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Low protein staple foods, nutritional status and disease management in children and adults with Phenylketonuria.

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MSc by Research - May 2014

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"This is to certify that the work reported in this document is all my own work
unless otherwise stated".

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Date: **12th October 2014**

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Scotland wide survey on the beliefs, perceptions and issues with the use of low protein foods in the management of Phenylketonuria.

Cochrane, Barbara[2], Schwahn, Bernd[2] Robinson, Peter[2], Jones, Jeremy[1] Gerasimidis, Konstantinos[1]

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Published paper:

A questionnaire survey on the usage of low protein staple foods by people with phenylketonuria in Scotland.

Cochrane, B., Schwahn, B., Galloway, P., Robinson, P. & Gerasimidis, K., *Journal of Human Nutrition and Dietetics.* Article first published online: 6 JAN 2014: DOI: 10.1111/jhn.12199

Abbreviation list:

BMI - Body Mass Index

CHO – Carbohydrate

EAR – Estimated Average Requirements

FFQ – Food Frequency Questionnaire

LPSF – Low protein staple foods

MRC - Medical Research Council

NDNS – National Diet and Nutrition Survey

NSPKU – National Society for Phenylketonuria

PAH - Phenylalanine Hydroxylase enzyme

Peds QL® – Paediatric Quality of Life inventory

Phe – Phenylalanine

PKU – Phenylketonuria

RNI – Reference Nutrient Intake

SDS - Standard Deviation Score

SIMD – Scottish Index of Multiple Deprivation

Summary

Introduction: Phenylketonuria (PKU) is an autosomal, recessive inborn error of metabolism caused by a deficiency of the enzyme Phenylalanine Hydroxylase (PAH). Reduced activity or absence of PAH results in increased concentrations of phenylalanine in plasma and brain, which if not addressed will result in severe disability and reduced quality of life.

The accepted management of PKU is to restrict intake of dietary phenylalanine and to adhere to a semi-artificial diet which consists of: a phenylalanine free protein substitute; measured amounts of phenylalanine containing foods; specially manufactured low protein staple foods (LPSF) and virtually unrestricted use of phenylalanine free natural foods. Dietary restriction of phenylalanine was first reported in the early 1950's and although the use, format and efficacy of the protein substitute has been well researched, evidence for the use of the LPSF is scarce.

Anecdotally the LPSF are believed to play a substantial role in PKU management and contribute to the nutritional requirements of patients. However, how patients and their carers perceive their usefulness and the role they play in the overall management of the disease has not been evaluated. Recommendations exist for the amount of LPSF required by patients has been produced by the NSPKU society, but are not evidence based.

Aim: To evaluate the contribution LPSF make to the typical diet of children with PKU. To explore any association between LPSF and nutritional status, quality of life, diet variety and any issues patients and carers may face when obtaining supplies.

Method: The use of LPSF in PKU management was evaluated in two ways: a) by a survey of adult patients and carers of children from metabolic centres throughout Scotland, to explore perception of the need for LPSF and any issues faced when obtaining supplies, b) a cross-sectional study using patients from one of the metabolic centres to determine the contribution these foods make to the nutritional status and quality of life. This was achieved by allocating patients into user groups dependent on daily use of LPSF using food diaries and and Food Frequency Questionnaire (FFQ).

Results:

Study 1: 178 questionnaires were sent to patients with PKU in Scotland returning a response rate of 46%. 97% of patients and carers who responded understood the need for LPSF in managing their condition, with 96% using the LPSF to control phenylalanine levels; 80% used the foods to provide diet variety and satisfy appetite. The most common foods requested were core LPSF of pasta and pasta products (77% of monthly prescriptions); flour mix (61%). Greater than 40% of respondents reported that the sensory properties of LPSF were good. However, 49% of respondents experienced comments from primary health care providers when requesting prescriptions, with 59% of these having a comment of a negative nature which affected the number of prescription requests made. Of the respondents 50% ordered less than the amount of LPSF recommended by the NSPKU society. When questioned about use of a home delivery service, 51% used this service for ordering LPSF and 68% had attended cookery workshops arranged by their dietitian. No information regarding disease severity was requested for this survey.

Study 2: 40 patients (children aged between 2-17 years: 56% (n=23) male) were recruited of whom 30 (75%) completed the seven-day food diary and FFQ. 67% (n=27) had classical PKU; 7.5% moderate (n=3) and 25% (n=10) having mild PKU (exchanges / day: 2-6; 7-8; 9-13). Of the children, 67% (n=27) had a SDS BMI within the normal range; 22% (n=9) were overweight; 7% (n=3) obese. Those of secondary school age (27%; n=11) took significantly less energy (%EAR) from the protein substitute than the younger children (p=0.007). When the subjects were allocated into LPSF user groups, no difference between SIMD score, quality of life domains, metabolic control or height was observed between the groups. However, the low users of the LPSF had a significantly higher SDS weight (p=0.047) and SDS BMI ((p=0.036). Children from the mildest disease category recorded the lowest amount of energy from the LPSF (p=0.012), and ingested significantly more energy from snack foods (which included both LPSF and regular snack foods) [high vs. medium vs. low: median (IQR): 12% (11); 14% (13); 36% (26) (p=0.010)]. All patients from the three LPSF user groups took less than 50% of non-protein energy (%EAR) from LPSF (0-49%). Low users in particular only took between 0-17% of the recommended number of units suggested by the NSPKU society. The use of food from groups as outlined in the NDNS survey was similar for children with PKU as with the general population however intake of all food groups was lower, especially in the potatoes and cereal (including LPSF) groups.

Conclusion: Patients and their carers have a positive attitude and perception on the use and need for LPSF in the management of their diet for PKU, but have issues in obtaining supplies. Low users of LPSF were in the milder disease category and had a greater intake of energy from snack foods, also a higher SDS weight and SDS BMI.

Low users of LPSF who have milder mutations of PKU should be encouraged by their metabolic specialists in the use of use some protein dense foods to provide their phenylalanine requirements. This would allow the use of a greater amount of LPSF to ensure that energy needs are met by the staple LPSF rather than regular high energy snack type foods. Support by specialists in the metabolic centres to aid parents and carers obtain sufficient supplies of LPSF in a timely manner is essential to promote use of product.

Further studies are needed to determine the perception of the usefulness of LPSF in the management of PKU by patients with mild disease and by low users of LPSF. In addition there is a need to explore whether it is low LPSF users who find it most difficult to obtain supplies. Studies with the adult PKU population exploring any association between obesity and use of LPSF are also necessary given the lack of information in this group of patients.

Introduction

Phenylketonuria

Phenylketonuria (PKU) is an autosomal recessive inborn error of metabolism caused by deficiency of the enzyme Phenylalanine Hydroxylase (PAH). PAH is essential for the conversion of the amino acid phenylalanine to tyrosine along with the co-factor Tetrahydrobiopterin (BH4). Reduced activity or absence of PAH results in increased concentrations of phenylalanine and reduced levels of tyrosine in plasma. PKU has an incidence of 1:10,000 per year in the UK (MRC Steering committee, 1981) and the prevalence is similar in European populations (Mitchell, Trakadis & Scriver 2011) although variations amongst ethnic groups are noted (Hardelid et al. 2008). Since 1969 PKU has been diagnosed through the U.K National Newborn Screening programme.

Historical overview

PKU was first described in 1934 by Asbjøn Følling (Følling 1994) a professor of nutritional medicine, physiology and biochemistry, who suggested that the presence of phenylketones in the urine as being the underlying cause of neurological deficits in two siblings Liv and Dag Egeland from Oslo. Their parents' persistence in wanting to find the cause of their children's mental retardation persuaded Følling to examine the children. The parents also noted that the urine of both children had an unusual odour. During the examination of the children, a routine urine check detected phenylpyruvic acid.

Følling speculated that the presence of the ketoacids in the urine was the result of an inability to metabolise the amino acid; phenylalanine. He then went on to test urine of other children with mental retardation and discovered another 8 children with urinary phenylpyruvate. All 8 children had similar characteristics notably: eczema, fair skin, a spastic gait and severe intellectual impairment. Følling described this condition as "imbecillitas phenylpyrouvica".

Following this discovery, Jervis (USA) and Penrose (UK) tested similarly affected individuals in their respective countries. In 1937, Jervis identified a deficiency of the enzyme PAH as the cause for the phenylketones present in the urine (Williams, Mamotte & Burnett 2008). The condition became known as "phenylketonuria", and is still commonly referred to as this, although hyperphenylalaninaemia more accurately identifies the range of conditions treated today. In 1950, Horst Bickel introduced a low phenylalanine diet which was found to be effective in lowering phenylalanine levels. Woolf (Woolf, Vulliamy 1951) suggested that the neurological damage characteristic in PKU was caused by an intoxication of phenylalanine and that the low phenylalanine diet would re-

lieve the symptoms of mental deficiency. Armstrong reported the first use of a synthetic diet using an amino acid mixture and low phenylalanine foods in 5 children with phenylketonuria (Armstrong, Tyler 1955a). The children who were aged between 7 months and 4 ½ years all showed improvement in behaviour, development and a lowering of seizure activity.

The detection, treatment and effect on intelligence of PKU were all described in the early 1950's by several authors (Woolf et al. 1958; Blainey, Gulliford 1956; Woolf, Griffiths & Moncrieff 1955; Gibbs, Woolf 1959).

A report in the British Medical Journal of 1958 suggested that by the time the condition was diagnosed and diet established, the mental rating was only able to be raised from "*idiot to imbecile level*" and noted that the effect of treatment varied with the age dietary restriction was started. Moncrieff suggested that early diagnosis from the age of 3 weeks and use of a low phenylalanine diet, intelligence could be improved (Moncrieff, Wilkinson 1961). Parents of these treated children also commented that their affected children were more manageable, less restless, fits ceased and their eczema cleared up when on diet.

Introduction of screening for PKU

In 1960, Allen described a method of screening patients identified with neurological defects who attended his paediatric clinic (Allen 1960). The test consisted of strips impregnated with ferric chloride which detected urine phenylpyruvic acid. The strips were given to the parents to test the urine in nappies and then return to him in the post. Robert Guthrie, a cancer researcher, whose niece had intellectual delay caused by PKU, developed the microbial inhibition technique (Guthrie, Susi 1963) used for mass screening of new-born's from the late 1960's.

In 1963, the report to the Medical Research Council (MRC) of the conference on Phenylketonuria gave recommendations on screening, diagnosis and treatment of Phenylketonuria (Anonymous 1963). This committee recommended that screening of infants be carried out in the fourth week of life by a urine test to detect phenylpyruvate acid. As a result of this conference, 131 local health authorities in England and Wales introduced routine urine testing, 5 planned to do so and 9 had no plans. In 1967, South Eastern Scotland introduced the first routine blood testing by the heel prick test using the Guthrie method at day 6. The tests were analysed by the bacteriology department at Stobhill Hospital and the laboratory subsequently became the National Screening Laboratory for Scotland. This test, commonly referred to as the Guthrie test, and the more modern tests using tandem mass spectrometry, now form the basis of neonatal screening for PKU in many countries around the world.

Newborn screening has been credited by Kyriakie Sarafoglou MD, Minnesota, as being "*one the most important public health advances of the 20th century, allowing children with rare disorders to live a better life"* (Kuchn 2013).

Current newborn screening

The newborn screening programme today uses a heel prick blood sample taken between day 5-8 (UK) from all newborn infants (99.9% uptake; *www.newbornbloodspot.screening.nhs.uk*) and now tests for a range of treatable conditions including: PKU, MCADD (Medium-chain-acyl-co-a-dehydrogenase-deficiency), Cystic Fibrosis (CF), Sickle Cell Disease, and Congenital Hypothyroidism (in Scotland). Tests for additional Inborn Errors of Metabolism were introduced as a pilot scheme in England and Wales (http://newbornbloodspot.screening.nhs.uk/expandedscreeningstudy) and will be incorporated into the screening programme from this year.

The test for PKU will identify abnormal phenylalanine and tyrosine levels. Normal phenylalanine levels are between 60-90 umol/l and the threshold for reporting abnormal samples is >240umol/l for phenylalanine and <240 umol/l for tyrosine for a diagnosis of PKU. Tyrosine levels ≥ 240 umol/l are reported as needing further investigation for conditions affecting tyrosine metabolism or liver dysfunction such as Galactosaemia. Screening and the prompt introduction of a phenylalanine restricted diet has enabled affected individuals to be identified and escape the severe neurological effects of untreated hyperphenylalaninaemia. Nowadays with early diagnosis and effective dietary control of the phenylalanine levels, individuals with PKU are expected to have the same ability and life chances as unaffected people (Spronsen et al. 1997; Weglage et al. 1996).

