	est	Std. dev	t-value	*p*-value
(Intercept)	644.427	44.814	14.380	0.000
Time	21.690	3.525	6.154	0.000
Gender (Male vs Female)	36.694	44.372	0.827	0.568
Treatment (PKU vs Control)	–72.867	40.121	–1.816	0.209
Time:Gender Interaction	5.233	3.554	1.473	0.319
Time:PKU Interaction	7.811	3.219	2.427	0.080

Table 10. Total energy intake as a percentage of DRV [25] for PKU and control groups and males vs. females (mean (range)).

	All		Males		Females	
	PKU	**Control**	**PKU**	**Control**	**PKU**	**Control**
4m	118 (72–153)	135 (109–166)	127 (105–153)	124 (109–153)	101 (72–120)	145 (124–166)
5m	121 (82–174)	117 (94–173)	126 (82–174)	114 (94–151)	110 (84–122)	119 (100–173)
6m	118 (92–164)	122 (96–160)	120 (95–164)	117 (96–148)	114 (92–128)	129 (112–160)
7m	116 (81–166)	120 (89–170)	116 (81–166)	115 (89–161)	115 (100–135)	127 (104–170)
8m	124 (91–192)	125 (88–168)	125 (91–192)	123 (88–156)	122 (100–168)	129 (94–168)
9m	136 (83–184)	137 (96–195)	134 (83–184)	136 (96–195)	140 (104–177)	140 (125–165)
10m	142 (85–218)	137 (98–165)	145 (85–218)	133 (98–165)	135 (111–173)	144 (121–165)
11m	140 (100–206)	150 (104–186)	140 (100–206)	151 (104–184)	140 (109–184)	149 (123–186)
12m	131 (81–186)	133 (91–179)	127 (81–186)	127 (91–167)	139 (110–172)	141 (114–179)
15m	155 (107–198)	145 (108–196)	159 (120–197)	141 (108–196)	146 (107–198)	150 (136–162)
18m	154 (83–198)	147 (111–208)	164 (123–198)	147 (111–208)	130 (83–181)	147 (115–183)
24m	134 (73–194)	123 (79–180)	136 (81–190)	122 (79–180)	131 (73–194)	126 (103–163)

APPENDIX 2: SUPPLEMENTARY DATA FOR CHAPTER 3A

Supplementary S1: FFQ SCRIPT (PKU)

- This questionnaire asks you questions about what you/your child eats.
- I would like you to tell me about all the foods and drinks you/your child eats regularly.
- The questionnaire lists 69 commonly eaten foods, food groups or drinks.
- For each food/food group there is a portion size suggested e.g. teaspoons, tablespoons, bowl, glass, slice.
- A portion size booklet is also provided. This will help you complete the food diary. *(SHOW THEM THE BOOKLET)*
  *(there is a * next to the portions on the FFQ for which there is a picture(s) - for tablespoons or teaspoons refer to the picture on the front of the portion size booklet, for 1 glass (200ml) refer to the Drinks pictures in the portion size booklet)*

- I will ask you to tell me the number of times a 'food portion' is taken each day.
- If you/your child's eats twice the portion size shown, we will count this as 2 portions or if you eat half the portion size shown we will count it as ½ a portion.
- Then I will ask you the number of days a week this amount is eaten.
- If a food is eaten weekly but not daily, tell me how many portions per week.
- If a food is eaten less than once a week we will record it as '0'.
- For food items made up of different ingredients e.g. jam sandwich, I will need to ask you about what ingredients are used e.g. 2 slices low protein bread, 1 teaspoon margarine, 2 teaspoons jam.
- If there are food or drinks you/your child has regularly that are not listed, we can add these in.

General Question (for all entries):

- This is the *(name of heading in table e.g. MILK)* group
- How much/many *(read out food word-for-word from FFQ)* would you/they have a day in terms of *(read out portion size units from questionnaire: i.e.ml/g/slices/pots/tablespoons/teaspoons/pieces/cups/bowls/glasses/serves/packets/exchanges)* (refer to portion size in booklet were applicable). This is *(amount & type of food in picture in portion size booklet)*, how much would you/your child have a day compared with this? More or less than this?
- Do you/your child have this amount every day?
- If you/your child doesn't have it every day how many times a week would you/your child have this much?

(Only put one number in each box NOT a range – ask them to indicate what happens most often e.g. rather than 3-4 times a week specify only 3 or 4. Portion sizes can be in halves or whole numbers).

- Are there any other foods in that group that you/your child eats?

Example 1:
This is the *MILK* group.
How much Milk – full fat, semi-skimmed, skimmed, condensed, soya (include on cereal, in drinks e.g. tea, in foods e.g. mash potato) would you have a day in terms of ml? *(show them the picture of a glass of milk in the portion size booklet)* This is a 200ml glass of milk, so in total how much would you have a day compared with this? More or less than this? If you don't have it every day how many times a week would you have this much?

Example 2:
This is the *PASTA, RICE AND POTATO* group.
How much low protein pasta, noodles, rice, couscous (cooked) would you have a day in terms of grams? *(show them the picture of the low protein pasta in the portion size booklet).* This is 80g and 160g of low protein pasta, how much would you have a day compared with this? More or less than either of these amounts? If you don't have it every day how many times a week would you have this much?

Example 3:
This is the *SAVOURY SNACKS* group.
How much Potato crisps, corn snacks, rice snacks, popcorn would you have a day in terms of a small packet (15-25g)? Would you have more or less than one packet a day? Or if not every day, how many times a week?

ADDITIONAL QUESTIONS
Read questions out but show them the responses so they can indicate their answers. Indicate that they need to answer based on <u>what usually happens on most days</u>.
E.g.
Q.1. If a certain meal/snack is not eaten every day, but more often than not, then still tick the box. If not, leave it blank.
Q.3. If salt is <u>usually</u> added to food then tick yes.
Q.8/10. If protein exchanges/protein substitute are not always eaten at a particular time of day, only tick the box if it is more often than not.

Supplementary S2: FFQ SCRIPT (CONTROL)

- This questionnaire asks you questions about what you/your child eats.
- I would like you to tell me about all the foods and drinks you/your child eats regularly.
- The questionnaire lists 69 commonly eaten foods, food groups or drinks.
- For each food/food group there is a portion size suggested e.g. teaspoons, tablespoons, bowl, glass, slice.
- A portion size booklet is also provided. This will help you complete the food diary. *(SHOW THEM THE BOOKLET)*
  *(there is a * next to the portions on the FFQ for which there is a picture(s) - for tablespoons or teaspoons refer to the picture on the front of the portion size booklet, for 1 glass (200ml) refer to the Drinks pictures in the portion size booklet))*

- I will ask you to tell me the number of times a 'food portion' is taken each day.
- If you/your child's eats twice the portion size shown, we will count this as 2 portions or if you eat half the portion size shown we will count it as ½ a portion.
- Then I will ask you the number of days a week this amount is eaten.
- If a food is eaten weekly but not daily, tell me how many portions per week.
- If a food is eaten less than once a week we will record it as '0'.
- For food items made up of different ingredients e.g. jam sandwich, I will need to ask you about what ingredients are used e.g. 2 slices white bread, 1 teaspoon margarine, 2 teaspoons jam.
- If there are food or drinks you/your child has regularly that are not listed, we can add these in.

General Question (for all entries):

- This is the *(name of heading in table e.g. MILK)* group
- How much/many *(read out food word-for-word from FFQ)* would you/they have a day in terms of *(read out portion size units from questionnaire: i.e.ml/g/slices/pots/tablespoons/teaspoons/pieces/cups/bowls/glasses/serves/packets)* (refer to portion size in booklet were applicable). This is *(amount & type of food in picture in portion size booklet)*, how much would you/your child have a day compared with this? More or less than this?
- Do you/your child have this amount every day?
- If you/your child doesn't have it every day how many times a week would you/your child have this much?

(Only put one number in each box NOT a range – ask them to indicate what happens most often e.g. rather than 3-4 times a week specify only 3 or 4. Portion sizes can be in halves or whole numbers).

- Are there any other foods in that group that you/your child eats?

Example 1:
This is the *MILK* group.
How much Milk – full fat, semi-skimmed, skimmed, condensed, soya (include on cereal, in drinks e.g. tea, in foods e.g. mash potato) would you have a day in terms of ml? *(show them the picture of a glass of milk in the portion size booklet)* This is a 200ml glass of milk, so in total how much would you have a day compared with this? More or less than this? If you don't have it every day how many times a week would you have this much?

Example 2:
This is the *PASTA, RICE AND POTATO* group.
How much pasta, noodles, rice, couscous (cooked) would you have a day in terms of grams? *(show them the picture of the pasta in the portion size booklet).* This is 80g and 160g of pasta, how much would you have a day compared with this? More or less than either of these amounts? If you don't have it every day how many times a week would you have this much?

Example 3:
This is the *SAVOURY SNACKS* group.
How much Potato crisps, corn snacks, rice snacks, popcorn would you have a day in terms of a small packet (15-25g)? Would you have more or less than one packet a day? Or if not every day, how many times a week?

ADDITIONAL QUESTIONS
Read questions out but show them the responses so they can indicate their answers. Indicate that they need to answer based on <u>what usually happens on most days</u>.
E.g.
Q.1. If a certain meal/snack is not eaten every day, but more often than not, then still tick the box. If not, leave it blank.
Q.3. If salt is <u>usually</u> added to food then tick yes.
Q.8/10. If protein exchanges/protein substitute are not always eaten at a particular time of day, only tick the box if it is more often than not.

Supplementary S3: SCRIPT FOR 24 DIETARY RECALL (PKU)

TO BE COMPLETED BY THE RESEARCHER BY INTERVIEW (FACE-TO-FACE OR TELEPHONE) WITH SAME PARENT EACH TIME (5-10Y CHILD) OR WITH THE CHILD ONLY (11-16Y)

We want to find out what you/your child eats and drinks on a typical day.

This includes all food, drinks, snacks and supplements eaten at home or outside the home including school (if not known can we ask your child?).

We also need to know how much was eaten – e.g. number of slices, tablespoons, teaspoons, cups, grams, ounces, ml or exchanges.

Only include the amount of food actually eaten, not what was offered.

We also need to know:

- brand names of products if known
- types of food (e.g. low protein, gluten-free or regular; type of milk: full fat/semi-skim/skim; type of spread: low fat/regular; type of bread: wholemeal/ white bread/half 'n half)
- how foods were cooked (e.g. fried, grilled, baked, boiled).
- If you can provide recipes or estimate quantities for home cooked foods.

We want to record everything you/your child had to eat or drink yesterday.

Was yesterday a normal day? i.e. no parties, celebration day, illness.

If yesterday was not normal can you tell me about the day before (or the last 'normal' day you can recall).

What day of the week was this? *(circle the day at the top of the diary page)*.

Starting with the beginning of the day, approximately what time was the first food or drink taken? *(record estimated time e.g. 8am)*.

What food/drink was taken first?

- **Brand**, **type**, **low protein/gluten-free**, **how cooked/prepared**, **amount** eaten, **recipe** if applicable?

What other food/drink was taken at that time?

- **Brand**, **type**, **low protein/gluten-free**, **how cooked/prepared**, **amount** eaten, **recipe** if applicable?

REPEAT ABOVE QUESTIONS UNTIL ALL FOOD/DRINK CONSUMED AT THAT TIME IS LISTED

Check: did you/they have any:

- cereal/toast/low protein milk (**Brand**, **type**, **low protein/gluten-free**, how **cooked/prepared**).
- spreads on bread/toast? (**Brand**, **type**, **amount)**.
- drinks e.g. drink/juice/water/milk/low protein milk? What type of cup, glass, mug or carton?
- anything added to food e.g. salt, seasoning, butter
- protein substitute (**Brand**, **amount** taken).

Any other comments about that meal? *(e.g. how they felt, eaten later/earlier than usual).*

REPEAT THE FOLLOWING SCRIPT FOR EACH SEPARATE MEAL/SNACK CONSUMED

When is the next time you/they have something to eat/drink? (record estimated time).

What food/drink was taken?

- **Brand**, **type**, **low protein/gluten-free**, **how cooked/prepared**, **amount** eaten, **recipe** if applicable?

What other food/drink was taken at that time?

- **Brand**, **type**, **low protein/gluten-free**, **how cooked/prepared**, **amount** eaten, **recipe** if applicable?

Did you/they have any:

- bread/vegetables/fruit/yoghurt/crisps/biscuits/pudding/other snacks/low protein milk (**Brand**, **type**, **low protein/gluten-free**, how **cooked/prepared**).
- spreads on bread/toast? (**Brand**, **type**, **amount)**.
- drinks e.g. juice/water/milk/low protein milk? What type of cup, glass, mug or carton?
- anything added to food e.g. salt, seasoning, butter
- protein substitute (**Brand**, **amount** taken).

Do you/they have any bedtime drinks or any drinks overnight?

Any other comments about that meal? e.g. how they felt, eaten later/earlier than usual.

Now I am going to read back what you have told me for each meal and snack, and if there is anything missing or if anything is incorrect please let me know.

READ THROUGH EACH MEAL/SNACK CHECKING FOOD/DRINK, <u>QUANTITY</u>, <u>BRAND</u>, <u>TYPE</u> (LOW PROTEIN/GLUTEN-FREE/REGULAR), <u>COOKING METHOD</u>. CHECK THAT ALL PROTEIN SUBSTITUTE AND LOW PROTEIN MILK IS RECORDED.