Classification of PKU

Severity of hyperphenylalaninaemia is a result of a mutation in the phenylhydroxylase (PAH) gene of which there are around 564 different mutations (Yu 1970a; Blau et al. 2011). Yu (Yu 1970a) in his review of phenylketonuria described 5 groups of hyperphenylalaninaemia severity based on the Guthrie screening test (Table 1). Classical phenylketonuria was defined as the fasting phenylalanine level being above 1,200 umol/l, after 6-10 days of age with phenylketones present in the urine, resulting from very low or absent PAH activity.

Atypical phenylketonuria was classed as having phenylalanine levels of 240-1200 umol/l with partial absence of the enzyme. Other groups included: maternal PKU, phenylalanine transaminase defects, and transient neonatal hyperphenylalaninaemia. Blau (Blau, van Spronsen & Levy 2010) classified severity of hyperphenylalaninaemia by the phenylalanine level based on newborn screening results before treatment has started. Those with blood phenylalanine concentrations of 120-

600 umol/l are described as having mild hyperphenylalaninaemia; concentrations between 600-1,200 umol/l as moderate hyperphenylalaninaemia and classical phenylketonuria is where the phenylalanine levels are above 1200 umol/l. Current UK classification as denoted by the UK Newborn Screening guidelines is noted in Table 1.

Table 1: Classification of PKU

Author	Number of categories of disease	Method of determination	Classification	Phenylalanine concentration umol/l
Yu 1970	5 groups	Based on screening result	Atypical Maternal Phe transaminase defects Transient neonatal Hyperphenylalaninaemia	240 – 1200
			Not reported	<240
Blau 2010	3 groups	Based on screening result	Monitored – no treatment Mild Hyperphenylalaninaemia Moderate Hyperphenylalaninaemia Classical PKU	240 - 400 400-600 600-1200 >1200
Guldberg 1998 **MRC working party**	3 groups	Based on phenylalanine tolerance	Severe Moderate Mild	250 - 350mg Phe/day 350 - 400mg Phe /day 400-600mg Phe / day
Current Newborn screening classification	4 groups	Based on screening result	Hyperphenylalaninaemia or Non-PKU Hyperphenylalaninaemia Mild PKU Moderate PKU Classical PKU	<600 600-900 900-1200 >1200

PKU register

In 1963 the MRC/DHSS Phenylketonuria Register was set up under the supervision of the MRC Committee for Phenylketonuria and later Dr Isabel Smith (Institute Child Health, London) to monitor the effectiveness of the dietary management of PKU. Patients, whose phenylalanine levels remained below 480 umol/l, and were on free diet, were excluded from the register. The register kept details of diagnosis, dietary management and intellectual ability of all patients diagnosed with PKU in the UK from 1964. This register allowed information to be gathered and analysed regarding the incidence and effectiveness of the low phenylalanine diet to aid the formation of treatment guidelines. The register was kept until 1998 and finally discontinued due to lack of funding.

Early dietary management

The early low phenylalanine diet was based on a low phenylalanine casein acid hydrolysate powder mixed with oil and sugar, with the phenylalanine being removed by charcoal treatment (Woolf, Griffiths & Moncrieff 1955). These protein substitutes included: Minafen (Truefood Ltd) and Lofenalac (Mead Johnston Ltd) used for infants and Cymogran (Allen & Hanbury Ltd) and Albumaid XP (Scientific Hospital Supplies) for older children. The remainder of the diet was based on: tinned fruit, some vegetables, vegetable margarine and specially prepared bread and biscuits made from wheat starch. Later, low phenylalanine preparations based on amino acids (Aminogran Food Supplement, UCB Pharma) were developed and had a more acceptable taste (Smith et al. 1975). The protein substitute was lower in fat and carbohydrate than previous substitutes, thereby allowing for the addition of a greater range of "normal" foods such as fruit and some vegetables into the diet. All the preparations required supplementation of vitamins and minerals to varying degrees.

The diet was considered complicated and children were accepted for treatment only if the parents were considered able to manage the diet at home following a long period in hospital stabilising phenylalanine levels (Woolf, Griffiths & Moncrieff 1955). Several reports mention the competency and intelligence needed by the parents in managing this complicated diet (Moncrieff, Wilkinson 1961; Woolf, Griffiths & Moncrieff 1955; Yu 1970b; Brimblecombe et al. 1961b).

Centerwall (Centerwall et al. 1961) described treatment and the dietary restrictions used in 10 cases of children aged 8 months to 3 years. He reported that the diet was well received and the children demonstrated an improvement in their development. All children

received daily vitamin and iron supplements and had weight in proportion to their height, but their height was below average.

The dangers of a low phenylalanine diet were reported by (Moncrieff, Wilkinson 1961) and included growth failure, weight loss, severe rash, skin lesions and anorexia in three treated infants. The affected infants, who were all fed different low phenylalanine preparations, responded to cow's milk being added to the diet. They consequently made a dramatic recovery and their blood when tested for phenylalanine concentrations showed that the infants were suffering from severe phenylalanine deficiency.

Early dietary management guidelines

In 1963, the report to the MRC (Anonymous 1963) of the conference on Phenylketonuria, gathered information from several countries on complications with the dietary management of PKU. It gave recommendations on screening, diagnosis and treatment of Phenylketonuria. The report from this conference concluded that infants should be given 25mg/kg phenylalanine from milk or cream and this adjusted as necessary to keep the blood phenylalanine levels just above the normal range for unaffected individuals.

The MRC also advised that 50% of the calories should come from carbohydrate and 35% from fat. The carbohydrate intake could be supplemented by the use of gluten free wheat starch and sugar. Recipes were developed to enable the wheat starch to be made into cakes and biscuits "*to make the diet interesting".* Yu (Yu 1970b) in a review of phenylketonuria reported the diet as being "*one of the most difficult and socially limiting of therapeutic diets*".

Included in the report were tables of natural foods which had their phenylalanine content estimated which allowed them to be incorporated into the diet. The estimated phenylalanine content was based on the assumption that animal and vegetable proteins contain between 4 -6% phenylalanine. Thereby these lists of foods could be used to aid variety in the diet and have formed the basis of the phenylalanine exchange system (50mg / 100g food) still in use today.

Consequences of PKU

Neurological consequences of PKU

As early as 1951 the mechanism of the damage to brain development caused by raised phenylalanine levels had been described (Woolf, Vulliamy 1951). The deficiency or reduced activity of PAH which results in high phenylalanine levels and consequently reduced tyrosine, leads to a shortage of dopamine, noradrenaline, and tryptophan, the precursor of serotonin. The raised phenylalanine levels restrict the transport of large neutral amino acids such as tryptophan and tyrosine, by competitive inhibition, across the blood–brain barrier interfering with the normal transport of neurotransmitter precursors. In addition, this insufficiency of large neutral amino acids may be linked to the mental impairment observed in phenylketonuria. A shortage of tyrosine is implicated in the cognitive and behavioural difficulties reported in early treated children who had higher than recommended phenylalanine levels (Van Spronsen, Ahring & Gizewska 2009).

With the introduction of newborn screening and prompt dietary therapy, the risk of severe neurological damage was eradicated. The focus then moved to determining: what degree of phenylalanine levels above normal (60-90 umol/l) require treatment; what are "safe" phenylalanine levels; how long the diet should be continued and whether or not the determined safe range is suitable for all ages (Waisbren et al. 2007; Pollitt 2012).

Griffiths (Griffiths, Campbell & Robinson 1998) compared executive function in eleven early treated primary school children with age matched controls and found no difference in the ability to maintain attention or make decisions when phenylalanine levels were kept under 300 umol/l. However, (Leuzzi et al. 2004) compared 14 early treated children (ages 8-13) and demonstrated impairment of executive function with similar phenylalanine levels (<400umol/l).

A review by Waisbren (Waisbren et al. 2007) discussed the reliability of using phenylalanine levels to predict outcomes in intelligence and the wide variation in accepted ranges of phenylalanine levels worldwide. The review noted that during childhood, for every rise of 100umol/l of phenylalanine there was a loss of between 1.3 and 3.1 IQ points. It concluded that levels ≤360 umol/l were optimal in preventing loss of IQ and that using of phenylalanine levels were a good indicator of future IQ. Gentile (Gentile, Ten Hoedt & Bosch 2010) highlighted the "hidden disability" experienced by well controlled patients with PKU and de-

scribed the emotional and social difficulties experienced by treated individuals which led to impact on quality of life.

Effect on growth

During the early days of dietary treatment, restricted growth was attributed in part to excessive protein restriction to limit phenylalanine intake (Fisch, Walker & Anderson 1966). This may not be the case at the present time as treatment has substantially improved (Spronsen et al. 1997). The increase in recommended protein intake from the protein substitutes (MRC 1993) has contributed to growth not being an issue with the majority of patients at the present time (Huemer et al. 2007).

Quality of life

Quality of life is a concept comprising many facets: mental, social, functional and psychological well-being. The complex nature of the dietary management for PKU such as: keeping track of natural protein intake; children requiring encouragement to take the protein substitute; regular blood tests and the clinic visits that are required by the clinicians managing the child, have been implicated in poor quality of life. The day to day management of PKU places a burden on parents and patients, especially the younger children (Vegni et al. 2010; Fidika, Salewski & Goldbeck 2013). Parental acceptance of the diagnosis has been shown to affect their levels of stress and coping abilities (Lord, Ungerer & Wastell 2008), although this did not seem to contribute towards any behaviour problems in the child with PKU.

Obesity

The trend towards obesity has been shown in particular with the older, female patients who have classical PKU (Burrage et al. 2012; Robertson et al. 2013). They also found that adults with PKU had a similar rate of obesity and overweight to the general population in the UK and demonstrated there was a correlation between high phenylalanine concentrations and rate of obesity. The aetiology behind this remains unclear, but may be related to lack of exercise, poor compliance with the diet or poor dietary choices. Physical activity and poorly controlled PKU is an area where there is little information and with the lack of knowledge of the general health of an aging PKU population is an area that is of interest for future study.

Micronutrient deficiencies

Although present in sufficient amounts in the protein substitute, Vitamin B12 is the most frequently reported vitamin deficiency (Walter 2011; Vugteveen et al. 2011; Lee et al. 1999; Hanley et al. 1996). Patients who are at most risk are those who are non-compliant with their protein substitute (Lee et al. 1999) or have discontinued their diet and have not completely normalised their dietary intake by using high protein foods such as meat, fish, eggs and dairy products (Robinson et al. 2000a).

Selenium is found mainly in foods that are restricted in the PKU diet, including: cereals, eggs, fish and Brazil nuts and was not routinely added to the protein substitute until the 1990s. Fruit and vegetables which are low in selenium because the soils of Europe generally tend to be depleted in selenium, make it essential that there is the addition of selenium to the protein substitute to provide requirements. A review of literature by Robert (Robert et al. 2013) suggests that while low serum selenium is common, reports of symptomatic selenium deficiency are rare in treated PKU (Greeves et al. 1990). Selenium deficiency has been linked to poorer performance in neuropsychology tests and altered antioxidant status (Gassió et al. 2008; van Bakel et al. 2000).

Early studies linked Zinc deficiency with poor growth (Acosta et al. 1982), but although nowadays low plasma zinc is frequently described (Fisberg et al. 1999b; Rohr, Munier & Levy 2001a; MacDonald et al. 2008), poor growth does not appear to be an issue.

Bone health and PKU is an issue currently attracting more attention with reduced bone mineral density and increased risk of fractures reported (Greeves, Thomas & Carson 1995; Greeves et al 1997), however, Mirás linked the development of bone disease with the reduced natural protein intake in well controlled, classical PKU (Mirás et al. 2013).

Use of Protein substitutes in PKU

Early protein substitutes

Low phenylalanine protein substitutes were commercially developed in the early 1950's. They were based on a protein hydrolysate rendered free from phenylalanine by passing it through a column of charcoal which removed phenylalanine along with tyrosine and tryptophan (Woolf, Griffiths & Moncrieff 1955).

This powder was made into a "soup" along with wheat starch, kosher margarine, glucose and water and given 4 or 5 times over the day. Additional vitamins, minerals and arachis oil were also added. As phenylalanine could not be converted to tyrosine, tyrosine therefore became an essential amino acid and was a necessary addition to the casein hydrolysate along with tryptophan. The first synthetic amino acid mixture was described by Armstrong (Armstrong, Tyler 1955a) and provided free by several companies for use in his experiments. This mixture required the addition of salts, vitamins, carbohydrate as sucrose and olive oil, made into a slurry and fed to infants and given as a paste to the older children. All children had watery diarrhoea, intestinal cramps and retching with the diet initially which settled down after a week or so.

The early "complete" protein substitutes were found to be deficient in zinc and selenium (Barretto et al. 2008; Fisberg et al. 1999a; Gokmen-Ozel et al. 2009), which prompted increased supplementation now contained in the substitutes used today. A report by Wilson and colleagues (Wilson, Clayton 1962) describes infants with PKU fed a low phenylalanine formula "Minafen" (Truefood Ltd) developing a severe skin rash and lack of weight gain due to micronutrient deficiencies. Such difficulties often led to the diet being stopped before the age of 8 or 10 years.

In 1970, Clayton and Frances trialled a new form of protein substitute as an amino acid formula (Aminogran, UCB Pharma) and demonstrated that this improved the palatability of the diet, improved acceptance and intake of early treated children with PKU. This new product required supplementation with vitamins and minerals, but a smaller amount of powder was needed to provide the required protein allowance was and was often recommended to be mixed with sugary, fizzy drinks such as cola or lemonade to make it acceptable.

Current use of protein substitutes

Macdonald described problems associated with taking the protein substitute in young children such as refusal to take the substitute (MacDonald et al. 1994). Over the years there have been many studies associated with the presentation and palatability of the protein substitute (MacDonald et al. 2004; MacDonald et al. 2006; Rohr, Munier & Levy 2001b). As the low phenylalanine diet is now recommended for life (NSPKU), protein substitutes have had to be adapted to become more acceptable to adults and consequently their lifestyle. This need has led to the development of tablets and "ready to drink" products in addition to changes in packaging which aim to aid convenience and reduce the medical appearance of the products and thereby improve adherence with the diet.

Another area of study has been on the amount of protein substitute and frequency it should be given. MacDonald (MacDonald et al. 2003; MacDonald et al. 2006) suggested that the more often the protein substitute is given over a 24 hour period, including overnight, the more stable the phenylalanine concentrations. However, the study acknowledged that this was generally impractical in reality.

The amount of energy supplied by the protein substitute has also been an area of interest (Gokmen-Ozel et al. 2011) with the trend towards lowering the carbohydrate content and therefore the satiety effects of carbohydrate to try to improve appetite without an adverse effect on phenylalanine control.

Design of the protein substitute and adherence to diet

As recommendations for "diet for life" have become the norm, there was a need to develop protein substitutes that were acceptable to adolescents and adults. Over the years, studies on the protein substitutes were designed to produce products concentrating on improving the taste, efficacy and acceptability using patients as their own controls (Bentovim et al. 1970; Macdonald et al. 2004; Yi, Singh 2008a). In addition, the focus also turned also to producing protein substitutes that were more convenient and easy to use (MacDonald et al. 2003; MacDonald et al. 2004; MacDonald et al. 2006). These protein substitutes became available in a variety of forms such as: ready to drink liquids, tablets, fat coated granules and bars.

Gokmen-Ozel in her study, reported on the modification of the protein substitute by the reduction of the carbohydrate content and the effect this had on phenylalanine control. Subjects again were their own controls and the ready to drink protein substitute was com-

pared with the protein substitute they were taking before the investigation and no effect on phenylalanine control was found (Gokmen-Ozel et al. 2011).

Specialist nutrition companies such as (Nutricia) SHS International Ltd, Vitaflo International Ltd and Milupa Ltd have strived to develop products designed to be more acceptable to the growing child and adolescent through to the adult with PKU. The companies aim to help maintain dietary control and attempt to increase adherence amongst this age group. The increased range of products has led to improved compliance with the protein substitute (Gokmen-Ozel et al. 2009; MacDonald et al. 2006; Yi, Singh 2008b; Rohr, Munier & Levy 2001b), however, the studies were short term and it is not known if the effect is sustained.

Compliance or adherence with treatment has been the subject of many reports (Alaei et al. 2011; MacDonald 2000; Bik-Multanowski et al. 2008). In a review of diet and compliance with treatment, MacDonald (MacDonald 2000) concluded that the concept of compliance was complex. Adherence was related to many factors, not only with taking the prescribed amount of protein substitute, but found that motivation and a positive attitude towards the diet was essential and support from all health professionals involved in the care of families and patients was paramount.

Current management practice

Biochemical Monitoring

Following the 1963 MRC report, Clayton and Francis published a very detailed, precise method for feeding and monitoring an infant with PKU and this formed the basis of the advice still used at the present time (Clayton, Francis & Moncrieff 1965). An intake of 20-45mg phenylalanine/kg (Centerwall, Centerwall 1969; Brimblecombe et al. 1961a) was suggested for infants in order to maintain serum phenylalanine levels between 180-480 umol/l (3-8mg/100ml). Although levels less than 600-720 umol/l (10-12 mg/100ml) were widely used during the 1970's (Yu 1970b), it was recognised that phenylalanine levels above 20mg / 100ml (1200 umol/l) were regarded as an upper safe limit in relation to the prevention of neurological impairment.

Safe limits for target phenylalanine levels advised for children as they grow into adulthood vary between countries (Table 2), as although the requirement for dietary management is undisputed, there is a lack of evidence as to the agreed goal of management.

Table 2: Target phenylalanine concentrations by age groups*

	UK	France	Germany	USA
Birth – 6 years	120-360	120-300	40-240	120-360
7-9years	120-480	120-300	40-240	120-360
10-12 years	120-480	120-600	40-900	120-360
13-15	120-700	120-900	40-900	120-600
>16	120-700	120-1200	40-1200	120-900

* adapted from Blau (2010)

New guidelines about to be published by the NSPKU (2014) will advise that all children with PKU up to the age of 16 should keep their phenylalanine levels below 480 umol/l and adults are recommended to maintain levels below 700 umol/l. However, guidelines to be published in the USA will state that phenylalanine levels for all ages should be kept between 12- 360 umol/l to maintain optimum health and wellbeing.

Frequency of monitoring

In addition to the variation of safe phenylalanine concentrations between countries, there is also variation in the recommendations of frequency of monitoring phenylalanine levels. Schweitzer-Krantz (Schweitzer-Krantz, Burgard 2000) reviewed practices in Europe and found that guidelines were very rarely updated and that the main variation between countries was the age at which less frequent blood spots were requested by the managing centres (Table 3).

Table 3: Variation in frequency of phenylalanine monitoring in Europe*

	UK 1993	Hungary 1998	Czech Republic 1998	Slovakia 1998	France 1996	Germany 1997	Poland 1996
Weekly	0-4 yrs.	0-4 yrs.	0-1 yr.	0-1 yr.	0-2 yrs.	0-1 yr.	0-6 mths.
Fortnightly	5-9 yrs.	5-9 yrs.			3-8 yrs.	1-9 yrs.	6-12 mths.
Monthly	> 9yrs	> 9yrs	1-6 yrs.	1-6 yrs.		10-15 yrs.	> 1 yr.
3 monthly			> 6 yrs.	> 6 yrs.	9-13yrs.	>15 yrs.	
Yearly					> 13 yrs.		

* adapted from Schweitzer-Krantz & Burgard 2000

In addition to the monitoring of phenylalanine levels, monitoring of growth, amino acids and clinical review varied widely between ages of patients at across Europe (Van

Spronsen, Ahring & Gizewska 2009) with clinical review decreasing from around 9 times a year for the under 1 year olds to yearly for patients over 12 years of age.

Several authorities recommended that children with PKU should be managed by specialists where expertise and support was available (Clayton, Heeley & Heeley 1970; McBean, Stephenson 1968; NIH, 2000). Current guidelines recommend that the specialist team consists of a consultant paediatrician in inherited metabolic disease, a paediatric dietitian with metabolic disease expertise, a metabolic nurse and suitable biochemical facilities. (.http://www.newbornbloodspot.screening.nhs.uk).

Current Dietary Practice

Current UK Dietary Guidelines

The management of PKU today is based on the 1993 recommendations and include:

1. The daily use of a phenylalanine free protein substitute containing all amino acids apart from phenylalanine, along with vitamins and minerals. This replaces protein containing foods such as meat, fish, nuts, soya, poultry, cereals and legumes, and these foods must be completely avoided. The protein substitute used should be tailored to the age requirements of the infant, child or adult with PKU.
2. The use of LPSF produced by specialist food companies which have negligible phenylalanine content. These low protein foods which are available on General Practitioner (G.P) prescription (UK), such as rice, bread and pasta, generally provide a main food ingredient around which a meal can be created and act as a substitute for "conventional" foods.
3. A daily phenylalanine intake provided by natural foods such as potatoes, breakfast cereals and some vegetables. These foodstuffs contain a known, measured amount of Phenylalanine which is 50 mg per 1g of naturally occurring protein and are generally referred to as protein exchanges.
4. Natural foods very low in phenylalanine e.g. most fruit and vegetables, fats, oils and sugars. These foods have had dietary analysis carried out by the National Society for Phenylketonuria (NSPKU) and are able to be given in moderate amounts without being counted in the phenylalanine allowance. These foods generally have a phenylalanine content of < 25mg phenylalanine / 100g.

Low protein manufactured foods

LPSF are specially manufactured alternatives to natural basic foods such as bread, pasta, biscuits and milk. The earliest described LPSF was a biscuit made from wheat starch, sugar and fat (Armstrong, Tyler 1955a; Woolf, Griffiths & Moncrieff 1955). A little later, bread became available in tins, but in Glasgow, a special low phenylalanine loaf and also biscuits were made by a local bakery and were reported as being a boon to patients (McBean, Stephenson 1968). These foods represent alternatives to foods eaten by the general population and serve to improve convenience for patients on a restrictive diet.

The LPSF have improved considerably in the variety, palatability and visual appeal over recent years. Nowadays, patients with PKU can be prescribed a wide range of products including: flour and cake mixes, vacuum-packed bread, rolls and pizza bases, along with burger mixes, several pasta shapes and dried ready meals which aim to improve variety and flexibility with the diet.

Recommendations from the medical advisory panel of the NSPKU (NSPKU-http://www.nspku.org/publications/publication/prescription-guidelines) are available which provide some guidance on the amount of the LPSF that should be prescribed, based on 50% non-protein energy requirements (EAR) (Table 4).

Table 4: Recommended maximum number of units of LPSF for each age group (NSPKU)

Age of patient with PKU	**Recommended maximum number of low protein items to prescribe each month***
4 months -3 years	15 units
4-6 years	25 units
7-10 years	30 units
1-18 years	50 units
Adults	50 units
Pre-pregnancy/Pregnancy	50 units

*This excludes low protein milk replacements and protein substitutes

The guidance from the NSPKU allows the G.P to have some indication whether or not the individual with PKU is having sufficient LPSF when dispensing prescriptions. Many patients will order a variable amount each month and anecdotal reports from patients and their carers is that they have difficulty obtaining sufficient product on prescription because they do not need to order the same amount each month.

When comparing the calorie content of the LPSF against their regular equivalents it can be seen from Table 5 that they are very similar (figures for regular foods taken from the website of a popular supermarket).

Table 5: Comparison of calorie content with regular foods and LPSF equivalent

Calories / 100g	Regular foods	LPSF equivalent
Dried pasta	360	363
Dried rice	355	366
Crackers	440	448
White bread	243	259
Semi skimmed milk / 100ml	50	40
Full cream milk / 100ml	65	66
Cheerios breakfast cereal	382	385

Access to dietary products

Although within Europe and the USA screening for PKU is offered to all new-borns, the treatment consisting of a low phenylalanine diet necessitating the use of LPSF and protein substitutes is not freely available to all (Camp, K et al. 2012). The cost borne by state and patients varies considerably between countries. In the United States, costs for screening and diagnosis are paid for by the state, but not treatment. Some states cover the cost of the protein substitute, but not the cost of the LPSF. In other states, medical insurance companies are required to cover the cost and policies vary between the states with no consensus of who should pay. Within Europe, the state or insurance companies bear the cost of the protein substitutes, however access to LPSF varies. In Belgium, Norway and Denmark, patients receive an annual allowance; in Poland patients are required to purchase LPSF over the internet, whereas in Germany, no allowance is made except for patients on state welfare (Bélanger-Quintana et al. 2011)

In the UK, the dietary products used in the management of PKU are prescribed by the G. P. and the expense borne by the primary care budget. The amount contributed by the patient varies between regions, with Scotland and Wales providing free prescriptions for all ages. Within the rest of the UK, patients over the age of 16 years pay a prescription charge, although a prepaid prescription can be bought which allows free prescriptions for a year. Consequently the cost of the protein substitute and LPSF foods adds a considerable amount to the budget of the UK primary health care service.