---

24 HOUR DIETARY RECALL

SUBJECT NO: _____

INITIALS: _____

DATE: _____

Please circle the correct day: **Mon/ Tue / Wed / Thu / Fri / Sat / Sun**

Time of day	**Amount eaten** g / oz / ml / slices / tbsp / tsp	**Type of food / drink / protein substitute** Include brand names, method of cooking, recipes, type (e.g. low protein, skim, low fat)	**Comments**

EXAMPLE:

Please circle the correct day: **Mon/ Tue / Wed / Thu / Fri / Sat / Sun**

Time of day	**Amount eaten** g / oz / ml / slices / tbsp / tsp	**Type of food / drink / protein substitute** Include brand names, method of cooking, recipes, type (e.g. low protein, skim, low fat)	**Comments**
e.g. 8am	174ml Small bowl 100ml	PKU Cooler 20 Low protein cereal (Loprofin strawberry flakes) Prozero	Breakfast later than usual
11am	1 47g 1 glass	Apple Yoghurt (Petit Filous) Sugar free squash	
1.00-1.30pm	2 slices 2 tsp 2 tsp 16g packet 174ml	Low protein bread (Mevalia) Butter jam Quavers PKU Cooler 20	Only ate half
3.30pm	2 200ml	Low protein biscuits (wafers) Sugar free squash	
5.30-6.00pm	1 1 ½ exchanges 60g 2 tbsp 174ml 1 glass	Low protein sausage Potato chips (oven baked) Broccoli Carrots PKU Cooler 20 water	
7pm	1	Mevalia fruit bar	

Supplementary S4: SCRIPT FOR 24 DIETARY RECALL (CONTROL)

TO BE COMPLETED BY THE RESEARCHER BY INTERVIEW (FACE-TO-FACE OR TELEPHONE) WITH SAME PARENT EACH TIME (5-10Y CHILD) OR WITH THE CHILD ONLY (11-16Y)

We want to find out what you/your child eats and drinks on a typical day.

This includes all food, drinks, snacks and supplements eaten at home or outside the home including school (if not known can we ask your child?).

We also need to know how much was eaten – e.g. number of slices, tablespoons, teaspoons, cups, grams, ounces or ml.

Only include the amount of food actually eaten, not what was offered.

We also need to know:

- brand names of products if known
- types of food (e.g. type of milk: full fat/semi-skim/skim; type of spread: low fat/regular; type of bread: wholemeal/ white bread/half 'n half)
- how foods were cooked (e.g. fried, grilled, baked, boiled).
- If you can provide recipes or estimate quantities for home cooked foods.

We want to record everything you/your child had to eat or drink yesterday.

Was yesterday a normal day? i.e. no parties, celebration day, illness.

If yesterday was not normal can you tell me about the day before (or the last 'normal' day you can recall).

What day of the week was this? *(circle the day at the top of the diary page).*

Starting with the beginning of the day, approximately what time was the first food or drink taken? *(record estimated time e.g. 8am).*

What food/drink was taken first?

- **Brand**, **type**, **how cooked/prepared**, **amount** eaten, **recipe** if applicable?

What other food/drink was taken at that time?

- **Brand**, **type**, **how cooked/prepared**, **amount** eaten, **recipe** if applicable?

REPEAT ABOVE QUESTIONS UNTIL ALL FOOD/DRINK CONSUMED AT THAT TIME IS LISTED

Check: did you/they have any:

- cereal/toast (**Brand**, **type**, **how cooked/prepared**).
- spreads on bread/toast? (**Brand**, **type**, **amount**).
- drinks e.g. juice/water/milk? What type of cup, glass, mug or carton?

- anything added to food e.g. salt, seasoning, butter

Any other comments about that meal? *(e.g. how they felt, eaten later/earlier than usual).*

REPEAT THE FOLLOWING SCRIPT FOR EACH SEPARATE MEAL/SNACK CONSUMED

When is the next time you/they have something to eat/drink? (record estimated time).

What food/drink was taken?

- **Brand**, **type**, **how cooked/prepared**, **amount** eaten, **recipe** if applicable?

What other food/drink was taken at that time?

- **Brand**, **type**, **how cooked/prepared**, **amount** eaten, **recipe** if applicable?

Did you/they have any:

- bread/vegetables/fruit/yoghurt/crisps/biscuits/puddings/other snacks (**Brand**, **type**, **how cooked/prepared**).
- spreads on bread/toast? (**Brand**, **type**, **amount**).
- drinks e.g. juice/water/milk/low protein milk? What type of cup, glass, mug or carton?
- anything added to food e.g. salt, seasoning, butter

Do you/they have any bedtime drinks or any drinks overnight?

Any other comments about that meal? e.g. how they felt, eaten later/earlier than usual.

Now I am going to read back what you have told me for each meal and snack, and if there is anything missing or if anything is incorrect please let me know.

READ THROUGH EACH MEAL/SNACK CHECKING FOOD/DRINK, QUANTITY, BRAND, TYPE, COOKING METHOD.

---

24 HOUR DIETARY RECALL

SUBJECT NO: _____

INITIALS: _____

DATE: _____

Please circle the correct day: **Mon/ Tue / Wed / Thu / Fri / Sat / Sun**

Time of day	**Amount eaten** g / oz / ml / slices / tbsp / tsp	**Type of food / drink / protein substitute** Include brand names, method of cooking, recipes, type (e.g. skim, low fat)	**Comments**

EXAMPLE:

Please circle the correct day: **Mon/ Tue / Wed / Thu / Fri / Sat / Sun**

Time of day	**Amount eaten** g / oz / ml / slices / tbsp / tsp	**Type of food / drink / protein substitute** Include brand names, method of cooking, recipes, type (e.g. low protein, skim, low fat)	**Comments**
e.g. 8am	200ml Small bowl 100ml	Orange juice cereal (Special K) Milk (Skim)	Breakfast later than usual
11am	1 47g 1 glass	Apple Yoghurt (Petit Filous) Sugar free squash	
1.00-1.30pm	2 slices 2 tsp 2 tsp 16g packet 200ml	bread (white) Butter jam Quavers water	Only ate half
3.30pm	2 200ml	biscuits (chocolate digestive) Sugar free squash	
5.30-6.00pm	2 15 60g 2 tbsp 200ml 1 glass	sausages Potato chips (oven baked) Broccoli Carrots Sugar free squash water	
7pm	1 square	Flap jack	

Table S1: Gender, age and mean z-scores for BMI, weight and height for PKU and control children

	PKU Group	Control Group	P value*
Gender	24M, 26F	24M, 26F	0.99
Mean age years (range)	9.3 (4.2-16.4)	9.3 (4.2-16.6)	0.99
Mean weight z-score (range)	0.76 (−2.0–2.4)	0.66 (−2.3–2.6)	0.54
Mean height z-score (range)	0.26 (−2.4–2.4)	0.60 (−2.1–2.8)	0.14
Mean BMI z-score (range)	0.87 (−1.0–2.9)	0.47 (−3.4–2.7)	0.17

NS=not significant; *Mann Whitney

Table S2: FFQ food items mean g/week FFQ1 vs FFQ2 (PKU and Control)

	No. children consuming FFQ1/ FFQ2 n=50	PKU FFQ1	PKU FFQ 2	Wilcoxon signed rank	Spearman rank correlation	FFQ1/ FFQ2 n=50	Control FFQ 1	Control FFQ 2	Wilcoxon signed rank	Spearman rank correlation
	n	Mean g/week	Mean g/week	p	r	n	Mean g/week	Mean g/week	p	r
Milk, cows	2/3	12.6	15	>0.99	0.82	48/46	1882	1782	0.41	0.71
plant milk	4/7	49	67.2	0.06	0.77	1/1	56	56	0.99	1.0
LP milk	42/40	1421	1509	0.54	0.92					
milk flavouring	6/8	3.3	6.3	0.07	0.52	18/16	14.1	14.3	0.51	0.68
cheese	3/3	3.7	1.8	0.50	0.99	47/45	88.2	87.4	0.75	0.60
soft cheese	5/4	6.6	5.9	0.62	0.89	9/15	6.8	12.8	**0.04**	0.67
LP cheese	31/34	66.4	80.4	0.10	0.66					
soya cheese	1/0	1.2	0	>0.99	-					
dairy desserts	18/17	70	51.6	0.22	0.78	44/41	254	182	**0.0002**	0.72
LP desserts	6/10	21.6	23.4	0.82	0.46					
icecream	12/15	22.8	20.4	0.48	0.60	31/27	46.8	49.2	0.37	0.61
LP ice cream	12/10	34.5	12.6	**0.03**	0.42					
milk based ice lolly	6/7	11.7	9	0.68	0.43	4/6	3.6	6.3	0.38	0.54
fruit based ice lolly	21/20	62.1	58.5	0.51	0.56	14/15	44.1	40.5	0.60	0.45
cream	2/2	1.2	1.8	>0.99	0.47	6/11	6.6	11.4	0.09	0.57
bread	2/3	14.4	19.8	0.50	0.82	49/44	281	242	0.12	0.61
rolls etc	3/1	3	2	>0.99	0.04	30/35	136	142	0.97	0.79
LP bread	45/45	441	421	0.80	0.78					
LP rolls etc	17/24	58	61	0.08	0.39					
GF bread	10/7	19.8	7.8	**0.04**	0.52					
GF rolls etc	4/7	9	9.5	0.98	0.50					
Breakfast cereal - wheat	14/20	23.1	22.4	0.60	0.76	34/30	97.2	73.2	**0.02**	0.73
Breakfast cereal - corn/rice/ oat	29/24	49.5	33.2	**0.04**	0.72	31/35	54.6	49.8	0.43	0.79
Oats/porridge						13/11	90.8	93.8	0.93	0.58
LP breakfast cereal	9/7	9.6	12.9	0.50	0.71					
sweet spreads	26/28	40.2	32.7	0.27	0.83	27/25	17.2	12.5	0.26	0.64
chocolate/nut spread	13/12	24.2	10.8	0.67	0.49	19/19	17.4	15.8	0.74	0.85
LP chocolate spread	9/8	6.2	6.4	>0.99	0.48					
sandwich spread	2/1	2	0.8	0.50	0.71	15/13	15.8	11.4	0.34	0.71
LP savoury spread	3/6	2.4	8.4	0.06	0.45					

butter/ margarine/ oil	47/44	86.9	84.9	0.92	0.64	50/47	50.1	43.6	0.42	0.31
Low fat spread	0/3	0	3.4	0.25	-	6/4	3.3	2.6	0.93	0.32
Pasta/ rice/ couscous	15/14	33.3	34.2	0.80	0.75	49/49	331	334	0.86	0.51
LP pasta/ rice/ couscous	43/43	369	376	0.77	0.81					
tinned pasta	17/9	56	26	**0.003**	0.50	5/8	17.6	27.2	0.86	0.43
LP ready meal / Ready meal	4/4	16	24	0.77	0.20	9/5	24	16	0.52	0.55
potato - boil/ mash/ jacket	42/36	201	189	0.69	0.57	41/44	234	222	0.55	0.50
potato - roast/ chips/ fried	44/46	207	186	0.26	0.51	47/44	176	151	0.34	0.39
potato - processed	28/27	60.3	54.6	0.63	0.67	19/18	25.8	27	0.83	0.94
table sauce	38/37	92.4	87.4	0.38	0.79	44/44	64.4	70.4	0.34	0.55
gravy	27/26	60	52	0.80	0.70	32/31	54.5	42	0.06	0.72
mayonnaise/ dressing	26/26	60.4	42.4	0.25	0.92	13/18	12.4	18.8	**0.007**	0.62
milk sauces	6/4	8.5	9	0.65	0.12	17/16	25	23	0.73	0.50
LP sauce	3/2	8	3	>0.99	0.38					
pasta sauce	32/34	187	152	0.58	0.38	35/39	123	132	0.32	0.51
soup	27/25	238	266	0.77	0.69	14/16	94	80	0.78	0.49
vegetables >100mg Phe/100g	30/34	59.4	73.5	**0.03**	0.68	49/48	263	275	0.48	0.68
vegetables 75-100mg Phe/100g	36/35	113	118	0.73	0.68	39/41	184	202	0.90	0.80
vegetables <75mg Phe/100g	50/49	625	805	**0.01**	0.76	50/50	554	563	0.70	0.59
legumes	15/16	31.8	24.8	0.77	0.85	37/37	154	140	0.80	0.82
fruit	45/44	915	941	0.79	0.74	48/50	939	954	0.62	0.65
dried fruit	17/16	35.4	33.9	0.78	0.35	18/21	31.8	33.9	0.78	0.52
meat						48/48	303	278	0.36	0.57
fish						35/33	78	72.6	0.43	0.81
processed meat/fish						50/49	198	182	0.31	0.49
ham/sandwich slices						33/35	37.7	38.7	0.83	0.76
LP burger/ sausage	23/26	135	168	0.95	0.82					
veg burger/sausage/tofu/quorn	13/15	52.5	36	0.90	0.66	6/8	6	9	0.53	0.50
eggs	0/0	0	0	-	-	29/27	74.5	65	0.49	0.84
Pizza	3/3	8.4	11.2	>0.99	0.65	41/36	107	77.2	**0.02**	0.52
LP pizza	30/31	108	103	0.75	0.69					
meat pie/ pastie	1/0	0.5	0	>0.99	-	26/25	29	30	0.95	0.33
vegetable pie/ pastie	7/6	13	10	0.68	0.25	1/2	1	2	>0.99	0.03
meat curry/ chinese/ lasagne	0/0	0	0	-	-	27/25	126	104	0.44	0.31
vegetable curry/chinese/lasagne	16/15	160	64	0.12	0.47	3/3	12	10	>0.99	0.30
water	47/45	4633	5096	0.73	0.48	43/46	3766	3700	0.62	0.69
fruit juice	28/26	648	520	0.75	0.57	27/22	568	572	0.27	0.75
smoothie	10/11	76	118	0.17	0.58	12/11	112	100	0.54	0.74
sweet drinks	33/32	1176	1686	**0.01**	0.64	17/15	372	305	0.35	0.76
sugar-free drinks	11/9	580	420	0.72	0.43	33/33	2252	2394	0.33	0.75
tea/ coffee	17/19	316	416	0.15	0.79	11/11	184	184	0.78	0.61
hot chocolate powder	3/1	0.9	0.3	0.50	0.57	17/13	9.3	16.2	0.16	0.66
non-dairy creamer	0/2	0	0.9	0.50	-	0/0	0	0	-	-
biscuits	15/15	13.5	22.5	0.21	0.38	43/43	87.2	77.4	0.26	0.66
cake	9/8	20.5	15	0.44	0.54	40/38	123	116	0.81	0.59
cereal bar	20/17	19.6	19.6	0.82	0.77	23/24	31.2	27.2	0.51	0.50
LP biscuit	30/27	58.8	50.1	0.52	0.59					
LP cake	29/30	139	118	0.23	0.66					
LP cereal bar	13/18	38.4	33.2	0.87	0.56					

puddings	1/3	1.6	14.4	0.38	0.04	22/27	81.6	76.8	0.82	0.52
LP puddings	10/9	40	32	0.91	0.24					
jelly	1/1	2	4	>0.99	0.02	10/10	36	38	0.94	0.75
LP jelly	17/23	118	108	0.89	0.71					
chocolate	9/6	12	6.3	0.12	0.65	44/45	76.2	76.2	0.88	0.52
LP chocolate	25/24	34.8	39	0.59	0.58					
gummy sweets	1/3	4.2	7.8	0.50	0.59	26/28	34.8	39.6	0.25	0.73
vegetarian gummy sweets	28/29	53.1	75.9	0.86	0.09					
other sweets	11/15	30.6	29.9	0.99	0.11	10/7	9.6	14.4	0.56	0.69
sugar	19/17	19.0	15.9	0.95	0.61	10/6	5.6	4.35	0.25	0.66
crisps	45/45	72.3	67.2	0.23	0.81	43/42	50.1	48.3	0.17	0.85
nuts/ seeds	0/0	0	0	-	-	9/9	3.8	2	0.18	0.44
LP crisps	4/2	7	2.5	0.63	0.66					
crackers	8/6	5.4	6.2	0.95	0.23	27/26	14.5	11	**0.02**	0.80
LP crackers	16/13	9.2	8.1	0.49	0.74					

n = number respondents consuming that food item; bule: PKU foods only; purple: Control foods only;
-: no; LP=low protein; GF=gluten free; Phe=phenylalanine;

Table S3: FFQ items mean g/week FFQ vs Dietary recalls (PKU and Control)

	No. children consuming food FFQ/ Recall	PKU FFQ average	PKU dietary recalls	Wilcoxon signed rank (p)	Spearman rank correlation (r)	No. children consuming food FFQ/ Recall	Control FFQ average	Control dietary recalls	Wilcoxon signed rank (p)	Spearman rank correlation (r)	Mann Whitney (p) FFQ average PKU vs Control
		Mean g/week	Mean g/week				Mean g/week	Mean g/week			
Milk, cows	3/4	13.8	18.5	0.37	0.87	48/45	1832	1639	0.06	0 79	**<0.0001**
plant milk	7/7	58.0	34.4	0.23	0.54	1/2	56	40	>0.99	0.69	0.06
LP milk	42/38	1465	1105	<0.0001	0.93						
milk flavouring	10/4	4.8	3.8	0.22	0.26	22/17	14.2	12.3	0.52	0 50	0.13
cheese	3/3	2.8	1.9	0.75	>0.99	48/44	87.8	90	0.95	0.39	**<0.0001**
soft cheese	5/4	6.2	12.0	0.19	0.90	16/12	9.8	12.1	0.84	0.29	**0.02**
LP cheese	35/31	73.4	102	0.18	0.77						
soya cheese	1/0	0.6	0	>0.99	-						
dairy desserts	21/21	60.8	86.1	0.16	0.71	46/42	218	238	0.59	0.61	**<0.0001**
LP desserts	12/6	22.5	24.9	0.95	0.01						
icecream	18/7	21.6	15.8	0.24	0.37	35/19	48.0	57	0.91	0.20	0.07
LP ice cream	16/7	23.6	18.2	0.26	0.47						
milk based ice lolly	10/4	10.4	10.2	0.72	0.05	7/15	5.0	40.9	**<0.0001**	0.52	0.36
fruit based ice lolly	27/10	60.3	26.3	**0.01**	0.40	21/7	42.3	26.8	0.13	0.42	**0.03**
cream	3/1	1.5	4.2	0.88	0.03	12/10	9.0	9.1	0.70	0.46	**0.01**
bread	3/9	17.1	28.0	**0.008**	0.65	50/48	261	309	0.06	0.48	**<0.0001**
rolls etc	4/0	2.5	0	0.13	-	35/40	139	204	**0.04**	0.49	**<0.0001**
LP bread	45/45	431	474	0.54	0.64						
LP rolls etc	28/15	59.5	47.7	0.66	0.46						
GF bread	12/7	13.8	20.4	0.67	0.56						
GF rolls etc	8/5	9.3	12.3	0.44	0.79						
Breakfast cereal - wheat	21/16	22.8	27.9	0.20	0.71	37/39	85.2	135	**0.05**	0.58	**0.002**
Breakfast cereal-corn/rice/oat	31/27	41.3	44.4	0.36	0.75	35/24	52.2	40	**0.02**	0.63	0.12
Oats/porridge						16/7	92.3	103	0.62	0.71	
LP breakfast cereal	10/7	11.3	14.7	0.77	0.38						
sweet spreads	30/24	36.5	31.0	0.19	0.78	31/31	14.8	18.5	0.46	0.45	**0.05**
chocolate/nut spread	18/6	17.5	10.1	0.27	0.26	21/16	16.6	16.3	0.69	0.61	0.63
LP chocolate spread	12/3	6.3	5.0	0.06	0.52						

sandwich spread	2/1	1.4	2.8	>0.99	0.71	17/6	13.6	7.4	**0.03**	0.41	**0.002**
LP savoury spread	7/0	5.4	0	**0.02**	-						
butter/ margarine/ oil	48/44	85.9	58.4	<0.0001	0.63	50/46	46.8	46.1	0.74	0.28	**<0.0001**
Low fat spread	3/0	1 7	0	0 25	-	8/1	3.0	0.5	**0.008**	0.38	0.61
Pasta/ rice/ couscous	18/11	33.8	46.7	0 85	0.68	49/44	332	344	0.70	0.31	**<0.0001**
LP pasta/ rice/ couscous	46/34	372	434	0 24	0.74						
tinned pasta	19/4	41	23.4	**0.009**	0 52	10/4	22.4	19.5	0.65	0.43	**0.04**
LP ready meal / Ready meal	7/1	20	5.3	0.14	0.06	10/2	20	5.6	**0.04**	0.41	0.66
potato - boil/ mash/ jacket	43/24	195	145	**0.005**	0 74	47/32	228	154	**0.004**	0.52	0.47
potato - roast/ chips/ fried	47/40	196	231	0.21	0.37	48/38	164	168	0.79	0.32	0.12
potato - processed	32/20	57.5	81.7	0.11	0 73	19/10	26.4	29.5	0.86	0.46	**0.02**
table sauce	39/33	89.9	56.6	**0.0003**	0.68	44/31	67.4	37.3	**0.0008**	0.27	0.55
gravy	30/14	56	44.1	0.13	0.41	34/26	48.3	44.9	0.49	0.47	0.64
mayonnaise/ dressing	28/25	51.4	30.9	**0.04**	0.67	21/16	15.6	13.5	0.39	0.48	**0.02**
milk sauces	9/4	8 8	10.4	0.96	0 25	23/5	24	13 2	**0.02**	0.42	**0.05**
LP sauce	4/3	5.5	2.1	0.44	0.53						
pasta sauce	40/33	170	173	0 84	0.43	45/27	128	97	0.10	0.01	0.75
soup	30/21	252	262	0 21	0 54	20/6	87	40.6	**0.01**	0.52	**0.001**
vegetables >100mg Phe/100g	36/19	66.5	29.2	**<0.0001**	0.61	49/42	269	163	**0.0001**	0.48	**<0.0001**
vegetables 75-100mg Phe/100g	37/21	116	86.5	**0.05**	0.60	44/25	193	71.7	**<0.0001**	0.55	**0.03**
vegetables <75mg Phe/100g	50/48	715	794	0.37	0.42	50/47	558	432	**0.002**	0.62	0.22
legumes	17/13	28.3	26.8	0.47	0 70	38/28	147	158	0.72	0.52	**<0.0001**
fruit	46/43	928	958	0 89	0 75	50/47	946	842	0.07	0.68	0.68
dried fruit	24/17	34.7	30.3	0.17	0.53	26/17	32.9	20	**0.01**	0.60	0.79
meat						48/48	290	314	0.22	0 50	
fish						38/15	75.3	54.0	**0.007**	0.52	
processed meat/fish						50/46	190	256	0.21	0.19	
ham/sandwich slices						37/30	38.2	45.9	0.61	0.56	
LP burger/ sausage	27/22	152	114	0.16	0.66						
veg burger/sausage/tofu/quorn	18/12	44.3	47.4	0.86	0 35	10/3	7.5	13.9	0.80	0.29	**0.03**
eggs	0/1	0	0.4	>0.99	-	31/22	69.8	85.6	0.92	0.48	**<0.0001**
Pizza	4/2	9.8	6.1	0.81	0.31	42/23	92.2	116	0.90	0.09	**<0.0001**
LP pizza	34/12	106	111	**0.04**	0 50						
meat pie/ pastie	1/0	0 3	0	>0.99	-	34/15	29 5	53.4	0.52	0.28	**<0.0001**
vegetable pie/ pastie	11/5	11.5	10.6	0.51	0.31	3/3	1 5	3.4	0 31	0.29	**0.01**

meat curry/ chinese/ lasagne	0/0	0	0	-	-	34/16	115	114	0.54	0.23	**<0.0001**
vegetable curry/chinese/lasagne	21/19	112	159	0.08	0.49	5/3	11	12.6	0.78	0.51	**<0.0001**
water	47/49	4865	3636	0.0005	0.60	47/44	3733	2560	**<0.0001**	0.60	**0.01**
fruit juice	32/25	584	433	0.29	0.52	28/29	570	460	0.31	0.78	0.23
smoothie	14/6	97	72	0.06	0.68	14/6	106	54.0	**0.05**	0.34	0.57
sweet drinks	39/43	1431	1701	0.08	0.65	18/23	339	495	**0.02**	0.64	**<0.0001**
sugar-free drinks	15/7	500	274	**0.005**	0.37	34/33	2323	1781	**0.01**	0.88	**<0.0001**
tea/ coffee	21/15	366	274	0.11	0.77	14/10	184	234	0.34	0.62	**0.02**
hot chocolate powder	3/0	0.6	0	0.25	-	20/9	12.8	8.8	0.08	0.48	**0.002**
non-dairy creamer	2/0	0.5	0	0.50	-	0/0	0	0	-	-	0.49
biscuits	22/16	18	19.8	0.95	0.54	46/44	82.3	115	**0.01**	0.50	**<0.0001**
cake	12/18	17.8	25.1	0.41	0.54	44/44	120	132	0.81	0.17	**<0.0001**
cereal bar	21/9	19.6	10.6	**0.04**	0.60	29/17	29.2	44.1	0.39	0.45	0.34
LP biscuit	34/22	54.5	38.6	0.29	0.33						
LP cake	35/27	129	118	0.40	0.59						
LP cereal bar	21/14	35.8	62.6	0.49	0.45						
puddings	4/1	8.0	1.4	0.25	0.46	32/11	79.2	34.7	**0.0004**	0.43	**<0.0001**
LP puddings	15/8	36	13.8	**0.02**	0.41						
jelly	2/0	3	0	0.50	-	12/10	37	45.5	0.62	0.69	**0.006**
LP jelly	25/8	113	51.1	**0.003**	0.53						
chocolate	10/8	9.2	13.7	0.50	0.65	47/36	76.2	74.1	0.41	0.22	**<0.0001**
LP chocolate	29/13	36.9	23.7	0.06	0.43						
gummy sweets	3/3	6.0	1.6	0.40	0.06	31/14	37.2	11.0	**0.0002**	0.19	**<0.0001**
vegetarian gummy sweets	38/10	64.5	9.1	**<0.0001**	0.21						
other sweets	22/21	30.2	28.1	0.34	0.64	11/10	12.0	9.8	0.89	0.37	**0.002**
sugar	23/21	17.5	17.3	0.54	0.50	11/13	5.0	5.9	0.30	0.56	**0.0004**
crisps	49/42	69.8	99.6	**0.004**	0.64	43/43	49.2	75.1	**0.0009**	0.66	**0.01**
nuts/ seeds	0/0	0	0	-	-	13/3	2.9	1.7	0.14	0.15	**0.003**
LP crisps	4/2	4.8	3.5	0.87	0.72						
crackers	12/12	5.8	15.5	**0.04**	0.69	29/15	12.8	14.8	0.66	0.29	**0.002**
LP crackers	18/8	8.7	10.3	0.59	0.46						

n = number respondents consuming that food; bule: PKU foods only; purple: Control foods only; -: no; LP=low protein; GF=gluten free; Phe=phenylalanine