Cost of dietary management

Woolf (Woolf, Griffiths & Moncrieff 1955) considered the economic cost of dietary treatment of PKU, quoting a perceived incidence of 4:100,000 and suggested that the untreated patient in 1955 would require care in an institution which would entail a cost of £5 per week. He also calculated the cost of the dietary constituents as 7s 4d per day, which included the casein hydrolysate, tryptophan, vitamins, liver extract and choline and therefore treating by diet would cost more than institutional care. Woolf concluded that if the patients were treated, they would be able to attain a level of education at normal schools although still be classed as educationally subnormal, but the cost of institutional care would be saved. The patients may then go on to be productive members of society. Consequently the cost of the diet should not be allowed to have an influence on the decision taken on whether or not to treat an affected individual.

Today, an average 8 year old child with PKU could cost around £6,700 per year for the protein substitutes and a further of £2,200 for LPSF based on the information to G.P's from the NSPKU. Guest and colleagues (Guest et al. 2013) calculated the expense of maintaining an early treated adult on diet up to the age of 35 years in 2007-2008 as between £21,000 and £149,000 dependant on the amount of prescribable products they received.

The cost of the protein substitute should not fluctuate significantly as patients are generally prescribed a specific daily dose to ensure their protein requirements are met. However, the use of LPSF will vary considerably between patients and the amount used is determined by: a) an individual's preference for the products, b) their compliance with the diet, c) the skills of the person managing the diet and d) the amount prescribed by the GP. Therefore, in times of increasing scrutiny of health service spending, the lack of evidence based guidelines may impact on the amount of LPSF a G.P may be willing to prescribe.

Duration of dietary treatment

There has been much debate as to how long an individual with PKU should remain on restricted diet. The MRC report (1963) did not give any recommendations. Clayton (Clayton, Moncrieff & Roberts 1967) suggested that although raised phenylalanine levels in the mature brain will not cause damage, a change was seen on electroencephalogram with loading doses of phenylalanine, therefore the committee advised not to stop dietary treatment.

McBean (McBean, Stephenson 1968) monitored the progress of a group of 31 children with PKU from Glasgow who were established on diet before the age of 7 years. Eight

of the children were commenced on diet before 3 months of age. Observations from the group included; the effect of stopping diet on intelligence and degree of dietary control required to improve intelligence. They used an upper limit of 4mg/100ml (240 microgram/l) as recommended by the MRC 1963 (Anonymous 1963) and noticed that levels above 12mg/100ml (720 microgram / l) was associated with lower intelligence. Their conclusion was that infants treated before 3 months would have normal intelligence provided phenylalanine levels were controlled below 12mg/100ml (720 microgram / l) and that results of stopping the diet in older children should be done with caution as intelligence may be affected. Due to the complexity of the diet and management of the children, they advised that infants detected by screening, should be managed in specialist centres.

Yu suggested that once brain growth was complete, then high phenylalanine levels would not cause damage and that stopping the diet in early treated children may be possible (Yu 1970a). However, the later diagnosed children who had problems with restlessness, irritability and autistic type behaviours and who were helped by the diet, may not benefit from the diet being stopped.

Smith (Smith, Beasley & Ades 1990) published a study of 599 early treated (treatment starting before 4 months of age) children detailing intellectual progress from 4-14 years using information collected from the UK PKU register. Children were divided into 2 groups: children born between 1964 and 1971 and the second group born between 1972 and 1978. Dietary control, IQ assessments using the Revised Standord-Binet Test for children under the age of 6.5 years or the Wechsler Intelligence Scale for Children (WISC) for older children (ages: 4,8,10,12 and 14 years), severity of PKU and social class were compared. This study concluded that optimal intelligence was achieved with phenylalanine levels maintained below 400umol/l in early childhood and that maintaining good biochemical control was more difficult with classical PKU, but effect on intellectual deterioration diminished as the child grew. The PKU register also revealed that 0.4% of children on the register developed neurological complications later in life and had lesions on subcortical white matter seen on MRI. The author concluded that patients with PKU should be advised to continue restricted diet indefinitely, but acknowledged the difficulties families faced in maintaining strict control.

The 1993 (Anonymous 1993) report from the MRC committee noted evidence of abnormal changes to myelin under MRI and the effect on the foetus of women on uncontrolled diet and advised promoting diet for life.

Maternal PKU syndrome

Mabry and colleagues (Mabry, Denniston & Coldweu 1966) reported on 3 cases of women with PKU having children who were mentally retarded of which one of the offspring, although severely retarded, subsequently went on to have a child of normal intelligence. This led them to postulate that it was high phenylalanine levels in the mother before conception that caused the defects rather than a genetic abnormality. A higher rate of miscarriage was also reported in women who had high phenylalanine levels at conception (Stevenson, Huntley 1967; Mabry, Denniston & Coldweu 1966). Allan (Allan, Brown 1968) reported a case of a woman being placed on diet during pregnancy and the infant consequently not developing any of the defects previously reported. By the age of 9 months the infant was reported to have had normal development. Lenke (Lenke, Levy 1980) identified a distinct range of symptoms associated with high maternal phenylalanine levels which included: facial dysmorphism, microcephaly, developmental delay, learning difficulties, and congenital heart disease (CHD). This became referred to as the "Maternal PKU Syndrome".

Smith (Smith, Beasley & Ades 1990) reported on data collected on 94 infants born to women with PKU from the UK PKU register. They found that the birth weight fell by 98g and head circumference decreased by 0.46cm with each 200 umol rise in phenylalanine concentration. The range of complications in offspring of these women with PKU who did not start diet before conception also included microcephaly, CHD and low birth weight.

Members of the MRC DHSS PKU register went on to set up a maternal PKU register and reported on data collected on 228 pregnancies of women with PKU between 1978 and 1997 (Lee et al. 2005). Of these, 18 (8%) did not have any dietary phenylalanine restriction during pregnancy, 91 (42%) started diet at some point during pregnancy and 110 (48%) started a low phenylalanine diet before conception and 2 had no recorded dietary information. Of the pregnancies of mothers who started diet before conception, the incidence of CHD was 2.4% and mean 4 year development quotient (DQ) was 108.9, whereas in women who started diet after conception, the incidence of CHD was 17% and DQ 96.8, both birth weight and head circumference were also significantly lower in women who did not start diet before conception.

The recommendation for phenylalanine levels prior to conception and during pregnancy is to maintain them between 60-250 umol/l (Smith et al. 1993). It is the aim of all units who manage women with PKU of childbearing age to ensure that they are aware of the need

for strict metabolic control before conception to prevent the complications associated with Maternal PKU Syndrome.

Integrative Statement

To date, the majority of studies on the nutritional management of PKU have concentrated on the use of the phenylalanine free protein substitute and its association with nutritional status of the patient. However, no studies have looked at the use of LPSF and their importance to the management of patients with PKU, although anecdotally they are believed to play a substantial role and contribute to the nutritional requirements of patients with PKU. There are also no evidence based guidelines or rationales for the exact amount of the LPSF required by patients with PKU.

Hypothesis

The use of LPSF is necessary to achieve energy requirements, optimal control of phenylalanine levels and aid compliance with the low phenylalanine diet and add to diet variety for children and adults with PKU

Aim of the study

The aim of this MSc project is two-fold:

1. First to determine the beliefs held by patients and their carers on the need for the LPSF in managing their condition and the issues they face with access and acceptability of the products.
2. Secondly, to assess the contribution the LPSF make to the dietary intake, nutritional status, diet variety, management of PKU, quality of life and compliance of PKU.

Study 1

A questionnaire survey on the usage of low protein staple foods by people with phenylketonuria in Scotland.

Summary

The first study explored beliefs, acceptability and issues around the use of LPSF by people with PKU or their carers. This was achieved by posting a semi-anonymous questionnaire to 178 people with PKU in Scotland (104 children 2-17 years and 74 adults). Questions explored: type and amount of LPSF ordered; perceptions on use and usefulness of LPSF; acceptability of the LPSF sensory properties (i.e. taste, smell, texture, appearance); support for the supply and use of LPSF and comments from primary care health professionals regarding dispensing and prescription.

Eighty two subjects returned the questionnaire and the majority perceived that the LPSF were useful for management of their diet. In addition, more than 85% reported LPSF important for phenylalanine control, satisfying appetite, and diet variety. The most common LPSF ordered were pasta/rice/cous cous, flour, biscuits and bread. The sensory properties of LPSF were well perceived. Nearly half of all respondents had received a comment from primary health care staff regarding the prescription or dispensing of their LPSF.

This part of the study concluded that there is a positive attitude and perception on the use and usefulness of LPSF in the management of PKU. Issues in supply and provision of LPSF within primary health care may indicate poor communication between specialists and primary care professionals or a lack of scientific evidence demonstrating their clinical effectiveness.

Introduction

Treatment of PKU relies on limiting dietary phenylalanine to maintain plasma phenylalanine concentrations within the recommended range as recommended by the MRC 1993 (Smith et al. 1993). As phenylalanine occurs in all protein containing foods, the conventional management approach is to restrict the intake of dietary protein and adhere to a partially "artificial" diet.

In UK, patients are generally managed in specialist metabolic centres under the care of a multidisciplinary management team encompassing a consultant in metabolic medicine, a specialist dietitian and nurse. To date, dietary management in PKU comprises: a) free consumption of natural foods low in protein, such as fruit and vegetables; b) use of phenylalanine-free amino acid substitutes containing micronutrients: c) a measured amount of natural protein, which is dependent upon the degree of residual PAH activity and d) use of LPSF with negligible phenylalanine content.

The LPSF are specially manufactured versions of common basic foods such as pasta, bread, rice and biscuits. In the U.K, these LPSF are only available on prescription from the G.P. and dispensed by pharmacists, unlike other specially manufactured products, such as gluten free foods for the management of coeliac disease, which are widely available in retail outlets. Although the dietary management of people with PKU is provided by the specialist health care professionals, the prescription and dispensing of the LPSF remain within the remit and budget of primary health care providers (e.g. G.P).

There have been several studies which have evaluated the use, clinical efficacy and acceptability of the phenylalanine free amino acid substitutes (Gokmen-Ozel et al. 2009; MacDonald et al. 2006; Yi, Singh 2008b), but similar evidence on the use of LPSF is scarce. From the earliest described LPSF; a biscuit made from wheat starch, sugar and fat (Woolf, Griffiths & Moncrieff 1955), much improvement has been made in the variety, palatability and visual appeal of these foods over recent years.

However, there is no scientific evidence which has formally assessed the use of LPSF in the management of PKU. To date, the use of LPSF (and free availability through national health services in some countries), relies on anecdotal evidence and clinical experience that they play an important role in the management of PKU, by providing energy, satisfying appetite, increasing food variety and improving overall compliance with the restricted dietary regimen. At present, the use of LPSF is determined by: a) an individual's preference for the products; b) the amount prescribed by their primary health care providers (e.g. G. P).

The cost of LPSF adds a substantial amount to health expenditure. The overall annual cost of care of patients with PKU in the UK is estimated at a total of £24 million per year (<0.1% of the NHS budget) of which 11% is attributed to the use of LPSF (Guest et al. 2013). Within Scotland and Wales, the cost of the prescription of LPSF is borne by the state for all ages; in England, adults but not children are required to pay for their prescriptions. Outside UK there are large differences in the availability, access and cost of the LPSF (Bélanger-Quintana et al. 2011; Berry et al. 2013; Camp, Lloyd-Puryear & Huntington 2012).

As public health funds are becoming increasingly limited and health services expenditure even more scrutinised, provision of LPSF through national health services requires both scientific and clinical evidence to support their use, efficacy and improvement of patient care. This study aimed to explore patients or their parents' beliefs, acceptability and any associated issues with the use of LPSF before we evaluate their clinical efficacy with clinical trials.

Study design and methods

A semi-anonymous (post code was disclosed) questionnaire survey (Figure 1) was designed to ask questions that would reflect issues discussed in the clinical setting by patients and their carers. Although the questionnaire was not piloted before being sent out to patients and their carers, the content was checked for face validity by the specialist metabolic dietitian's from the four recruitment centres and for readability by lay people

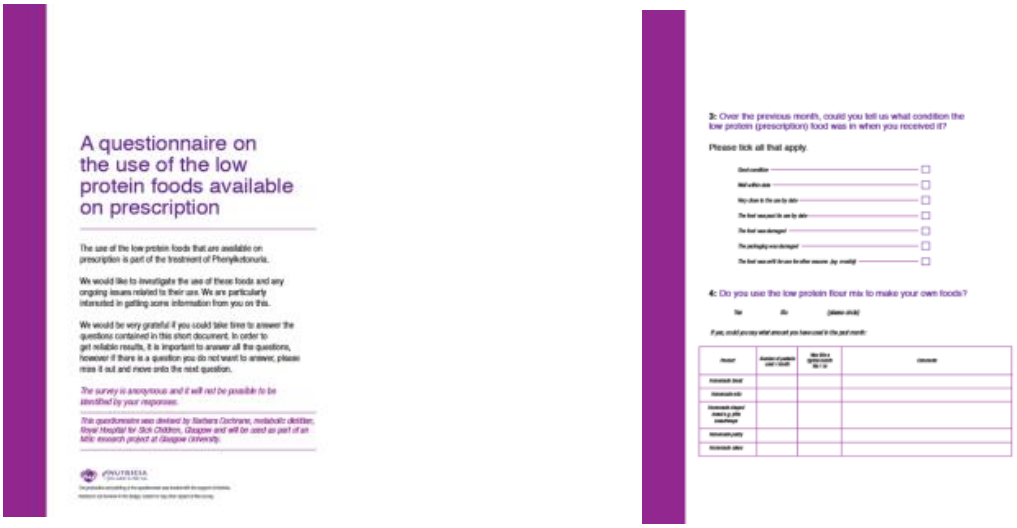
The questionnaire was posted to all people with PKU who were actively receiving care by one of the four metabolic clinical services in Scotland (Glasgow, Edinburgh, Aberdeen and Dundee). A stamped addressed envelope was provided for the return of the questionnaire. A reminder was sent out two months later to encourage response.

Families with children under the age of two years were excluded from the survey as children of this age generally have a low intake of LPSF. Patients who did not engage with the clinical PKU service were also excluded. Ethical approval was discussed with the West of Scotland Research Ethics Service but was not considered necessary for this study.

The questionnaire asked 11 questions, both open ended and multiple-choice (Appendix 1). Questions explored: comments received regarding the dispensing and prescription of LPSF in primary health care; participants' (people with PKU or their parents/carers) perceptions on the use and usefulness of the LPSF; the acceptability of their sensory properties (e.g. smell, taste and texture); and current available support for the supply and use of LPSF. The latter included questions about the use of a free (to recipients) LPSF home delivery service and the attendance at regular interactive cookery workshops organised by the dietitian's from the four metabolic clinical centres. Participants were also asked about the type and amount of LPSF ordered within the previous month. The units of LPSF ordered were compared against the NSPKU recommendations for the monthly allowance. (http://www.nspku.org/publications/publication/prescription-guidelines).

Basic demographic data were also collected. Participants' comments on the prescription and dispensing of the LPSF were given a positive, neutral or negative attribute by the investigator, based on any remark made by a member of the primary health care staff to the respondent when requesting or collecting a prescription.

Figure 1: A questionnaire on the use of LPSF available on prescription



Statistical analysis

Statistical analysis was carried out using Minitab version 16. Categorical responses were presented with numbers and percentages. Differences between groups (e.g. paediatric vs. adult users) were explored with Fisher's exact test, although small sample size and multiple testing precluded statistical analysis for some responses.

Results

A total of 178 questionnaires were posted to 74 adult and 104 paediatric (age 2-17 years) patients attending the four departments of inherited metabolic diseases in Scotland. Of these, 82 (46% response) questionnaires were returned providing information on 87 patients (five questionnaires each provided information on two patients). The majority of respondents originated from Glasgow, currently the largest metabolic centre in Scotland. Questionnaire return rates were [number returned/number posted, (% of number returned); Glasgow: 60/106 (57%); Aberdeen: 3/8 (38%); Dundee: 5/18 (28%); Edinburgh: 12/46 (26%); two respondents did not disclose their postcode]. The questionnaires were completed by: 47 (59% of respondents; 50% of eligible patients including five questionnaires providing information for two patients) parents of children [mean (SD) child age: 6.6 (4.1) y] and 33 (41% of respondents; 45% of eligible including adults looked after by carers) adult [mean (SD) adult age: 26.6 (9.7) y] patients. Two respondents did not specify. Because the numbers of carers were small,

we excluded them from subgroup comparative analysis. Not all questions were completed by all respondents.

Beliefs and importance attributed by participants on the use of LPSF

The large proportion of respondents (n=75/77; 97%) perceived that the LPSF were useful for their condition. There was no significant difference between adults with PKU (n=27; 100%) and parents of children with PKU (n=43; 98%). Most respondents reported that they used LPSF to help control phenylalanine levels (n=78; 96%) and because most of the LPSF could be used without restriction in the diet (n=74; 94%). Similarly, more than 80% reported that they used the LPSF to aid diet variety and help satisfy their appetite. Although not significant, a higher proportion (p=0.07) of parents (93%) than adult patients (78%) claimed to use LPSF to provide variety in the diet (Table 6). Of the parents of children with PKU, 91%, valued the LPSF for helping satisfy the appetite, compared with 74% of adults with PKU (p=0.09) (Table 6).

When the respondents were asked about the taste of the LPSF, a higher proportion of parents of children with PKU compared with adult patients [Parents of children with PKU vs. adults with PKU: 31/41 (76%) vs. 12/26 (46%); p=0.02] believed that the LPSF had an acceptable taste (Table 6). Both groups placed equal importance on unrestricted use of LPSF in the diet (Table 6). Approximately 50% of both adult patients and parents [Parents of children with PKU vs. Adults with PKU: 22/41 (54%) vs. 13/25 (52%); p=1.00], used the LPSF because they were advised to by health professionals (Table 6).

Table 6: Reasons and importance attributed to the use of LPSF by a cohort of parents of children and adults with PKU

n (%)*	Important or very important			Neither important or unimportant			Unimportant or unimportant		
	Parent	Adult patient	All	Parent	Adult patient	All	Parent	Adult patient	All
To give variety in the diet	42 (93)	21 (78)	63 (88)	1 (2)	3 (11)	4 (5)	2 (5)	3 (11)	5 (7)
Because LPSF taste good	31 (76)	12 (46)	43 (64)	7 (17)	10 (39)	17 (25)	3 (7)	4 (15)	7 (11)
LPSF can be used unrestricted in the diet	44 (98)	25 (93)	69 (96)	0 (0)	2 (7)	2 (3)	1(2)	0(0)	1 (1)
The respondent has been told to use LPSF by health professionals	22 (54)	13 (52)	35 (53)	13 (32)	9 (36)	22 (33)	6 (14)	3 (12)	9 (14)
The LPSF satisfy the appetite	39 (91)	20 (74)	59 (84)	3 (7)	6 (22)	9 (13)	1 (2)	1 (4)	2 (3)
LPSF help control phenylalanine levels	45 (98)	27 (96)	72 (98)	0 (0)	1 (4)	1 (1)	1 (2)	0 (0)	1 (1)

* percentage of group (i.e. parents of PKU children or adults with PKU or all respondents)

LPSF monthly orders

Parents of children with PKU and patients were asked about the amount and type of LPSF they had ordered in the previous month before completing the survey (Table 7). Seventy six (93%) of all respondents had ordered some LPSF in the previous month. The three most common LPSF ordered were pasta, rice and cous cous; (n=63; 77%), followed by flour mix, (n=50; 61%) and biscuits (n=37; 45%) (Table 7). A higher percentage of parents ordered biscuits and pizza bases than adult patients with PKU (Table 7). The total number of LPSF units ordered was expressed as a percentage of the NSPKU recommended allowance. Fifty per cent of the respondents who used LPSF, ordered less than 51% of the recommended unit number with wide inter-quartile range (Q1: 30.5% to Q3: 104).

Acceptability of the sensory properties of LPSF

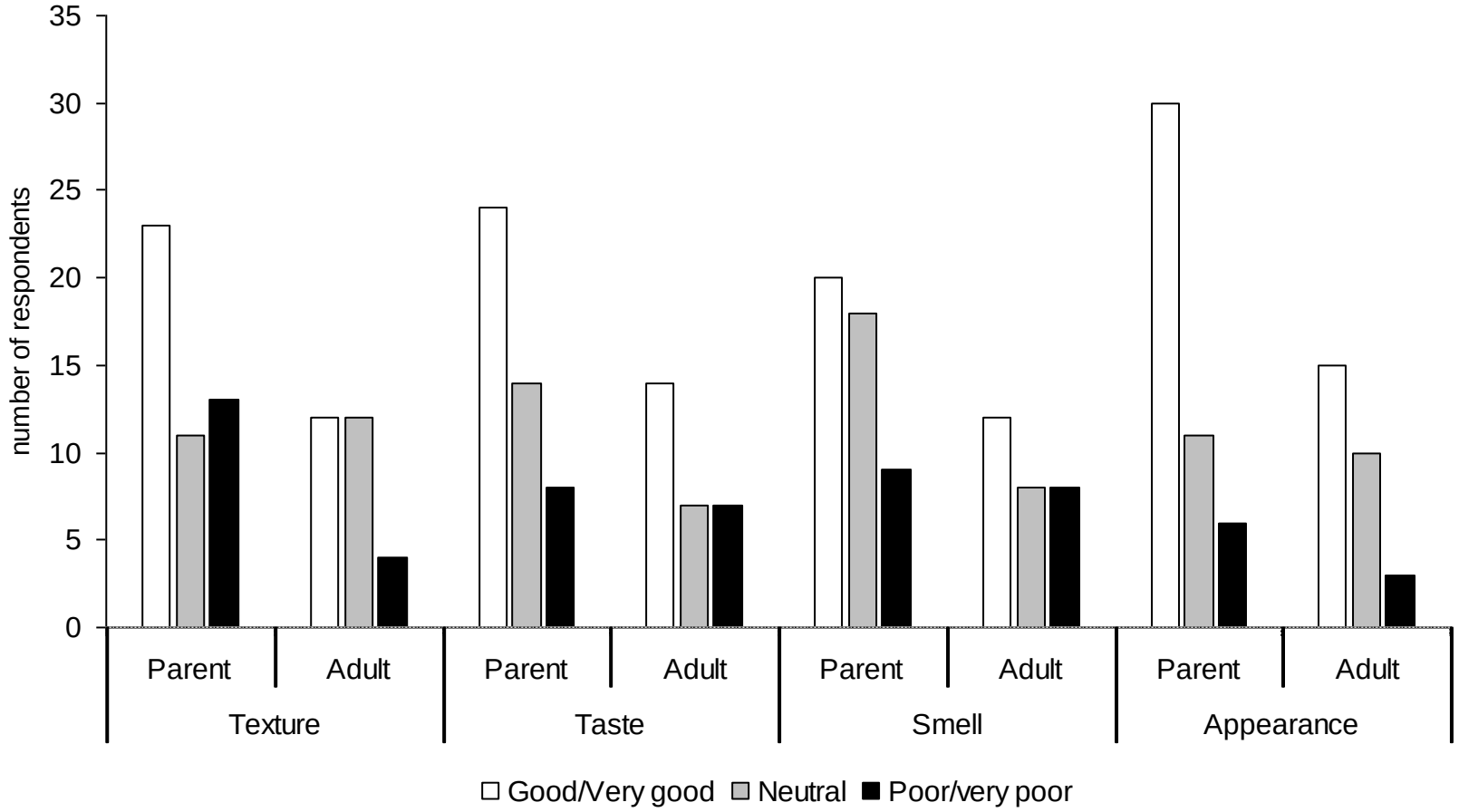
Approximately 40-50% of the respondents reported LPSF sensory properties as good or very good, whereas less than a quarter of them perceived these as poor or very poor (Figure 2). There were no significant differences between parents of children with PKU and adults with the condition (Figure 2).