Table S4: Kappa coefficient statistics FFQ vs Dietary Recalls

	Kappa Score	P-value	Level of Agreement
Energy kJ	0.35	<0.001	Fair
Carbohydrate	0.51	<0.001	Moderate
Protein	0.49	<0.001	Moderate
Fat	0.21	0.03	Fair
Starch	0.52	<0.001	Moderate
Fibre	0.33	<0.001	Fair
Sugars	0.44	<0.001	Moderate

LOW PROTEIN

FOOD FREQUENCY QUESTIONNAIRE

DATE: _____

SUBJECT NUMBER: _____

INITIALS: _____

DOB: _____

Version 1: 21.9.17

DATE:
SUBJECT NO:
INITIALS:

FOOD FREQUENCY QUESTIONNAIRE

- **This questionnaire asks you questions about what you/your child eats.**
- **We would like you to tell us about <u>all</u> foods/drinks <u>regularly</u> eaten by you/your child.**
- **The questionnaire lists 90 commonly eaten foods, food groups or drinks.**
- **For each food/food group there is a portion size suggested e.g. teaspoon, tablespoon, bowl, glass, slice.**
- **A portion size booklet is also provided. This will help you complete the food diary.**

PLEASE MAKE SURE YOU PUT <u>A NUMBER IN EVERY BOX ON EVERY LINE</u> IN THE QUESTIONNAIRE.

- Write the number of times a 'food portion' is taken each day in the <u>'in a DAY'</u> box.
- If you/your child's <u>portion size</u> is double the suggested size, count this as 2 portions or if their portion size is smaller count it as ½ a portion.
- Then write the number of days a week this amount is eaten in the <u>'per WEEK'</u> box.
- If a food is eaten <u>less than once a week</u> then put a '0' in both boxes for this food.
- For food items made up of different ingredients e.g. jam sandwich, divide into individual ingredients e.g. 2 slices white bread, 1 teaspoon margarine, 2 teaspoons jam.
- If there are food/drinks you/your child has regularly that are not listed, add these in the last section of each food group under 'other'.

DATE:
SUBJECT NO:
INITIALS:

FOR EXAMPLE:

If you/your child has:

- **½ a small pot of yoghurt once a day,**
- **a jam sandwich (2 slices of bread with butter and jam) 5 days a week,**
- **fruit once every 2 weeks**

your answers should look like this:

Food	Portion size	Number of portions in a DAY	Number of times per WEEK
Regular Flavoured or plain yoghurt	1 small pot (60g)	½	7
Regular Bread – sliced, toast	1 slice (30-40g)	2	5
Butter/margarine/oils	1 teaspoon (5g)	2	5
Sweet spreads e.g. Jam/marmalade/honey/icing	2 teaspoons (10g)	1	5
All fresh, canned or frozen fruit	1 piece (80-100g)	0	0

DATE:
SUBJECT NO:
INITIALS:

MILK AND MILK REPLACEMENTS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Regular Milk – full fat, semi-skimmed, skimmed, condensed, soya (include on cereal, in drinks e.g. tea, in foods e.g. mash potato)	30ml*		
Other plant milks – coconut, oat, rice, almond, hemp (include on cereal, in drinks e.g. tea, in foods e.g. mash potato)	200-250 ml		
Low protein milk replacement e.g. Prozero, Sno Pro, Loprofin, Dalia (include on cereal, in drinks e.g. tea, in foods e.g. mash potato)	200-250 ml*		
Milk flavouring e.g. chocolate, strawberry, hot chocolate powder	1 tablespoon (15ml)		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DAIRY PRODUCTS AND ALTERNATIVES			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Regular Cheese e.g. cheddar, Edam, Emmental	15g (1 small cube)*		
Regular soft cheese, cream cheese, cheese spread	15-20g (1 tablespoon)		
Low protein cheese e.g. Violife, Tesco Free-From	1 slice (20g)*		
Soya cheese	15g (1 small square)		
Regular flavoured or plain yoghurt, drinking yoghurt, soya yoghurt, fromage frais, dairy desserts, custard, mousse, instant dessert	Small pot (40-60g)*		
Low protein yoghurt (e.g. coconut yoghurt), protein-free custard, mousse, dessert mix	1 small serve (60g)*		
Regular ice cream	30g*		
Low protein ice cream/sorbet e.g Alpro coconut icecream	30g		
Ice lolly – milk based	1 lolly (45-80ml)		
Ice lolly – fruit/juice flavour	1 lolly (45-80ml)		
Cream (all types)	30ml (1 tablespoon)		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DATE:
SUBJECT NO:
INITIALS:

BREADS AND CEREALS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Regular Bread – sliced, toast, small wrap	1 slice (30g-40g)*		
Regular bread roll, English muffin, crumpet, pitta, naan, ciabatta, large wrap	1 small (50-70g)		
Low protein bread- sliced, toast, small wrap	1 slice (30g-40g)*		
Low protein bread roll, English muffin, crumpet, pitta, naan, ciabatta, large wrap	1 small (50-70g)*		
Gluten-free bread- sliced, toast, small wrap	1 slice (30g-40g)		
Gluten-free bread roll, English muffin, crumpet, pitta, naan, ciabatta, large wrap	1 small (50-70g)		
Regular Breakfast cereals – **wheat based** e.g. muesli branflakes, multigrain, shredded wheat, Weetabix, gluten-free	10g*		
Regular or gluten-free Breakfast cereal – **corn, rice or oat based** e.g. Cornflakes, Rice krispies, Cheerios, Sugar puffs, Frosties, oats/porridge (cooked)	15-20g*		
Low protein breakfast cereal	1 small bowl (30g)*		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

SPREADS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Sweet spreads e.g. Jam/marmalade/honey/icing	2 teaspoons (10g)		
Regular chocolate/nut spread	1 tablespoon (20g)		
Low protein chocolate spread	1 tablespoon (20g)		
Regular Savoury spreads/sandwich fillers e.g. Peanut butter/hummus/cheese spread	1 tablespoon (20-30g)		
Low protein savoury spreads e.g. Violife cream cheese, sandwich spread	1 tablespoon (20-30g)		
Butter/margarine/oils (added to food or in cooking)	1 teaspoon (5g)		
Low fat spreads (added to food or in cooking)	1 teaspoon (5g)		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DATE:
SUBJECT NO:
INITIALS:

PASTA, RICE AND POTATO			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Regular & gluten-free pasta, noodles, rice, couscous (cooked)	Rice cooked (45g)* pasta/ noodles/couscous cooked (15g)		
Low protein pasta, noodles, rice, couscous (cooked)	Cooked 80-100g*		
Tinned pasta e.g. spaghetti in sauce/pasta shapes	2 tablespoons (50g – 70g)*		
Low protein ready meal e.g. snack pot, pot noodles	1 small bowl/1 pot		
Potato (boiled, mashed, jacket, baked)	80g*		
Potato cooked in fat (roast, chips, fried)	Roast (55g), chips (45g)*		
Processed potatoes – waffles, croquettes, wedges, Alphabites, hash browns, Smiley faces, potato salad	1 piece or 30g- 40g		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

SAUCES & SOUPS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Regular table top sauces, **ketchup**, fat-free dressings, chutney, pickles, mint sauce, vinaigrette	1 tablespoon (20-30g)		
Gravy	3 tablespoons (50ml)*		
Mayonnaise, salad cream, oil based dressings	1 tablespoon (20-30g)		
Other milk based sauces (including homemade) e.g. cheese, white, parsley, carbonara	2 tablespoons (50ml)		
Low protein sauce e.g. LP cheese sauce	2 tablespoons (50ml)		
Cook-in/meal based/pasta sauces (including homemade) e.g. curry, sweet & sour, tomato, vegetable	5 tablespoons (100ml)*		
Soup	1 small bowl (200 ml)*		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DATE:
SUBJECT NO:
INITIALS:

VEGETABLES (fresh, raw, cooked, frozen and canned)			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Broad beans, peas, spinach, Spring greens, sweetcorn, mixed vegetables	Broad beans (20g), peas (25g)*, spinach (25g), Spring greens (35g), sweetcorn (35g)*, mixed vegetables (30g)		
Asparagus, beansprouts, broccoli*, Brussel sprouts, cauliflower, okra, yam, sugar snap peas, mange tout	60g*		
All other vegetables e.g. carrots*, sweet potato, tomatoes, mushroom*, cucumber*, peppers, salad	60g*		
Legumes/pulse e.g. baked beans*, Lentils, chickpeas, dried peas/ beans	20g cooked*		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING
REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

FRUIT			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
All fresh, canned or frozen fruit	1 piece (80-100g)*		
Dried fruit e.g. apricot, dates, cranberries, banana chips, mango, raisins, sultanas, fruit bars	2 tablespoons (30-40g)*		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING
REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DATE:
SUBJECT NO:
INITIALS:

MEAT AND ALTERNATIVES			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Low protein sausage/burger	1 (80g)*		
Vegetarian burger/sausages, tofu, Quorn	1 (50g)		
Eggs – boiled, poached, fried, scrambled, omelette	1 (50-60g)		
Regular Pizza	1 slice (40-50g)		
Low protein pizza	1 slice (40-50g)*		
Meat based pie, sausage roll, pastie, samosa, pakora, spring roll	1 small (50 -80g)		
Vegetable based pie, sausage roll, pastie, samosa, pakora, spring roll	1 small (50-80g)		
Meat based curry/Chinese takeaway/lasagne	200g		
Vegetable based curry/Chinese takeaway/lasagne	200g*		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DRINKS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Water (tap, mineral)	1 glass (200 ml)		
Natural fruit juice (no added sugar)	1 glass (200 ml)		
Fruit smoothie	1 glass (200ml)*		
Regular sweet drinks, squash, fizzy drinks, coke	1 glass (200 ml)		
Sugar-free drinks, squash, fizzy drinks, coke	1 glass (200 ml)*		
Tea/coffee	1 small cup (200 ml)		
Powdered hot chocolate	15g (1 tablespoon)		
Non-dairy creamer e.g. coffee whitener	15g (1 tablespoon)		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DATE:
SUBJECT NO:
INITIALS:

SWEET SNACKS/PUDDINGS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Regular biscuit/cookie/rice cakes	1-2 (15-20g)*		
Regular cake, muffin, doughnut, tart, scone, pastry	1 (50-70g)		
Regular cereal bar	1 (20-40g)		
Low protein biscuit/cookie	1-2 (15-20g)*		
Low protein cake, muffin, doughnut, tart, scone, pastry	1 (50-70g)*		
Low protein cereal bar	1 (20-40g)		
Regular Puddings – cheesecake, crumble, pie, tart, sponge, trifle, rice	Small serve (80-100g)		
Low protein Puddings – cheesecake, crumble, pie, tart, sponge, trifle, rice	Small serve (80-100g)		
Regular Jelly with gelatine	100g*		
Low protein jelly	100g		
Regular chocolate/chocolate bars	3-5 squares (15-25g)*		
Low protein chocolate/chocolate bars	1 bar (25g)		
Gummy sweets or marshmallows (contain gelatine)	4-8 sweets (30g)		
Vegetarian gummy sweets/low protein marshmallows	4-8 sweets (30g)*		
All other sweets e.g. boiled, chewy, mints, candy	4-6 sweets (30g)		
Sugar added to drinks or cereals	1 teaspoon (5g)*		
Other: please list			

PLEASE MAKE SURE THERE IS <u>A NUMBER IN EVERY BOX ON EVERY LINE</u> BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

SAVOURY SNACKS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Potato crisps, corn snacks, rice snacks, popcorn	1 small packet (15-25g)		
Nuts and seeds	1 tablespoon (10-15g)		
Low protein crisps	1 packet (25g)		
Regular crackers, crispbreads, breadsticks, rice cakes	2 (10-15g)		
Low protein crackers, crispbreads, breadsticks, rice cakes	2 (10-15g)*		
Other: please list			

PLEASE MAKE SURE THERE IS <u>A NUMBER IN EVERY BOX ON EVERY LINE</u> BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DATE:
SUBJECT NO:
INITIALS:

ADDITIONAL QUESTIONS:

1. **Tick** all meals/snacks (including e.g fruit) that are eaten regularly every day.