Table 7: Type of LPSF ordered by respondents in the month prior to the survey

N (%)	Parents of PKU	Adults with PKU	Total*
Pasta/rice/cous cous	36 (72)	25 (83)	63 (77)
Bread/bread rolls	18 (36)	14 (47)	34 (41)
Flour	33 (66)	16 (53)	50 (61)
Breakfast cereals	13 (26)	4 (13)	18 (22)
Pasta in sauce meal	14 (28)	5 (17)	19 (23)
Snack pot	13 (26)	4 (13)	18 (22)
Pizza bases	17 (31)†	3 (10)	21 (26)
Burger/ sausage mix	15 (30)	8 (27)	24 (29)
Biscuits	27 (54)†	8 (27)	37 (45)
Cakes	6 (12)	3 (10)	10 (12)
Energy bars	6 (12)	4 (13)	10 (12)
Egg replacer	12 (24)	4 (13)	16 (19)
Dessert mix/hot breakfast	9 (18)	3 (10)	13 (16)
Spread	0 (0)	0 (0)	0 (0)

* Two respondents did not disclose if they were a parent, carer or patient; † $p<0.05$ between groups

Figure 2: Perception of sensory properties of LPSF by adults with PKU and parents of children with PKU



Issues with the dispensing and prescription of LPSF in primary health care

Thirty nine out of 79 (49%) recalled that they received a comment over the previous 12 months from a member of staff within a primary health care setting regarding the prescription or dispensing of LPSF. Fifteen of these (38%) received comments from more than one member. Twelve of these comments were made by a GP, 22 from staff within this area (GP manager: n=8; GP receptionist: n=14) and 20 were made within a pharmacy service (pharmacist: n=16; counter assistant: n=4) (Table 8). Of the 39 participants who received comments, 23 (59%) received negative comments; 10 (26%) neutral comments and five (13%) received positive comments. One gave no explanation. The majority of the comments on the use of LPSF made within a GP surgery were negative [GP: 8/12 (67%); GP manager: 6/8 (75%); GP receptionist: 13/14 (93%)] (Table 8). In contrast less than 50% of the comments made within a pharmacy practice were perceived as negative [pharmacist: 6/16 (37%); counter assistant: 2/4 (50%)] (Table 8). Of those who received a negative comment, 81% felt anxious about asking for future LPSF prescriptions. Comments quoted by the patients included: *"always asked why I need these prescriptions and how do I need so much"* and *"the GP hoped that it would not put his prescribing budget up"*.

Table 8: Comments received from staff within primary health care regarding the prescription and dispensing of LPSF to people with PKU

	G.P	**G.P receptionist**	**G.P manager**	**Pharmacist**	**Pharmacy counter assistant**
Comments received n (%)*	12 (22)	14 (26)	8 (15)	16 (30)	4 (7)
Negative comments n (%)†	8 (67)	13 (93)	6 (75)	6 (37)	2 (50)

* Percentage of all comments received (some responders received comments from more than one comments); † Percentage of comments given an negative attribute

Use of LPSF home delivery and cookery workshops

When participants were asked if they had used the LPSF home delivery service, 41/80 (51%) had used this service on a regular basis. Of these 28 (76%) reported that they found this more convenient and it helped with issues regarding supply of LPSF. One patient quoted: *"it has made my time better managed as the food is delivered straight to my work"*; and another said *"pharmacist had problems getting things in, so numerous trips to and from pharmacy"*. Fifty four (68%) had attended a cookery workshop session, with 44 (86%) of them agreeing that attendance had increased the variety of LPSF they used.

Discussion

This survey looked at beliefs, acceptability and issues associated with the use of the LPSF by people and carers of children with PKU. The majority of respondents perceived that the LPSF helps them or their children control their phenylalanine levels. Although the LPSF do not taste, smell or have the same appearance as the regular products they represent such as pasta or bread, this was not a major concern for the majority of the respondents and was independent of their age. This may indicate that people with PKU or their carers recognize the importance of LPSF in the management of their condition. Also sensory properties may not be a major concern in a population adhering from birth, to a lifelong, artificial, relatively unpalatable diet. In contrast, young people with conditions not diagnosed through national newborn screening programmes but with similar dietary management (e.g. Homocystinuria) might find it more difficult to adjust to the less appealing organoleptic characteristics of the LPSF, as they have been used to unmodified conventional foods.

Despite the acceptability and positive attitude to the use of LPSF, carers of children and adults with PKU frequently receive negative criticism by their primary health care staff, in particular from the staff within GP surgeries with regard to prescription requests and the supply of these products. This was in accordance to our original expectations, as the cost of these products is borne by the GP budget within the UK. Similar patterns might also be expected in Europe and the US, where the state may not reimburse such expenses. In a substantial proportion of patients, negative criticism was reported to influence requests for future prescriptions. Whether this has a negative impact on the management of PKU remains unknown, as is also the clinical efficacy of LPSF in the management of PKU. However, it is noteworthy that a large majority of parents and adults with PKU rated the LPSF as important

in controlling phenylalanine levels. Future studies should address this question with a prospective design.

As expected the most common LPSF ordered by the respondents were the staple foods used by the UK general population. Although there were no major differences in the type of products ordered between children and adults with PKU, a higher proportion of children ordered biscuits and pizza bases compared with adults. Although this may indicate similarities in eating patterns of children without PKU, health professionals managing children with PKU should be aware of this in the light of the obesity epidemic in the general population.

The use of a home delivery service and cookery workshops have been well received by parents, carers and people with PKU, and might help them with the use of LPSF. This could have implications for dietary compliance and therefore overall management of their condition. Cookery workshops are organised on a voluntarily basis and therefore, if use of the LPSF increases, there could be an argument that health service policy makers and budget holders should support such initiatives.

This study is not without its limitations. The response rate was modest and it may be possible that non-respondents have a different view than participants on aspects around the use of LPSF. However, we tried to encourage participation using reminders and a semi-anonymised questionnaire. The number of participants in this study may be considered small, but PKU is a very rare condition and we managed to capture responses from a substantial number of participants from an entire geographically defined area within the same health service system. Future studies should explore differences between countries or between states with different health services within a country. Moreover, the sensory properties of LPSF were only self-reported and these need to be explored with proper organoleptic characteristic studies.

Conclusion

People with PKU and their carers have a positive experience, attitude and perception on the use of LPSF and their usefulness in the management of their condition. However, they seem to confront barriers to both the supply and provision of LPSF in primary health care. This may indicate a lack of understanding of the management of PKU in primary care or poor communication between specialists and primary care health professionals. Alternatively this may simply reflect the lack of scientific evidence demonstrating the usefulness and effectiveness of LPSF in the management of people with PKU. This aspect should be addressed in future studies.

Study 2

Dietary intake and usage of low protein staple foods in children with phenylketonuria.

Justification for second study

The first study in this thesis examined the patients and/or carers perceived benefits and issues with the use of LPSF. Yet there is no research evidence to demonstrate their usefulness in aspects of the management of PKU and their user's health and wellbeing.

Studies on the nutritional management of PKU have concentrated mainly on the use of the phenylalanine free amino acid protein substitutes (Bentovim et al. 1970; Smith et al. 1975), their effect on growth and phenylalanine control (Acosta, Yannicelli 1994, Acosta et al. 2003; Gokmen-Ozel et al. 2011).

In contrast, there are no such studies which have looked at the use of LPSF and the role they play in the management of PKU. Anecdotally these products are believed to play a substantial part and contribute to the energy requirements, food variety, quality of life and phenylalanine control. Although no such studies have evaluated the use of the LPSF, guidelines have been developed by the NSPKU society, for the amount of the LPSF needed in the diet of children and adults with PKU in the UK and are based on the dietary recommendations for the amount of energy that carbohydrates should provide in a diet (NSPKU-http://www.nspku.org/publications/publication/prescription-guidelines). The NSPKU guidelines use units of LPSF centred on the age of the patient and aim to provide sufficient LPSF to supply 50% of the of the non-protein daily energy requirements (EAR). Yet it remains unknown how much of the recommended energy the LPSF actually provide in the PKU diet. Anecdotally these products are believed to play a substantial part and contribute to the energy requirements, food variety, quality of life and phenylalanine control.

In order to obtain robust scientific evidence of the usefulness of LPSF in aspects of the management of PKU, an intervention clinical trial is required whereby patients would be randomised, either to the use of or not of LPSF. However, as LPSF have been part of the standard management of PKU since the diet was first introduced in 1955 (Woolf, Griffiths & Moncrieff 1955) such study design would be inappropriate to perform for ethical reasons. An alternative approach would be to assess the current intake of LPSF using a cross-sectional study design and explore differences between people with different degrees of use of LPSF.

The second chapter of this dissertation aimed to: a) look at the dietary intake of the LPSF and how this compares with the NSPKU recommendations; b) compare the eating patterns of children with PKU with the diet consumed by the general population using the data from the NDNS (National Diet and Nutrition Survey (NDNS- 2008/2009) survey; c) explore

whether or not patient's use of LPSF is associated with their nutritional status, phenylalanine control, diet variety and quality of life.

Introduction

Phenylketonuria was identified as a disorder that could be managed by a restricted diet more than 50 years ago. Since then, there have been many developments in the specialist products to aid dietary management. It was recognised that the only way of reducing phenylalanine in the diet was to remove all protein containing foods. This however, meant that patients were left with very little to eat. There was a need to replace high protein foods and this was achieved with a phenylalanine free protein substitute containing amino acids and micronutrients in a form that patients could find palatable. The research into the palatability and efficacy of protein substitutes is an area that has been extensively studied over the years (Bentovim,A. 1970; Gokmen-Ozel, Hulya 2011; MacDonald,A. 2006)

Protein free "filler" foods (LPSF) were used from the earliest days of dietary restriction when patients given wheat-starch biscuits (Woolf,L.I. 1955; Armstrong,M.D. 1955). However, although such foods are used as ''fillers'', the value they contribute to diet management has been overlooked. Although these foods are recommended for clinical use, their role and efficacy has always been assumed rather than proven through evidence based studies.

In the first study we explored how people with PKU feel about the use of LPSF. The foods were perceived to help with phenylalanine control to satisfy their appetite and provide variety in the diet (Cochrane et al. 2014). However, similar findings have not been evaluated in any other studies and are a widely held belief, not just by patients and their carers, but also by health professionals who manage these patients.

The previous study also highlighted many of the issues patients and their carers face when trying to obtain product on prescription mainly with primary health care. As LPSF are only available on prescription, and incur a substantial cost to NHS, it is important to collect evidence to support their use and benefits in the management of PKU.

The variety and palatability of the LPSF has expanded greatly over the years and now the range encompasses foods that might also be classed as luxury or convenience foods e.g. energy bars, sausage mixes, cakes, crackers, crisps, and recently launched breakfast bars. The LPSF give patients the opportunity to produce a diet that can be varied and become similar to the rest of the family. Due to the relatively small numbers of patients requiring the LPSF,

they, unlike the expansion of gluten free foods for the management of coeliac disease into supermarkets, are not generally bought "over the counter", as are gluten free products and must be prescribed through the patient's G.P. Therefore this cost is added to the prescribing budget, and is sometimes seen as an additional burden to the workload of a busy G.P practice.

Aim of the study

The aim of this study is:

To evaluate the contribution the LPSF make to the diet of children and adults with PKU and compare this to the NSPKU guidelines.

In addition, the study will also

- Compare the low phenylalanine diet with the diet of the general population.
- Explore whether the use of LPSF is associated with nutritional status, diet variety, quality of life and the metabolic control of PKU

Material and Methods

Eligible participants & study design

This was a cross-sectional study where eligible participants were adults and children aged between 2 – 18 years, diagnosed with PKU through the newborn screening programme. The patients attended the outpatient clinics at: The Royal Hospital for Sick Children in Glasgow, the PKU adult clinics at the Southern General Hospital and Glasgow Royal Infirmary and were recruited between May 2010 and August 2011. Although this is a wide range of ages of patients, the management is essentially the same for all age groups. The NSPKU recommendations for the proportion of energy obtained from use of the LPSF is the same for all ages. Healthy controls were recruited from the general population.

Exclusion criteria

Children under the age of 2 years were excluded as children below this age are in the process of weaning from infant formula onto solid food and are less likely to receive significant amounts of LPSF. In addition, their carers are experimenting with the range of special medical foods and developing the necessary skills to use the products effectively, therefore the range of products used may not be representative of the usual diet for the management of PKU. From the age of two years, the majority of children with PKU are weaned from infant formula and are established on solid food. Children with hyperphenylalaninaemia who had phenylalanine levels monitored, but not on any dietary restriction were also excluded. Although we intended to recruit adults with PKU and they were included in the study proposal, due to time constraints and an alternative study taking place at the same time in the same population, we decided to omit them from recruitment.

Controls

Healthy children over the age of 2 years were recruited as controls. Healthy siblings of children with PKU were also invited to participate. Children from the general population were recruited by the display of posters in public areas and word of mouth. Siblings were invited to participate at the same time as the affected subjects. Controls were classified into siblings and other controls.

Recruitment

Eligible candidates were identified from the dietetic records of patients under the care of the dietetic department at the Royal Hospital for Sick Children in Glasgow and approached to take part. They were sent a letter of invitation to participate in the study during the 3 month period before their annual nutritional screen, or if aged between 2 and 4 years, before their subsequent clinic review.

The letter of invitation was sent to the parent or carer along with: a study information leaflet (Appendix 6), a reply slip and stamped addressed envelope. On receipt of a positive reply whereby the parent or carer agreed to receive further information before the appointment at PKU clinic, a copy of the Food Frequency Questionnaire (FFQ) (Appendix 5), a universal tube for urine collection and a foil bowl was posted out to their home along with instructions to return the FFQ and urine sample at the clinic visit.