Breakfast	Mid-morning snack	Midday meal	Mid-afternoon snack	Evening meal	Bedtime snack

2. How often is a meal missed/not eaten? Please **tick one box**.

Never/ rarely/ less than once a week	Once a week	Several times a week	Once a day	More than once a day

3. Is salt added to food (**tick** yes or no):

   a. during cooking? Yes _____ No _____
   b. at the table? Yes _____ No _____

4. Are any vitamin, mineral or energy (e.g. glucose polymer) supplements taken?

Yes _____ No _____ If yes, please list the type of supplement and dose:

Name: _____

Amount/day: _____

5. How many times a week are meals eaten outside the home e.g. restaurant/cafe?

_____

DATE:
SUBJECT NO:
INITIALS:

6. How many protein exchanges (1g protein = 1 exchange) are taken each day?

_____

7. How are the protein exchanges usually measured? Please **tick** one.

I rarely/ never measure them	I usually estimate/guess	I usually use scales/measuring cups/spoons/packets

8. Is a protein exchange eaten at each meal?

Yes _____ No _____

Tick all the meals/snacks that protein exchanges are given:

Breakfast	Mid-morning snack	Midday meal	Mid-afternoon snack	Evening meal	Bedtime snack

9. Please enter the protein substitute name and the amount per day:

Name: _____

Amount/day: _____

10. How many times a day is protein substitute taken? _____

Please put one **tick** for each time of day that protein substitute is taken.

Breakfast	Mid-morning	Midday meal	Mid-afternoon	Evening meal	Bedtime

11. Do you usually take the full amount of protein substitute each day?

Yes _____ No _____

If no, how often is some left/not taken?

_____

Picture Portion Size Guide

for

FOOD FREQUENCY QUESTIONNAIRE

Low Protein Diet

This booklet contains pictures of food portion sizes to use as a guide with the Low Protein Food Frequency Questionnaire.

Pictures marked with an asterisk (*) show food displayed on a dinner size plate to give an indication of the size of the food. All other plates are on smaller 'side plates.'

Tablespoons and teaspoons are sometimes used to describe portion sizes in the Food Frequency Questionnaire.

1 tablespoon, 1 teaspoon

MILK AND MILK REPLACEMENTS

30ml (1 exchange)
regular milk

200-250ml
low protein milk

1 exchange = 50mg phenylalanine/1g protein

DAIRY PRODUCTS AND ALTERNATIVES

15g (small cube)
regular cheese*

15g grated regular
cheese*

20g (1 slice)
low protein cheese

1 small serve (60g)
low protein dessert

1 small pot (55g)
yogurt/dairy dessert

30g regular or low
protein ice-cream

BREADS AND CEREALS

40g (1 slice) regular bread + 1 teaspoon butter/margarine

20g (1 slice) Mevalia low protein bread + 1 teaspoon butter/margarine

48g (1 slice) Promin low protein bread + 1 teaspoon butter/margarine

60g (1 slice) Fate low protein bread (homemade) + 1 teaspoon butter/margarine

1 whole (100g) low protein Mevalia baguette

65g Mevalia ciabatta

10g (1 exchange) wheat-based cereal

10g (1 exchange) wheat-based cereal

15g (1 exchange) rice-based cereal

1 small bowl (30g) protein-free cereal

1 exchange = 50mg phenylalanine/1g protein

PASTA, RICE AND POTATO

80g cooked
low protein pasta*

160g cooked
low protein pasta*

45g (1 exchange)
cooked regular rice*

80g cooked
low protein rice*

55g (1 exchange)
tinned pasta*

McDonalds chips
small (80g=1.5 exchanges)
medium (115g=2.5 exchanges)
large (150g=3.5 exchanges)

80g (1 exchange)
potato*

80g (1 exchange)
mashed potato*

55g (1 exchange)
roast potato*

45g (1 exchange)
chips*

1 exchange = 50mg phenylalanine/1g protein

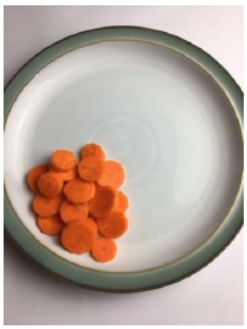
VEGETABLES (fresh, raw, cooked, frozen and canned)

25g (1 exchange) peas*

35g (1 exchange) sweetcorn*

60g broccoli*

60g brussel sprouts*



60g carrots*

20g (1 exchange) baked beans*

60g cooked mushrooms *

60g cucumber*

FRUIT

40g (2 tablespoons) dried apricots

40g (2 tablespoons) raisins

80g grapes

80g strawberries

1 exchange = 50mg phenylalanine/1g protein

MEAT AND ALTERNATIVES

80g (1) low protein sausage*

80g (1) low protein sausage*

80g regular burger + 100g roll*

50g slice low protein pizza*

200g vegetable curry*

200g low protein lasagne*

SOUPS & SAUCES

3 tablespoons (50ml) gravy

5 tablespoons (100ml) cook-in sauce

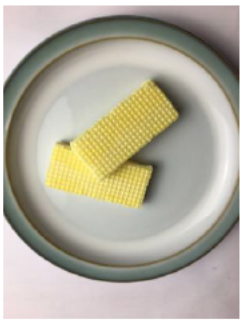
200ml soup

SWEET SNACKS

20g regular biscuit

20g low protein biscuit

20g Aminex low protein rusk

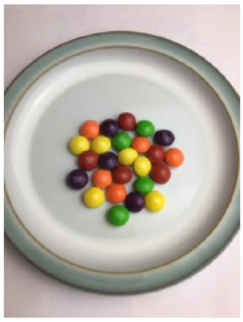


20g Loprofin low protein wafers

50g low protein cupcake

25g regular chocolate

30g low protein gummy sweets



30g Skittles

30g low protein mallows

100g low protein jelly

1 teaspoon sugar

SAVOURY SNACKS

15g low protein crackers

40g low protein crackers

DRINKS

200ml squash

200ml fruit smoothie

FOOD FREQUENCY QUESTIONNAIRE

CONTROL

DATE: _____

SUBJECT NUMBER: _____

INITIALS: _____

DOB: _____

STUDY TITLE: Dietary pattern analysis in children with PKU Version 1: 21.9.17

DATE:
SUBJECT NO:
INITIALS:

FOOD FREQUENCY QUESTIONNAIRE

- **This questionnaire asks you questions about what you/your child eats.**
- **We would like you to tell us about all foods/drinks regularly eaten by you/your child.**
- **The questionnaire lists 69 commonly eaten foods, food groups or drinks.**
- **For each food/food group there is a portion size suggested e.g. teaspoon, tablespoon, bowl, glass, slice.**
- **A portion size booklet is also provided. This will help you complete the food diary.**

PLEASE MAKE SURE YOU PUT A NUMBER IN EVERY BOX ON EVERY LINE IN THE QUESTIONNAIRE.

- Write the number of times a 'food portion' is taken each day in the 'in a DAY' box.
- If you/your child's portion size is double the suggested size, count this as 2 portions or if their portion size is smaller count it as ½ a portion.
- Then write the number of days a week this amount is eaten in the 'per WEEK' box.
- If a food is eaten less than once a week then put a '0' in both boxes for this food.
- For food items made up of different ingredients e.g. jam sandwich, divide into individual ingredients e.g. 2 slices white bread, 1 teaspoon margarine, 2 teaspoons jam.
- If there are food/drinks you/your child has regularly that are not listed, add these in the last section of each food group under 'other'.

DATE:
SUBJECT NO:
INITIALS:

FOR EXAMPLE:

If you/your child has:

- **½ a small pot of yoghurt once a day,**
- **a jam sandwich (2 slices of bread with butter and jam) 5 days a week,**
- **fruit once every 2 weeks**

your answers should look like this:

Food	Portion size	Number of portions in a DAY	Number of times per WEEK
Flavoured or plain yoghurt	1 small pot (60g)	½	7
Regular Bread – sliced, toast	1 slice (30-40g)	2	5
Butter/margarine/oils	1 teaspoon (5g)	2	5
Sweet spreads e.g. Jam/marmalade/honey/icing	2 teaspoons (10g)	1	5
All fresh, canned or frozen fruit	1 piece (80-100g)	0	0

DATE:
SUBJECT NO:
INITIALS:

MILK			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Milk – full fat, semi-skimmed, skimmed, condensed, soya (include on cereal, in drinks e.g. tea, in foods e.g. mash potato)	200-250ml*		
Other plant milks – coconut, oat, rice, almond, hemp (include on cereal, in drinks e.g. tea, in foods e.g. mash potato)	200-250 ml		
Milk flavouring e.g. chocolate, strawberry	1 tablespoon (15ml)		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DAIRY PRODUCTS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Cheese e.g. cheddar, Edam, Emmental	20g (1 slice)*		
Soft cheese, cream cheese, cheese spread	20g (1 tablespoon)		
Flavoured or plain yoghurt, drinking yoghurt, soya yoghurt, fromage frais, dairy desserts, custard, mousse, instant dessert	Small pot (40-60g)*		
Ice cream	30g*		
Ice lolly – milk based	1 lolly (45-80ml)		
Ice lolly – fruit/juice flavour	1 lolly (45-80ml)		
Cream (all types)	30ml (1 tablespoon)		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DATE:
SUBJECT NO:
INITIALS:

BREADS AND CEREALS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Bread – sliced, toast, small wrap	1 slice (30g-40g)*		
Bread roll, English muffin, crumpet, pitta, naan, ciabatta, large wrap	1 small (50-70g)*		
Breakfast cereals – **wheat based** e.g. muesli branflakes, multigrain, shredded wheat, Weetabix (1), gluten-free	20-30g*		
Breakfast cereal – **corn or rice based** e.g. cornflakes, rice krispies, Cheerios, sugar puffs, Frosties,	20-30g*		
Oats - porridge/Ready Brek	20-30g uncooked* 150-200g cooked*		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

SPREADS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Sweet spreads e.g. Jam/marmalade/honey/icing	2 teaspoons (10g)		
Chocolate/nut spread	1 tablespoon (20g)		
Savoury spreads/sandwich fillers e.g. Peanut butter/hummus/cheese spread	1 tablespoon (20-30g)		
Butter/margarine/oils (added to food or in cooking)	1 teaspoon (5g)		
Low fat spreads (added to food or in cooking)	1 teaspoon (5g)		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DATE:
SUBJECT NO:
INITIALS:

PASTA, RICE AND POTATO			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Pasta, noodles, rice, couscous (cooked)	80-100g*		
Tinned pasta e.g. spaghetti in sauce/pasta shapes	3 tablespoons (80g – 100g)*		
Ready meal e.g. Pot noodle	Noodles (80-100g dry)		
Potato (boiled, mashed, jacket, baked)	80-100g*		
Potato cooked in fat (roast, chips, fried)	80-100g*		
Processed potatoes – waffles, croquettes, wedges, Alphabites, hash browns, Smiley faces, potato salad	1 piece or 30g- 40g		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

SAUCES & SOUPS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Table top sauces, **ketchup**, dressings, chutney, pickles, mint sauce, vinaigrette	1 tablespoon (20-30g)		
Gravy	3 tablespoons (50ml)*		
Mayonnaise, salad cream, oil based dressings	1 tablespoon (20-30g)		
Other milk based sauces (including homemade) e.g. cheese, white, parsley, carbonara	2 tablespoons (50ml)		
Cook-in/meal based/pasta sauces (including homemade) e.g. curry, sweet & sour, tomato, vegetable	5 tablespoons (100ml)*		
Soup	1 small bowl (200 ml)*		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DATE:
SUBJECT NO:
INITIALS:

VEGETABLES (fresh, raw, cooked, frozen and canned)			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Broad beans, peas*, spinach, Spring greens, sweetcorn*, mixed vegetables	60g*		
Asparagus, beansprouts, broccoli*, Brussel sprouts, cauliflower, okra, yam, sugar snap peas, mange tout	60g*		
All other vegetables e.g. carrots*, sweet potato, tomatoes, mushroom*, cucumber*, peppers, salad	60g*		
Legumes/pulse e.g. baked beans*, Lentils, chickpeas, dried peas/ beans	80-100g cooked*		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

FRUIT			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
All fresh, canned or frozen fruit	1 piece (80-100g)*		
Dried fruit e.g. apricot, dates, cranberries, banana chips, mango, raisins, sultanas, fruit bars	2 tablespoons (30-40g)*		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DATE:
SUBJECT NO:
INITIALS:

MEAT AND ALTERNATIVES			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Meat e.g. beef, lamb, pork, veal, chicken, turkey	1 small piece (60-80g)*		
Fish e.g. tuna*, salmon, cod, haddock, kippers, mackerel, sardines prawns, crab, scallops, mussels	1 small piece (60-80g)*		
Processed meats/ fish e.g. **sausage(1)*, chicken nugget(4-5), fish finger(2)**, burger(1)*, battered/crumbed fish(1), scampi(4-5), bacon(3)	60-80g*		
Ham*/Sandwich slices	15-20g		
Vegetarian burger/sausages, Tofu, Quorn	1 (50g)		
Eggs – boiled, poached, fried, scrambled, omelette	1 (50-60g)		
Pizza	1 slice (40-50g)*		
Meat based pie, sausage roll, pastie, samosa, pakora, spring roll	1 small (50 -80g)		
Vegetable based pie, sausage roll, pastie, samosa, pakora, spring roll	1 small (50-80g)		
Meat based curry/Chinese takeaway/lasagne	200g*		
Vegetable based curry/Chinese takeaway/lasagne	200g		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DRINKS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Water (tap, mineral)	1 glass (200 ml)		
Natural fruit juice (no added sugar)	1 glass (200 ml)		
Fruit smoothie	1 glass (200ml)*		
Sweetened drinks, squash, fizzy drinks, coke	1 glass (200 ml)		
Sugar-free drinks, squash, fizzy drinks, coke	1 glass (200 ml)*		
Tea/coffee	1 small cup (200 ml)		
Powdered hot chocolate	15g (1 tablespoon)		
Non-dairy creamer e.g. coffee whitener	15g (1 tablespoon)		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DATE:
SUBJECT NO:
INITIALS:

SWEET SNACKS/PUDDINGS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Biscuit/cookie (all types)	1-2 (15-20g)*		
Cake, muffin, doughnut, tart, scone, pastry	1 (50-70g)*		
Cereal bar	1 (20-40g)		
Puddings – cheesecake, crumble, pie, tart, sponge, trifle, rice	Small serve (80-100g)		
Jelly	100g*		
Chocolate/chocolate bars	1 bar/ 6-8 squares (30-50g)*		
Gummy sweets or marshmallows (contain gelatine)	4-8 sweets (30g)*		
All other sweets e.g. boiled, chewy, mints, candy	4-6 sweets (30g)		
Sugar added to drinks or cereals	1 teaspoon (5g)*		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

SAVOURY SNACKS			
Food	**Portion size**	**Number of portions in a DAY**	**Number of times per WEEK**
Potato crisps, corn snacks, rice snacks, popcorn	1 small packet (15-25g)		
Nuts and seeds	1 tablespoon (10-15g)*		
Crackers, crispbreads, breadsticks, rice cakes	2 (10-15g)*		
Other: please list			

PLEASE MAKE SURE THERE IS A NUMBER IN EVERY BOX ON EVERY LINE BEFORE CONTINUING

REMEMBER TO INCREASE OR DECREASE PORTION SIZE IF YOU EAT MORE OR LESS

DATE:
SUBJECT NO:
INITIALS:

ADDITIONAL QUESTIONS:

1. **Tick** all meals/snacks (including e.g fruit) that are eaten regularly every day.