A further explanation and written information on the study (Appendix 2 and 3) was given by the researcher at the clinic appointment, written consent (Appendix 4) was then obtained and the FFQ and urine sample returned. Routine blood tests were taken from all participants over the age of four years and an additional 4-6 ml blood taken for study purposes. Participants were given a copy of the 7 day food diary to take home, food scales if required and the PedsQL® questionnaires. A stamped addressed envelope was given to return the diaries and questionnaires on completion.

Disease Management

The clinical management of PKU was based on the results of phenylalanine levels from routine bloodspot cards posted by the participant or carer to the biochemistry laboratory at Yorkhill hospital. A finger-prick sample of blood is used in routine clinical practice to determine phenylalanine levels once a week. The results are used by the clinical team to determine the degree of phenylalanine control. Participants were not asked to take more tests than those normally requested by the clinical team.

Analysis of the bloodspot cards was performed by tandem mass spectrometry according to standard NHS laboratory protocols. Participants were advised that in addition to the results from their nutritional screen, the five results prior to and five results post completion of food diary would be documented. The median of the 5 blood spots prior to the study and of the 5 bloodspots post study was recorded and the percentage abnormality of tests outside the target range for each age group were determined as proxies of PKU clinical management con-

trol. This would give a more reliable reflection of the long-term management of the disease and rule out bias of improved compliance prior to the clinical appointment.

Assessment of eating patterns and dietary intake

The frequency of use and type of LPSF was established using a self-administered semi-quantitative Food Frequency Questionnaire (FFQ) and 7-day weighed food diary. The food diary assessed dietary intake (energy and nutrient intake) and the contribution the LPSF make to the diet composition. Participants were asked to weigh and record all food and drink taken. The food diary is a record of all food and drinks taken over a 7 day period. Food weighing scales were provided by the researchers if required. The food diary was given to the participants at the clinic visit and participants and parents were asked to complete the diary on the 7 days following the clinic visit. They were given a stamped addressed envelope and asked to return the completed diary by post.

Although recording intake for 7 days placed a burden on the parent and child, it was considered that recording intake for the 7 days would give a more accurate reflection of the range of LPSF taken. Recording for less than 7 days, may overlook use of some of the categories of LPSF used less frequently.

Snack type foods can make a significant contribution to the energy intake of the diet and this was of interest to the researcher for this study. The researcher made a decision to include both low protein foods such as biscuits, cake and energy bars, in addition to regular foods such as crisps, sweets and sugary beverages. Some of these foods therefore also featured in the assessment of the daily protein allowance and total energy intake and the results would therefore reflect this inclusion. The total energy intake from snack foods was calculated as a percentage of the Estimated Average Requirements (EAR).

In order to determine the role that the LPSF played in the management of PKU, patients were categorised into three groups: low, medium and high users of LPSF as described below. The NSPKU society suggests that 50% of non-protein calories in the diet should come from the LPSF. This figure was used when estimating the contribution the LPSF make to the energy content of the diet and how close the children with PKU meet these requirements. (http://www.nspku.org/sites/default/files/publications/Prescription_Guidelines_Oct_2009).

The assessment of food variety

The FFQ was adapted from a validated Scottish Collaborative Group Food Frequency Questionnaire to include the special medical foods and protein supplements regularly used by

children with PKU (Tsiountsioura, M 2014). Foods were grouped into LPSF, protein substitutes and then groups of foods based on the groups used in the National Diet and Nutrition Survey (NDNS- 2008/2009: *http://www.natcen.ac.uk/media/175123/national-diet-and-nutrition-survey-years-1-2-and-3.pdf*). The NDNS is a rolling programme of surveys designed to assess the diet, nutrient intake and nutritional status of the general population from the age of 1.5 years who live in private households in the UK and is jointly funded by the Department of Health (DH) in England and the UK Food Standards Agency (FSA). The study is carried out by a consortium of three organisations: Nat Cen Social Research (Nat Cen), MRC Human Nutrition Research (HNR) and the University College London Medical School (UCL).

The participant was asked to record the frequency of their intake of the foods over the period of a month. This allows an estimation of how often a certain type of food item is taken and what foods are omitted from the diet. This gives a depiction of the participant's dietary habits and intake (Tsiountsioura et al. 2014). The participants were asked to hand back the completed FFQ to the researchers on the day of recruitment. For the purpose of analysis, the LPSF from the FFQ were also categorised into "staple foods" which consisted of foods such as: flour mix, pasta, bread and rice and milk substitute and also "convenience" type foods which included items such as: burger mix, pasta in sauce and cakes. This was in order to gain an insight into the proportion of energy obtained from staple foods and convenience type foods in the diet of our subjects.

Anthropometric data

Weight and height measurements (using electronic Seca chair scales, SECA Hamburg, Germany and wall mounted stadiometer, Holtain Ltd, Crymych, UK) were taken at the beginning of the study from controls and those with PKU and converted to z-scores based on the UK 1990 data. These measurements are taken regularly as part of the clinical management of the patient during the regular out-patient review. Body composition was measured with the foot to foot bioelectrical impedance technique (TBF -300 Tanita, Portable Body composition monitor, Japan). This method may not be as sensitive as other methods, but is a method that can readily be used in the out-patient clinic and without placing an additional burden on the patient and family during the out-patient consultation (Tsiountsioura, et al.2014). For the healthy controls measurements took place within the joint premises of the Royal Hospital for Sick Children and Human Nutrition Section, University of Glasgow.

BMI class was used to define the participants as: thin, normal, overweight or obese as defined by the following criteria (*www.who.int/**child**growth/standards/**bmi**_for_age*):

- BMI 1st to 4th percentile: Thin
- BMI 5th to 84th percentile: Healthy Weight
- BMI 85th to 94th percentile: Overweight
- BMI >95th percentile: Obese

Quality of life assessment

Quality of life was assessed with the validated questionnaire PedsQL® generic core scales (Paediatric Quality of life Inventory: version 4.0). On the day of recruitment the participants and parents were given the questionnaire to complete and return along with the 7 day food diary by post. The PedsQL® report assesses how much a problem carers and subjects have with different aspects of their life by scoring from 0-4 on how much of a problem the child experiences in four separate domains. The report is available for the following age groups: Parent report for toddlers (2-4 years) and Parent and Child reports for ages: 5-7; 8-12; 13-18. One domain covered physical functioning with 8 categories from which a mean was taken from the total score. The psychosocial scale was taken from 3 domains which included: emotional functioning, social functioning and school functioning, each with 5 categories. The scores from these three domains were added and a mean taken and categorised into the psychosocial scale. The parents or carers and child were provided with separate reports and asked to score each scale from: never (0) to almost always (4), with regard as to how much of a problem they judge that PKU impacts on themselves or their child. Written permission for the use of the Inventory was granted from the MAPI Research Trust.

Micronutrient status

All children reviewed at the PKU clinic at Yorkhill, over the age of 4 years, have blood taken annually for a nutrition screen to check macro and micronutrient status. The diet of children under the age of 4 years is generally under the control of their carers and therefore they are deemed less at risk of developing nutritional deficiencies. Where there is concern about the nutritional status due to non-compliance, children would have a nutritional screen before the age of 4 years.

The micronutrient status of patients with PKU was assessed using objective indices in venous blood samples and a spot urine sample. An additional 4-8 mls was collected in addi-

tion to the routine screening blood of 17 ml approximately. Twenty additional micronutrients were measured in the Trace Element and Micronutrient Reference Unit at Glasgow Royal Infirmary. The results were compared with in house reference intervals. Participants were asked for written permission to use any redundant bloods available following routine biochemical and haematological analysis. This reduced the extra volume of blood required for research purposes. The participants were also asked to provide a spot urine sample on the day of recruitment.

Although urine and redundant bloods were collected, the data collected from them were not used in this MSc study due to time constraints and the amount of data already presented for the purposes of an MSc programme. However, information collected from this and also the results from the annual nutritional screen will be used in future studies and publications.

Demographic information

Information on demographics, gender and age was collected from the patients' dietetic notes and the case recording form for the controls. Postcodes were given a score from the Scottish Index of Multiple Deprivation (SIMD) (2012) and socioeconomic class was ranked from the most deprived (score: 1) to least deprived (score 5) and allowed consistency in identifying areas of multiple deprivations.

Classification of subject's disease severity

Subjects were classified into mild, moderate and classical PKU depending on their tolerance to phenylalanine. This tolerance determines the level of dietary phenylalanine required to keep the plasma phenylalanine levels within the target range recommended by the MRC working party (1993) and are classified as: severe; 250-300mg Phenylalanine/day; moderate: 350 – 400mg / day and mild: 400-600mg /day (Guldberg et al. 1998). A system of 50mg phenylalanine food exchange lists (equivalent to 1g natural protein) allows a consistent approach to dietary control. Each subject was then classified according to how many daily exchanges (mg phenylalanine) they were prescribed.

For the purposes of analysis, the moderate and mild disease patients were grouped together due to the small numbers of these patients recruited.

Sample size

The sample size for the number of subjects required for the study was computed by calculating a mean difference of 100 units in phenylalanine with a SD of 90 units between low, medium and high free protein users, with a power of 80% and an error of 5%. 17 participants will be required for each group giving a total of 51 subjects and it was decided to aim to recruit 60 to account for dropouts.

Data handling and statistical analysis

The estimation of Estimated Average Requirements (EAR) for energy intake and Recommended Nutrient Intake (RNI) for protein intake was used for determining the total energy intake and protein intake (Department of Health 1991).

Patients were grouped into low, medium or high users of LPSF by first ranking them on the energy from LPSF as a percentage of their EAR and then dividing into three equal groups. The Ksruskall Wallis test was used to determine any significance in the variables of interest between the groups.

The energy being provided by the protein substitute, regular foods and snack foods was similarly calculated as a percentage of EAR. Regular foods were those foods in the diet that were either used as exchange food (e.g. potatoes, breakfast cereal) or freely allowed low protein foods (e.g. fruits, fats and oils, preserves). The energy provided by snack foods was determined by calculating the energy from individual foods and which was then taken as a percentage of the overall energy intake. As described previously, the snack foods included foods such as biscuits and cake as well as sugary drinks, crisps and sweets, both regular and low protein.

Results were collated on Microsoft Excel and analysed by the Minitab 16.0 package (Minitab Ltd). Dietary analysis used the WinDiets 2005 dietary analysis program. WinDiets includes the complete UK database as published in the food tables and supplements. The LPSF, protein substitutes and other regional foods were added to the programme as "local foods". The nutrient values for the local foods were taken from published specialist company information and web based manufacturer's information. For the food diary analysis, data were entered into the programme and an average of 7 days intake was recorded and analysed.

In order to obtain data on the variety of foods taken, the FFQ data was entered into the programme as a proportion of food intake taken over a month and expressed as intake per day (g). Food portion sizes were based on individual portions as sold or taken as an average por-

tion based on published food portion sizes (*Ministry of Agriculture, Fisheries and Food 1988).*

Ethical Approval

The study protocol was approved by the West of Scotland Research Ethics Service. REC reference number: 11/AL/0077 R&D reference: GN11DT042 and every participant and carer (when appropriate) provided written informed consent.

Results

Subject Recruitment

Of the children with PKU in the patient cohort at Royal Hospital for Sick Children, 60 were identified as being eligible to take part in the study. Four declined the invitation, 14 did not respond to the invitation and for social reasons were not approached at clinic, 40 (68%) patients were recruited and gave written consent to take part in the study. Of these; 23 (56%) were male [mean (IQR) age 7.8 (8.0) yr.] and 17 (44%) were female [mean (IQR) age 7.3 (7.25) yr.]. Six were from three families with more than one child with PKU. Eight (20%) were aged between two and four years and therefore did not have blood taken for nutritional screen. Of the subjects, the majority 41% (n=17) attended primary school; 29% (n=12) were preschool and 27% (n=11) were at secondary school.

Healthy controls

Of the healthy controls recruited 41 adults and children were recruited. Of these, 13 withdrew from the study and did not complete the food diaries or FFQ. Nine adults completed the seven day food diary, but as no adults with PKU were able to be recruited, the data from the healthy adult controls have not been included in this MSc thesis.

Of the 19 healthy children, 10 were male [mean (IQR) age 8.3 (10.25) yr.]; nine female [mean (IQR) age 8.8 (6.5) yr.). Of these nine (47%) were siblings of children with PKU (five male). Of the control children, 32% (n=6) were pre-school; 42% (n=8) attended primary school and 26% (n=5) were at secondary school.