Breakfast	Mid-morning snack	Midday meal	Mid-afternoon snack	Evening meal	Bedtime snack

2. How often is a meal missed/not eaten? Please **tick one box**.

Never/ rarely/ less than once a week	Once a week	Several times a week	Once a day	More than once a day

3. Is salt added to food (**tick** yes or no):

   a. during cooking? Yes _____ No _____
   b. at the table? Yes _____ No _____

4. Are any vitamin, mineral or energy (e.g. glucose polymer) supplements taken?

Yes _____ No _____ If yes, please list the type of supplement and dose:

Name: _____

Amount/day: _____

5. How many times a week are meals eaten outside the home e.g. restaurant/cafe?

_____

Picture Portion Size Guide

for

FOOD FREQUENCY QUESTIONNAIRE

Control

This booklet contains pictures of food portion sizes to use as a guide with the Control Food Frequency Questionnaire.

Pictures marked with an asterisk (*) show food displayed on a dinner size plate to give an indication of the size of the food. All other plates are on smaller 'side plates.'

Tablespoons and teaspoons are sometimes used to describe portion sizes in the Food Frequency Questionnaire.

1 tablespoon, 1 teaspoon

MILK

200ml milk

DAIRY PRODUCTS

20g (1 slice) cheese*

20g (small cube) cheese*

1 small pot (55g) yogurt/dairy dessert

30g regular ice-cream

BREADS AND CEREALS

40g (1 slice) bread + 1 teaspoon butter/margarine

1 small (60g) roll

100g baguette

1 small (60g) ciabatta

20g cereal

30g cereal

30g oats/porridge (uncooked)

200g oats/porridge (cooked)

PASTA, RICE AND POTATO

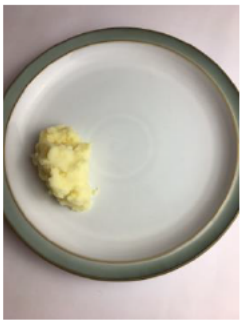
80g cooked pasta*

160g cooked pasta*

80g cooked rice*

80g tinned pasta*

80g potato*



80g mashed potato*

80g roast potato*

80g chips*

McDonalds chips
small (80g), medium (115g), large (150g)

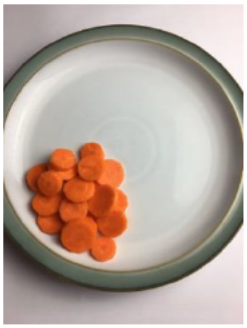
VEGETABLES (fresh, raw, cooked, frozen and canned)

60g peas*

60g sweetcorn*

60g broccoli*

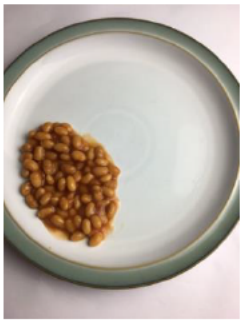
60g brussel sprouts*



60g carrots*

60g cooked mushrooms*

60g cucumber*



80g baked beans*

FRUIT

40g (2 tablespoons) dried apricots

40g (2 tablespoons) raisins

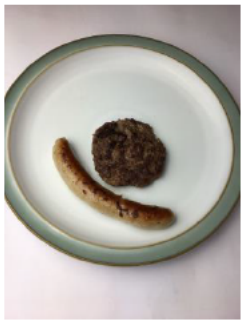
80g grapes

80g strawberries

MEAT AND ALTERNATIVES

60g slice of meat*

60g tuna*



70g (1) burger or sausage*

60g burger + 100g roll*

50g slice pizza*

200g curry*

200g lasagne*

15g slice ham

SOUPS & SAUCES

3 tablespoons (50ml) gravy

5 tablespoons (100ml) cook-in sauce

200ml soup

SWEET SNACKS

20g biscuit

50g cup cake

45g chocolate

30g gummy sweets

30g marshmallows

100g jelly

1 teaspoon sugar

SAVOURY SNACKS

15g crackers

15g breadsticks
(mini =1.4g each)

15g peanuts

DRINKS

200ml squash

200ml fruit smoothie

APPENDIX 3: SUPPLEMENTARY DATA FOR CHAPTER 4B

APPENDIX A:
LOW PROTEIN LABELLING CONSENSUS STATEMENTS FOR PEOPLE WITH PKU ON ≤10G/DAY OF NATURAL PROTEIN

Foods best avoided

FOOD GROUPS	STATEMENTS	% agreement
Meat (lamb, beef, pork, ham, bacon, chicken. turkey, duck, game, beef jerky, sausages, beef or chicken burgers, meat paste, meat pies). Offal (liver, kidney, tongue). Canned meats (e.g. corned beef)	High in protein and are best avoided.	83
Fish (all varieties including shellfish, frozen or tinned fish)	High in protein and are best avoided.	83
Eggs – boiled, poached, fried, scrambled, omelette	High in protein and are best avoided. However, any foods with eggs as an ingredient (e.g. cakes or sweets), must be calculated/weighed as an exchange food if they contain protein >0.5g/100g.	100
Cheeses (all dairy based cheese and cheese spreads)	High in protein and are best avoided. However, any foods with cheese as an ingredient (e.g. in baby foods or sauce), must be calculated/weighed as an exchange food if they contain protein >0.5g/100g.	100
Nuts	High in protein and are best avoided. **Exception:** whole chestnuts	82
Aspartame (E951) or neotame containing food and drinks	All foods, drinks and tabletop sweeteners containing aspartame or neotame are best avoided.	100
Seeds	High in protein and so are best avoided. However, any food products with seeds as an ingredient (e.g. cereal bar) must be calculated/weighed as an exchange food if they contain protein >0.5g/100g.	100
Soya based foods	High in protein and are best avoided.	100
Tofu	High in protein and best avoided.	100
Quorn based foods	High in protein and are best avoided.	100
Goji berries	High in protein and are best avoided.	100
Yeast extract spreads	High in protein and are best avoided.	90
Pork crackles/pork scratching	High in protein and are best avoided.	82

Exchange-free foods

Definition: Any food given without protein measurement/restriction is referred to as an ***exchange-free food.***

FOOD GROUPS	STATEMENTS	% agreement
Fresh, canned, frozen or dried fruit & vegetables ≤75mg Phe/100g	Fruits and vegetables (except potatoes) containing a Phe content **≤75 mg/100g** weight of fruit & vegetable are exchange-free.	100
Sugar – granulated, white, brown, demerara, muscovado, castor, icing sugar, glucose, fructose, lactose, maltose, maltodextrin	Exchange-free **excluding tabletop sugars containing aspartame.**	100
Syrup – golden, maple, agave, flavoured syrups	Exchange-free if they contain exchange-free ingredients only.	100
Black Treacle	Exchange-free as it contains exchange-free ingredients only. The protein content appears higher than other sugars due to its processing. Treacle is made from the 'remaining' syrup after sugar is refined.	100
Fruit syrups/fruit sauces	Exchange-free if they contain exchange-free ingredients only.	100
Sweet spreads e.g. Jam/marmalade/honey	Exchange-free if they contain exchange-free ingredients only.	100
Artificial sweeteners e.g. acesulfame, cyclamate, erythritol, fructose, isomalt, lactitol, maltitol, maltodextrin, mannitol, saccharin, sorbitol, stevia, sucralose, thaumatin, xylitol	All artificial sweeteners/sugar substitute **except aspartame or neotame**, are exchange-free. Aspartame and neotame are best avoided.	96
Fats (butter, margarine, low fat spreads, oils, vegetable fats, oil sprays)	Exchange-free, irrespective of the protein content on the food ingredient label, due to the small quantities consumed.	96
Vinegar-based sauces: Worcester sauce, mint sauce/jelly, vinegar	Exchange-free if they contain exchange-free ingredients only or are used in small quantities.	80
Natural fruit juice (no added sugar)	Exchange- free if they contain exchange-free/aspartame-free ingredients only.	100
Fruit smoothie	Exchange-free if made with exchange-free, aspartame-free fruit/vegetable juice.	100
Soft drinks e.g. squash, fizzy drinks, coke	Exchange-free, providing aspartame-free.	100
Sugar-free drinks e.g. squash, fizzy drinks, cola	Exchange-free, providing aspartame-free.	100
Tea - black, green, mint, camomile, fruit, herbal, and iced.	Exchange-free, providing aspartame-free.	100
Chewing gum/Bubble gum	Chewing gum/bubble gum that is aspartame-free is exchange-free. Chewing gum that contains aspartame is best avoided.	95
Coffee - black ground, instant, expresso	Exchange-free if it contains exchange-free ingredients only. White coffee (including dried reconstituted from sachets), frappuccino's, cappuccino, lattes, coffee pods/capsules with added milk, iced coffee, macchiato must be calculated/measured as exchange drinks.	100
Herbs, spices, seasonings (fresh or dried), salt & pepper	All herbs, spices and seasonings fresh or dried (irrespective of the protein content on the food ingredient label) are exchange-free, due to the small quantities consumed.	100

Custard powder containing exchange-free ingredients only	Custard powders containing exchange-free ingredients only (e.g. cornflour, colour, flavourings) are exchange-free. **Prepare with protein-free milk.**	100
Milkshake powders/syrups containing exchange-free ingredients and aspartame-free.	Milkshake syrups or powders containing exchange-free ingredients only (e.g. cornflour, colour, flavourings) are exchange-free. **Prepare with protein-free milk.**	100
Food colouring (gel, liquid or paste) and flavouring/essences (e.g. vanilla, peppermint, almond)	Exchange-free if they contain exchange-free ingredients only.	100
Flours - arrowroot, cornflour/maize starch/corn starch, cassava/tapioca starch, sago	Exchange-free. Note: Cornmeal is high in protein and is an exchange food. Ready-to-serve milk puddings made with tapioca and sago are not exchange-free.	100
Baking ingredients – bicarbonate of soda, baking powder, cream of tartar	Exchange-free.	100
Fibres/gums – psyllium fibre, psyllium husks, xanthan gum	Exchange-free.	100
Konnyaku	Exchange-free.	100
Cassava crisps	Exchange-free if ingredients are all exchange-free.	100

Foods that require measuring/weighing as an exchange food if they contain protein >0.1g/100g or 0.1g/100ml. If they have a protein content no more than 0.1g/100g or 0.1g/100ml, they are exchange-free.

FOOD GROUPS	STATEMENTS	% agreement
Milk (animal based)– full fat, semi-skimmed, skimmed, condensed, soya	Any milk product (plant or animal) containing protein >0.1g /100ml must be measured/calculated as an exchange food.	80
Plant milks e.g. coconut, oat, rice, almond, hemp Includes coffee with these added e.g. dried reconstituted from sachets, frappuccino's, cappuccino, lattes, coffee pods/capsules with added milk, iced coffee, macchiato	Any milk product (plant or animal) containing protein >0.1g /100ml must be measured/calculated as an exchange food. Coffee either containing or made with animal/plant milk must be calculated/measured as an exchange drink. Exchange-free if it contains exchange-free ingredients only.	80

Foods that require measuring/weighing as an exchange food if they contain protein >0.5g/100g. If they contain exchange-free ingredients only or have a protein content no more than 0.5g/100g, they are exchange-free.

FOOD GROUPS	STATEMENTS	% agreement
General food groups		
Gluten-free foods	Gluten-free foods with a protein content >0.5g/100g must be calculated/weighed as exchange foods.	96
Gelatine containing foods	All foods containing gelatine and with a protein content >0.5g/100g must be calculated/weighed as exchange foods.	93
Dairy products and alternatives		
Vegan, free-from or soya/plant cheese or regular cheese as an ingredient	Any foods with added regular cheese as an ingredient (e.g. in baby foods or sauce), and with a protein content >0.5g/100g, must be calculated/weighed as an exchange food. Any vegan or 'free-from' cheese is exchange-free if the protein content is ≤0.5 g/100g or if they contain exchange-free ingredients only. Any with exchange ingredients and a protein content >0.5g/100g must be calculated/weighed as an exchange food.	94
Soft cheese, cheese spread, cream cheese	Soft, cream cheeses with a protein content >0.5g/100g must be calculated/weighed as an exchange food. Any vegan or free-from soft cheese is exchange-free if the protein content is ≤0.5 g/100g or if they contain exchange-free ingredients only. If they contain protein >0.5g/100g and contain exchange ingredients, they must be calculated/weighed as exchange foods.	87
Ice-lolly milk based	Exchange-free if the protein content is ≤0.5g/100g of food. Any with exchange ingredients and a protein content >0.5g/100g must be calculated/weighed as an exchange food.	96
Ice-lolly fruit/juice based	Exchange-free if the protein content is ≤ 0.5g/100 of food. Any with exchange ingredients and a protein content >0.5g/100g must be calculated/weighed as an exchange food.	96
Cream e.g. double, single, clotted, cream alternatives, sour, spray cream	Any cream with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Ice-cream (dairy and dairy-free)	Any ice-cream (dairy or dairy-free) with a protein content >0.5g/100g must be calculated/ weighed as an exchange food. **Weight** rather than the volume of ice-cream must be used to calculate the protein exchange amount.	100
Dairy or soya yogurt e.g. flavoured or plain, drinking, soya	Any yoghurts with a protein content >0.5g/100g must be calculated/weighed as an exchange food. However, if they contain exchange ingredients and protein ≤0.5g/100g, it is best to limit to 1 portion/pot per day.	100
Dairy desserts e.g. fromage frais, dairy desserts, custard, mousse, instant dessert, rice pudding	Any desserts with a protein content >0.5g/100g must be calculated/weighed as an exchange food. However, if they contain exchange ingredients and protein ≤0.5g/100g, it is best to limit to 1 portion/pot per day.	97

Coconut-based desserts	Any coconut desserts with a protein content >0.5g/100g must be calculated/weighed as an exchange food. If they contain ≤0.5g/100g, they are considered exchange-free. However, if they contain exchange ingredients and protein ≤0.5g/100g, it is best to limit to 1 portion/pot per day.	97
Coconut products	Dried coconut products with a protein content >0.5g/100g protein must be calculated/weighed as an exchange food.	93
Breads & cereals		
Bread e.g. bread roll, English muffin, bagels, crumpet, pitta, naan, ciabatta, wrap, breadsticks, croissants, brioche (including gluten-free)	All breads and bread products, including gluten-free varieties, with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Breakfast cereals – **wheat based** e.g. muesli branflakes, multigrain, shredded wheat, Weetabix and **corn, rice or oat based** e.g. Cornflakes, Rice krispies, Cheerios, Sugar puffs, Frosties, oats/porridge (cooked), including gluten-free	All breakfast cereals and breakfast oats (including gluten-free varieties) with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Cereal grains e.g. wheat, bulgar wheat, quinoa, barley, oats, corn, rice, semolina, millet sorghum, buckwheat, quinoa, chia, triticale, amaranth	All cereals with a protein content >0.5g/100g must be calculated/weighed as exchange foods.	100
Yorkshire pudding, stuffing balls, pancakes, waffles (including gluten-free)	All Yorkshire puddings, pancakes, waffles, stuffing balls, (including gluten-free varieties) with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Spreads & dips		
Lemon curd	All lemon curd with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Chocolate spread	All chocolate spread with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Nut spread/chocolate nut spread e.g. peanut butter, Nutella	All nut spreads with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	87
Hummus	Hummus with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Dips – sweet and savoury	Any sweet or savoury dips (e.g. sour cream, Tzatziki, nacho cheese dip) require measuring/weighing as exchange foods if they contain protein >0.5g/100g and contain exchange ingredients. If they contain exchange-free ingredients only or have a protein content no more than 0.5g/100g, they are exchange-free.	100
Sauces, dips, gravy & soup		
Gravy	Exchange-free if the protein content is **≤ 0.5 g/100 ml when prepared.** Any with exchange ingredients and a protein content >0.5g/100g must be calculated/measured as an exchange food.	100
Cheese or milk-based sauces e.g. cheese, white, parsley, carbonara	All cheese sauces must be calculated/measured as part of exchange system unless dry powder ingredients are exchange-free and are made up with water or protein-free milk.	100
Herby/spicy rubs or coatings	Exchange-free if the protein content is ≤0.5g per serving. Any that contain exchange ingredients with a protein content >0.5g/100g per serving must be calculated/weighed as an exchange food.	96

Pesto, mustard, tapenade, plant-based vegetable spreads	Any pesto, mustard, tapenade or plant-based spread, including homemade, with a protein content >0.5g/100g must be calculated/weighed as an exchange food (unless it contains exchange-free ingredients only).	100
Soup	If soups contain exchange ingredients and their protein content is >0.5 g/100 g, then they must be calculated/measured as an exchange food (unless they contain exchange-free ingredients only).	79
Pasta & rice		
Regular & gluten-free pasta, rice, noodles, couscous, gnocchi, tinned pasta, pasta shapes	All regular or gluten-free pasta, rice or pasta products with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Pot Noodles	All pot noodles with a protein content >0.5g/100g when reconstituted, must be calculated/weighed as an exchange food.	100
Meat & Alternatives		
Vegan meat e.g. vegetarian burger/sausages, *Hooba* mushroom sausages	Any vegan meat replacements must be calculated/weighed as an exchange food if it contains protein >0.5g/100g and contains protein exchange ingredients.	83
Vegan fish	Any vegan fish replacements must be calculated/weighed as an exchange food if it contains protein >0.5g/100g and contains protein exchange ingredients.	83
Vegan Eggs, including aquafaba	Any vegan egg replacements must be calculated/weighed as an exchange if it contains protein >0.5g/100g and contains protein exchange ingredients.	100
Legumes/pulses e.g. Lentils, chickpeas, dried peas, split peas. Beans – baked, red & black, kidney, haricot, pinto, cannellini, turtle, butter, adzuki	All pulses and beans with a protein content >0.5g/100g must be calculated/weighed as an exchange food. **Exception**: French green beans are exchange-free.	95
Drinks		
Hot chocolate, malted milk powders, milkshake powders or liquids	Flavoured milk powders, milkshake powders/liquids and hot chocolate powders/liquids with a protein content >0.5g/100g must be calculated/measured as an exchange food. These are best prepared with protein-free milk.	94
Sweet snacks		
Biscuit/cookie	Biscuits with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Cake, muffin, doughnut, tart, scone, pastries, brownies, flapjack, fruit loaves, fruit pies, scones, cupcakes, cake bars, teacakes (including gluten-free varieties).	Cakes and pastries, including gluten-free varieties, with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Breakfast or cereal bars	Breakfast and cereal bars with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Fruit bars	Any fruit bars with a protein content >0.5g/100g must be calculated/weighed as an exchange food (unless they contain exchange-free ingredients only).	97
Puddings – cheesecake, crumble, pie, tart, sponge, trifle, meringue, sponge	Any desserts with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Frozen puddings e.g. pies, pastries, crumbles (including gluten-free varieties).	All frozen pastry, pies, crumbles (including gluten-free varieties) with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100

Jelly (aspartame-free)	Exchange-free if the protein content is ≤0.5g/100g. Any with exchange ingredients and a protein content >0.5g/100g must be calculated/weighed as an exchange food.	97
Chocolate/chocolate bars, fudge, toffee	Exchange-free if the protein content is ≤0.5g/100g. All with exchange ingredients and a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Gummy sweets, vegetarian gummy sweets (aspartame-free)	Exchange-free if the protein content is ≤0.5g/100g. Any with exchange ingredients and a protein content >0.5g/100g must be calculated/weighed as an exchange food.	91
Marshmallows	Exchange-free if the protein content is ≤0.5g/100g. Any with exchange ingredients and a protein content >0.5g/100g must be calculated/weighed as exchange foods. **Note:** only gelatine-free mallows are likely to contain ≤0.5g/100g protein.	95
All other sweets e.g. boiled, chewy, mints, candy (aspartame-free)	Exchange-free if the protein content is ≤0.5g/100g. Any with exchange ingredients and a protein content >0.5g/100g must be calculated/weighed as an exchange food.	91
Liquorice	Liquorice with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Savoury snacks		
Potato crisps	All potato crisps with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Vegetables crisps	All vegetable crisps with a protein content >0.5g/100g must be calculated/weighed as an exchange food. **Exceptions**: Cassava crisps are exchange-free if ingredients are all exchange-free. Vegetable crisps containing exchange-free vegetables are calculated/weighed as exchange foods due to the concentration of protein associated with cooking methods; their protein content per 100g must be used to determine the exchange amount.	100
Corn and rice-based snacks	All corn and rice-based snacks with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Popcorn	All popcorn with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Pretzels	All pretzels with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Crackers, crispbread, breadsticks	Crackers, crispbreads and breadsticks with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Rice, corn or oat cakes	Rice, corn or oat cakes with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Flours and Starch		
Flour and starch e.g. white, self-raising, wholemeal, chapatti, bread mixes, gram flour, gluten-free, coconut flour, rye flour, spelt flour, almond flour, nut flours, rice flour, peasemeal, chestnut flour, sorghum flour, atta flour, maida flour, spelt flour, cornmeal	All flours with a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Sugars		

Icing – e.g. Buttercream, fondant icing	All icings with a protein content ≤0.5g/100g or if they contain exchange-free ingredients only, are considered exchange-free. Any with exchange ingredients and a protein content >0.5g/100g must be calculated/weighed as an exchange food.	100
Marzipan	Marzipan with a protein content >0.5g/100g must be calculated/weighed as an exchange food	100

Foods that require measuring/weighing as an exchange food if they contain protein >1g/100g. If they contain exchange-free ingredients only, or have a protein content no more than 1g/100g, they are exchange-free.

FOOD GROUPS	STATEMENTS	% agreement
Sauces & dressings		
Table top sauces, brown sauce, tomato ketchup, BBQ, chilli, fat-free dressings, chutney, pickles, mint sauce, salad dressings, vinaigrette, Horseradish, and tartare.	Require measuring/weighing as exchange foods if they contain protein >1g/100g and contain exchange ingredients. If they contain exchange-free ingredients only or have a protein content no more than 1g/100g, they are exchange-free.	80
Mayonnaise, salad cream, oil-based dressings	Require measuring/weighing as exchange foods if they contain protein >1g/100g and contain exchange ingredients. If they contain exchange-free ingredients only or have a protein content no more than 1g/100g, they are exchange-free.	91
Cook-in/pour over or liquid sauces e.g. Italian, curry, sweet & sour, tomato, vegetable	Require measuring/weighing as exchange foods if they contain protein >1g/100g and contain exchange ingredients. If they contain exchange-free ingredients only or have a protein content no more than 1g/100g, they are exchange-free.	87
Sugars		
Cake decorations	All cake decorations are exchange-free if they contain exchange-free ingredients only or if the protein content is ≤1g/100g. If they contain >1g/100g and contain exchange ingredients, they must be calculated/weighed as exchange foods.	97

Foods that require measuring/weighing as an exchange food if they contain protein >1.5g/100g. If they contain exchange-free ingredients only or have a protein content no more than 1.5g/100g, they are exchange-free.

FOOD GROUPS	STATEMENTS	% agreement
Soya sauce	Exchange-free if it contains protein ≤1.5g/100g. Any with a protein content >1.5g/100g must be calculated/measured as an exchange food	79

Special low protein food containing Phe >25 mg/100g and containing exchange ingredients such as milk or seeds

FOOD GROUPS	STATEMENTS	% agreement
Low protein special foods (including bread, bread rolls, baguettes, pizza bases, breakfast cereals, chocolate spread, pasta, noodles, rice, couscous, sausage/burger mix, snack pots, biscuits/cookies, cakes, cereal bars, puddings, chocolate, crackers, sauces, flour, cake mix, dessert mix)	Exchange-free if they contain exchange-free ingredients only. If low protein special prescribed foods contain exchange ingredients but contain Phe **≤25mg Phe/100g** of prepared product, they are exchange-free. If they contain exchange ingredients and contain Phe **≥26/100g** of prepared product, they are an exchange food.	83

Low Protein Milk Replacements

FOOD GROUPS	STATEMENTS	% agreement
Low protein milks replacements (available on prescription)	Any low protein special milk that provides a Phe intake of **>5mg/100ml** must be calculated/measured as an exchange food.	80

Fruit and Vegetables as exchange foods

FOOD GROUPS	STATEMENTS	% agreement
Fresh fruit & vegetables 76-100mg Phe/100g	Fruit and vegetables containing a Phe content **76–99 mg/100g** weight of fruit and vegetable, are calculated/weighed as exchange foods A standard exchange amount of **60g** for any fruit or vegetables containing Phe between 76-99 mg/100 g is used.	80
Fresh fruit & vegetables ≥100mg Phe/100g	Fruits and vegetables with a Phe content of **≥100 mg/100g** weight of fruit and vegetable, are calculated/weighed as exchange foods. Their phenylalanine content is used to calculate exchange amounts.	100
Manufactured fruit & vegetables e.g. frozen, canned, passata, tomato puree	Any manufactured fruit or vegetables (e.g. frozen or canned products) that are exchange-free in their fresh form, are exchange-free foods if they contain exchange-free ingredients only, e.g. herbs, oil. However, if they contain added ingredients that are protein containing (e.g. milk or wheat), they are calculated/weighed as exchange foods.	96
Dried fruit e.g. apricot, dates, cranberries, banana chips, mango, raisins, sultanas, fruit bars	Any dried fruits that are exchange-free in their fresh form, are exchange-free foods. However, if they contain added ingredients that are protein containing (e.g. milk or wheat), they are calculated/weighed as exchange foods.	100
Potato and potato products		
Potato - boiled, mashed (no milk), jacket, baked	**80g = 1 exchange of protein, based on phenylalanine analysis.** Fresh/frozen potatoes are calculated/weighed as exchange foods. If potato products contain additional exchange ingredients (e.g. wheat flour, or milk), the protein analysis on the packet is used to determine its exchange amount.	96
Canned potato	**100g = 1 exchange of protein, based on phenylalanine analysis.** If potato products contain additional exchange ingredients (e.g. wheat flour, or milk), the protein analysis on the packet is used to determine its exchange amount.	96
Roast potato (fresh/frozen)	**55g = 1 exchange of protein, based on phenylalanine analysis.** If potato products contain additional exchange ingredients (e.g. wheat flour, or milk), the protein analysis on the packet is used to determine its exchange amount.	96
Chips/fries (fresh/frozen)	**45g = 1 exchange of protein, based on phenylalanine analysis.** If potato products contain additional exchange ingredients (e.g. wheat flour, or milk), the protein analysis on the packet is used to determine its exchange amount.	96
Instant mashed potato	**10g (dry weight) = 1 exchange of protein based on phenylalanine analysis.** If potato products contain additional exchange ingredients (e.g. wheat flour, or milk), the protein analysis on the packet is used to determine its exchange amount.	96
Processed potatoes – waffles, croquettes, wedges, Alphabites, hash browns, Smiley faces, potato salad	Fresh/frozen potato products are calculated/weighed as exchange foods. If Phe analysis is unavailable, the protein content is used to calculate exchange amounts. If potato products contain additional exchange ingredients (e.g. wheat flour, or milk), the protein analysis on the packet is used to determine its exchange amount.	96

General statements*

	STATEMENTS
Rounding values up or down if protein is <0.5g or >0.5g/100g	**Definition: 1g protein exchange = the amount of food that is calculated/measured to provide 1g protein from the protein food analysis.** Food 'exchange' amounts for food portions must be rounded up or down based on the 'rule of maths' (Table 1* below for guidance).
General guidance	Patients/caregivers are advised to read ingredient lists as well as the protein content per 100g on food labels. As guidance: - If ingredients are protein containing (but the protein analysis appears very low), it is important to establish more accurate protein analysis before consuming. - If ingredients are 'clearly' exchange-free, then it is appropriate to give as an exchange-free food even if the protein analysis is unavailable on the label. - If there is no nutritional analysis on the product but it contains ingredients that are exchange foods, then further food protein analysis must be obtained before consuming. - If a label states protein content is 0 g but one or more ingredients is a protein source (e.g. gelatine), then an accurate food protein analysis must be obtained before consuming.

*previously reported in: Evans S, Ford S, Adam S, Adams S, Ash J, Ashmore C, et al. Development of national consensus statements on food labelling interpretation and protein allocation in a low phenylalanine diet for PKU. Orphanet J Rare Dis. 2019;14:2.

Table 1: Calculating food protein exchanges from protein analysis on the food label

Protein content per item when calculated from label	**Calculated exchange**
0g protein per food portion	Exchange free
0.1g protein per food portion	Exchange free (if total volume consumed is ≤ 0.5g protein)
0.2g protein per food portion	Exchange free (if total volume consumed is ≤ 0.5g protein)
0.3g protein per food portion	Exchange free (if total volume consumed ≤ 0.5g protein). Suggest 1 portion is exchange-free, 2 portions is ½ exchange*
0.4 to 0.7g protein per food portion	½ exchange protein
0.8 to 1.2g protein per food portion	1 exchange protein
1.3 to 1.7g per food portion	1.5 exchange protein
1.8 to 2.2g per food portion	2 exchange protein
2.3 to 2.7g per food portion	2.5 exchange protein
2.8 to 3.2 per food portion	3 exchange protein

*This may apply to ice cream lolles, gluten-free cakes, very small packets crisps, sweets

APPENDIX 4: SUPPLEMENTARY DATA FOR CHAPTER 4C

SUBJECT NUMBER:
INITIALS:
DATE:

LOW PROTEIN LABEL READING

For the following questions, circle the answer you think best fits that product.
Please <u>answer all questions</u>.

This product contains protein 1.3g/packet.

How many exchanges would you count for this product?

a. Not allowed
b. 1 exchange
c. 1½ exchanges
d. 2 exchanges
e. Exchange-free so I don't need to count it
f. Other amount (write here) _____
g. Unsure

These mushrooms contain protein 2.1g/100g.

How many exchanges would you count for this product?

a. Not allowed
b. 2 exchanges
c. 2½ exchanges
d. Exchange-free because mushrooms are exchange-free.
e. Other amount (write here) _____
f. Unsure

Ingredients:
Italian tomatoes (76%), tomato purée, onion (3%), sugar, carrot (2%), water, extra virgin olive oil, Basil, Salt, acidity regulator: lactic acid.

This jar of sauce contains protein 1.2g/100g.

How many exchanges would you count for this product?

a. Not allowed
b. 1 exchange
c. 1½ exchanges
d. 2 exchanges
e. Exchange-free because all the ingredients are exchange-free.
f. Other amount (write here) _____
g. Unsure

SUBJECT NUMBER:
INITIALS:
DATE:

	Typical analysis/100g	Typical analysis/ 39g lolly
Protein	0.6g	<0.5g

How many exchanges would you count for 1 ice lolly?

a. Not allowed
b. ½ exchange
c. 1 exchange
d. Exchange-free if eat one only per day; ½ exchange if eat 2 per day.
e. Exchange-free
f. Other amount (write here) _____
g. Unsure

	Per oatcake	Per 100g
Protein	1.0g	11.2g

How many exchanges would you count for 1 oatcake?

a. Not allowed
b. ½ exchange
c. 1 exchange
d. 1½ exchanges
e. Exchange-free
f. Other amount (write here) _____
g. Unsure

	Per 100g	Per portion (25g)
Protein	1.0g	<0.5g

Can you to eat these sweets as an exchange-free food?

a. Yes Why? _____
b. No Why not? _____
c. Unsure

Ingredients:
Sugar, glucose syrup, palm fat, humectant: sorbitol syrup, acid: citric acid, gelatine, flavouring.

SUBJECT NUMBER:
INITIALS:
DATE:

Ingredients:
Sweeteners: xylitol, sorbitol, mannitol, aspartame, acesulfame K, gum base, thickener: gum arabic, flavourings, humectant: glycerol, emulsifier: soybean lecithin, acids: citric acid, malic acid, colour E172, glazing agent carnuba wax, antioxidant BHA, colours E100, E133.

The label says the protein content of these sweets is 0g/100g.

Are these okay for you to eat?

a. Yes Why? _____
b. No Why not? _____
c. Unsure

Ingredients:
Sugar, maize, rice, glucose syrup, flavourings, colours: beet red, copper chlorophyllin, lutein, paprika.

There was no protein content on the label of this product. Could you eat this?

a. Yes it's okay
b. Not allowed.
c. I would ask my dietitian
d. Could eat a small amount
e. I would count it as an exchange
f. Other: (write here)_____
g. Unsure

	Per 100ml
Protein	0.4g

If you had 200ml of this product how many exchanges would you count for this?

a. Not allowed
b. ½ exchange
c. 1 exchange
d. Exchange-free
e. Other amount (write here) _____
f. Unsure

SUBJECT NUMBER:
INITIALS:
DATE:

Ingredients:
Water, coconut oil (18%), modified starch, cranberry (9%), starch, sea salt, sunflower kernel grounded, flavourings, olive extract, colour: B-carotene, vitamin B12.

This vegan cheese contains protein 0.6g/100g.

If you had 70g of this how many exchanges would you count for this?

a. Not allowed
b. ½ exchange
c. 1 exchange
d. Exchange-free
e. Other amount (write here) _____
f. Unsure

Ingredients:
Water, coconut milk (17%), strawberries (7%), sugar, thickeners (modified maize starch, pectin, guar gum), dextrose, glucose fructose syrup, concentrated strawberry juice, calcium phosphate, salt, Vitamin D2, natural flavouring, vitamin B12, colour (strawberry concentrate, carotene), non-dairy live cultures (S.Thermophulis, L.Bulgaricus).

This coconut yogurt contains protein 0.6g/100g and one tub is 125g.

How many exchanges would you count for 1 tub?

a. Not allowed
b. ½ exchange
c. 1 exchange
d. Exchange-free
e. Other amount (write here) _____
f. Unsure

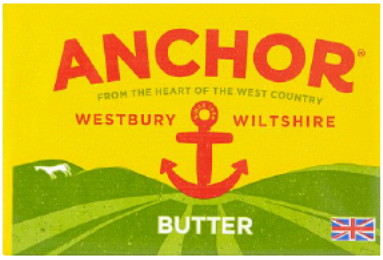
Ingredients:
Avocado, tomato, soured cream, onion, concentrated lime juice, coriander, antioxidant (ascorbic acid), garlic puree, red chilli, salt, rice starch.

This dip contains protein 1.4g/100g.

How many exchanges would be in 50g?

a. Not allowed
b. ½ exchange
c. 1 exchange
d. 1 ½ exchanges
e. Exchange-free
f. Other amount (write here) _____
g. Unsure

SUBJECT NUMBER:
INITIALS:
DATE:



This butter contains protein 0.6g/100g.

Could you eat this?

a. Yes it's exchange-free
b. Not allowed
c. Could eat a small amount
d. I would count it as part of my exchanges
e. Other: (write here)_____
f. Unsure

Ingredients:
Tomatoes, spirit vinegar, sugar, salt, spice and herb extracts, spice.

This ketchup contains protein 1.2g/100g.

Could you eat this?

a. Yes it's exchange-free because all the ingredients are exchange-free
b. Not allowed
c. Could eat a small amount
d. I would count it as part of my exchanges
e. Other: (write here)_____
f. Unsure

These gravy granules contains protein <0.5g/100 ml.

Could you eat this?

a. Yes it's exchange-free
b. Not allowed
c. Could eat a small amount
d. I would count it as part of my exchanges
e. Other: (write here)_____
f. Unsure

This soy sauce contains protein 1.3g/100g.

Could you eat this?

a. Yes it's exchange-free
b. Not allowed
c. Could eat a small amount
d. I would count it as part of my exchanges
e. Other: (write here)_____
f. Unsure

SUBJECT NUMBER:
INITIALS:
DATE:

Ingredients:
Tomatoes (75%), onions, fresh cream (4%), sunflower oil, sugar, modified maize starch, white wine, tomato paste (1%), butterfat, cheddar cheese, salt, basil, white wine vinegar, lactose, acidity regulator (lactic acid), natural flavouring (celery), palm fat, herbs, spices, milk proteins, stabiliser (xanthan gum), antioxidant (rosemary extract).

This jar of sauce contains protein 1.1g/100g.

How many exchanges would be in 125g?

a. Not allowed
b. 1 exchange
c. 1½ exchanges
d. Exchange-free
e. Other amount (write here) _____
f. Unsure

	Per 100g	Per waffle (58g)
Protein	2.5 g	1.4g

Ingredients:
Potato (87%), rapeseed oil, potato granules, potato starch, salt, stabiliser (E464), white pepper.

How many exchanges is 1 waffle (58g)?

a. Not allowed
b. 1 exchange
c. 1½ exchanges
d. 2 exchanges
e. 2.5 exchanges
f. Exchange-free
g. Other amount (write here) _____
h. Unsure

How much of this vegetable provides one exchange?

a. It's exchange-free
b. Not allowed
c. _____ grams

SUBJECT NUMBER:
INITIALS:
DATE:

Ingredients:
Mixed root vegetables (parsnip, beetroot, carrot, 66% sunflower oil, sea salt.

These crisps contain protein 4.1g/100g.

If you ate 25g, how many exchanges would this be?

a. Not allowed
b. 1 exchange
c. 1½ exchanges
d. 4 exchanges
e. Exchange-free because all the ingredients are exchange-free.
f. Other amount (write here) _____
g. Unsure

Ingredients:
White wine vinegar (sulphites), dried garlic.

This garlic provides protein 4.7g/100g.

Can you eat this?

a. Yes it's exchange-free because all the ingredients are exchange-free and I will only use a small amount
b. Not allowed
c. Could eat a small amount
d. I would count it as part of my exchanges
e. Other: (write here)_____
f. Unsure

Ingredients:
Maize starch, salt, flavouring, colour.

	Per 100g as prepared with semi-skim milk	Per portion (164g) as prepared with semi-skim milk
Protein	3.0 g	4.9g

Can you have this custard powder?

a. Yes it's exchange-free if made up with low protein milk, because all the ingredients are exchange-free.
b. Not allowed
c. Could eat a small amount
d. I would count it as part of my exchanges
e. Other: (write here)_____
f. Unsure

What would you add to this product to make it up?

_____