The information gained from the healthy controls, especially the siblings, can provide an insight into the differences between the diet of children with PKU and their peers. However, as with the adults, insufficient numbers of healthy child controls, either siblings or other controls could be recruited to allow this comparison. Therefore, data from controls has not been used in this study. It is the intention to continue to recruit controls, particularly more siblings in order to make this comparison possible in a future study.

Demographics

Eleven (28%) of the children with PKU came from the most deprived area with a score of one and 12.8% (n=5) came from the least deprived area with a score of five (Table 9). One patient had moved to England at the time of the study, therefore did not have an SIMD score.

Disease severity of children with PKU

The children with PKU were categorised according to disease severity by the number of protein exchanges required to keep their phenylalanine levels within the target range for their age. Twenty seven (67.5%) were classed as having severe or classical PKU (2-6-exchanges / day); 7.5% (n=3) (7-8 exchanges / day) as having moderate PKU and 25% (n=10) (9-13 exchanges / day) as having mild PKU (Table 9). For the purpose of analysis, the mild and moderate group were combined.

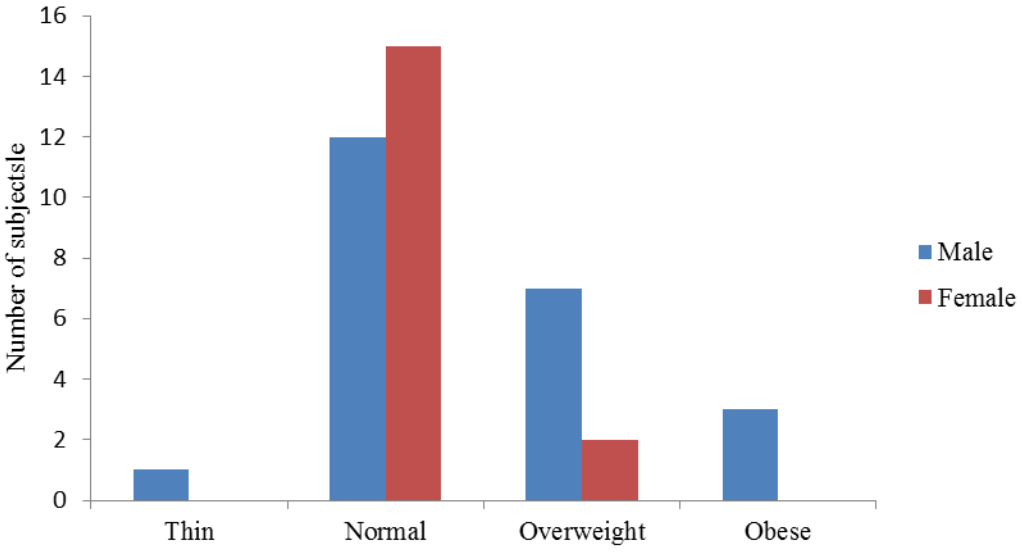
Anthropometric characteristics of children with PKU

Twenty seven (67%) children with PKU had a BMI SDS within the normal range; one child (2%) was underweight; 22% (n=9) overweight and 7% (n=3) were obese (Figure 3). 83% (n=15) of the girls and 52% (n=12) of the boys were classed as within the normal BMI range (Table 9). No girls were obese, however, 18% (n=3) of the boys were obese. One boy was categorised as thin and 11% (n=2) girls and 30% (n=7) boys classed as overweight (Figure 3).

Table 9: Patient characteristics associated with gender

Patient Characteristic		Male N (%)	Female N (%)	Total N (%)
SIMD score	1	6 (27.3)	5 (29.4)	11 (28.2)
	2	7 (31.8)	1 (5.9)	8 (20.5)
	3	4 (18.2)	5 (29.4)	9 (23.1)
	4	3 (13.6)	3 (17.6)	6 (15.4)
	5	2 (9.1)	3 (17.6)	5 (12.8)
Stage of schooling	Pre-school	7 (30.4)	5 (29.4)	12 (30.0)
	Primary school	10 (43.5)	7 (41.2)	17 (42.5)
	Secondary school	6 (26.1)	5 (29.4)	11 (27.5)
Classification	Classical	15 (65.2)	12 (70.6)	27 (67.5)
	Moderate	2 (8.7)	1 (5.9)	3 (7 .5)
	Mild	6 (26.1)	4 (23.5)	10 (25.0)
BMI	Thin	1 (4.3)	0 (0.0)	1 (2.5)
	Normal	12 (52.2)	15 (88.2)	27 (67.5)
	Overweight	7 (30.4)	2 (11.7)	9 (22.5)
	Obese	3 (13.0)	0 (0.0)	3 (7.5)

Figure 3: BMI distribution between male and female children with PKU

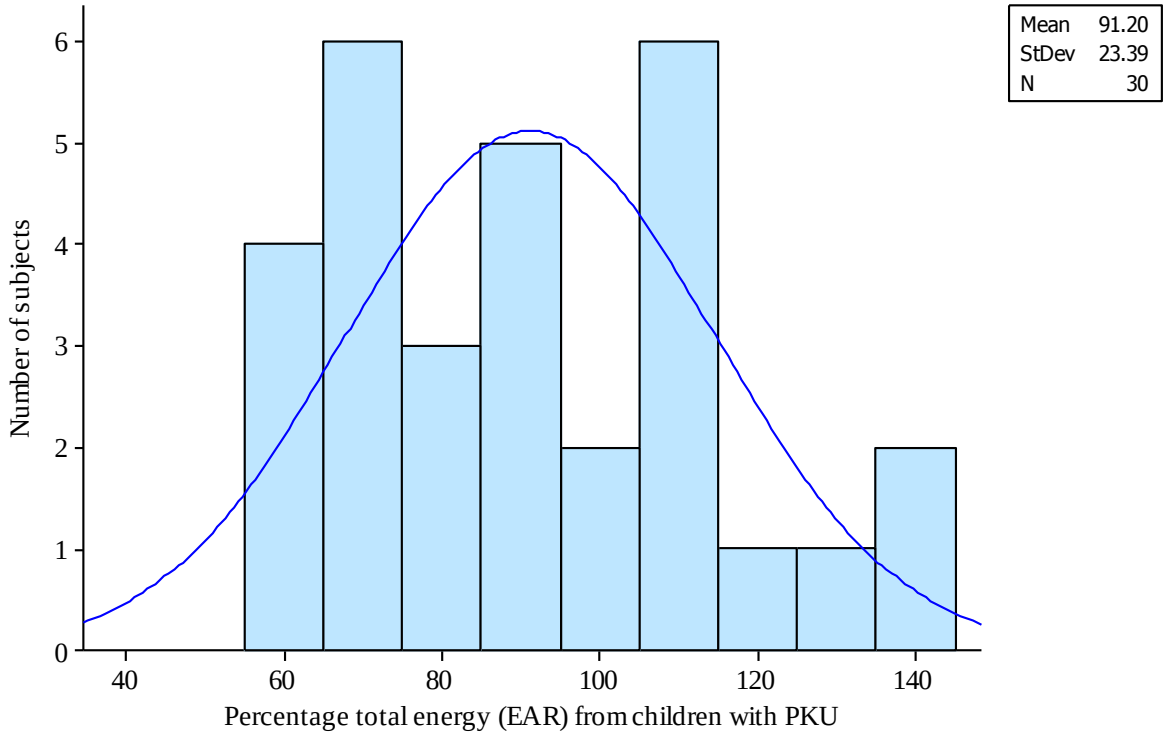


Results from the analysis of the 7 day weighed food records

Patients with PKU: Analysis of 7 day weighed Food diary

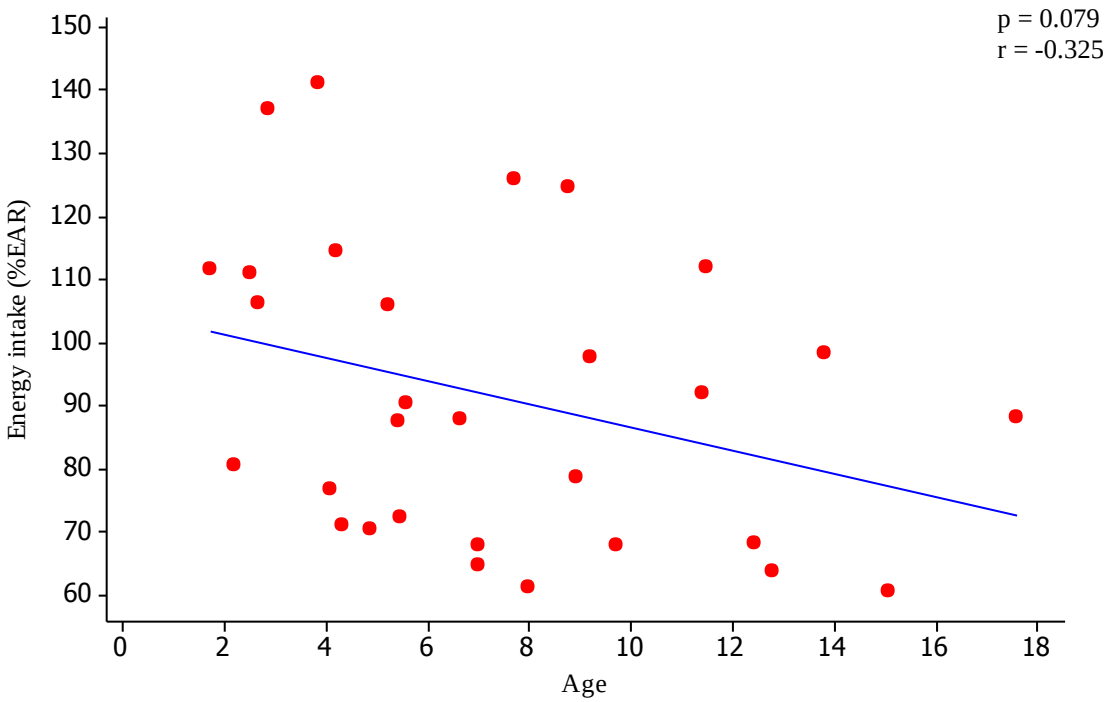
Of the 40 subjects recruited into the study, 30 (75%) completed the seven day food diary which was analysed to determine the energy and protein content of the PKU diet. One third took more than 100% (n=10: 5 male and 5 female) and 66% (n=20: 9 female and 11 male) had an intake less than EAR (Figure 4).

Figure 4: Energy intake of children with PKU



As can be seen from Figure 5, there is a tendency for a decrease in energy intake (%EAR) as the subjects become older (Figure 5).

Figure 5: Correlation between of energy intake (%EAR) with age



Most of the dietary energy came from regular foods (i.e. phenylalanine exchange foods) and LPSF (%EAR) [LPSF vs. protein substitute vs. regular foods: median (IQR): 20 (14.2); 14 (4.1); 49 (36.7)] (Table 10a).

Similarly, the majority of the protein intake was from the protein substitute: [LPSF vs. protein substitute vs. regular foods (% RNI): median (IQR): 3 (3.0); 137 (55.3); 41 (37.1)] (Table 10b). No subject received a protein intake less than 100% of the RNI for their age. 36% (n=15) took between 100-200% RNI and 27% (n=11) consumed over 200% RNI and 10% (n=4) consumed more than 300% RNI. The median protein intake was above the recommended intake of 12% energy from protein [median (IQR): 14.1 (3.8)] (Table 10b).

When the intake of energy from carbohydrate and fat were assessed, the energy from fat was lower than the FAO/WHO recommendations of 35% with a median intake of 18% [%EAR; median (IQR): 18 (46)] and the carbohydrate slightly higher [%EAR; median (IQR): 57 (23)] (Table 10a).

Table 10a: Energy distribution from 7 day food diary analysis

Dietary characteristics of subjects - % EAR* (% of total energy intake**)	N	Median	IQR	Minimum	Maximum
Energy intake	30	88	41.3	61	141
Energy intake from LPSF foods	30	20 (27.3)	14.2 (17.1)	0 (0.0)	49 (56.2)
Energy intake from Regular Foods	30	49 (55.5)	36.7 (13.5)	15 (22.3)	110 (88.7)
Total energy from Snack foods	30	16 (19.9)	22.0 (15.9)	3 (5.0)	52(48.7)
Energy intake from Protein Supplement	30	14 (18.0)	4.1 (8.4)	10 (10.0)	30 (37.1)
Total energy from CHO	30	57 (64.0)	23.0 (6.5)	34 (56.6)	91 (73.3)
Total energy from Fat	30	18 (24.2)	12.0 (7.1)	11(15.5)	46 (33.3)

***** % EAR provided by dietary characteristics; ** % of the energy intake provided by the dietary component

Table 10b: Energy distribution from protein intake

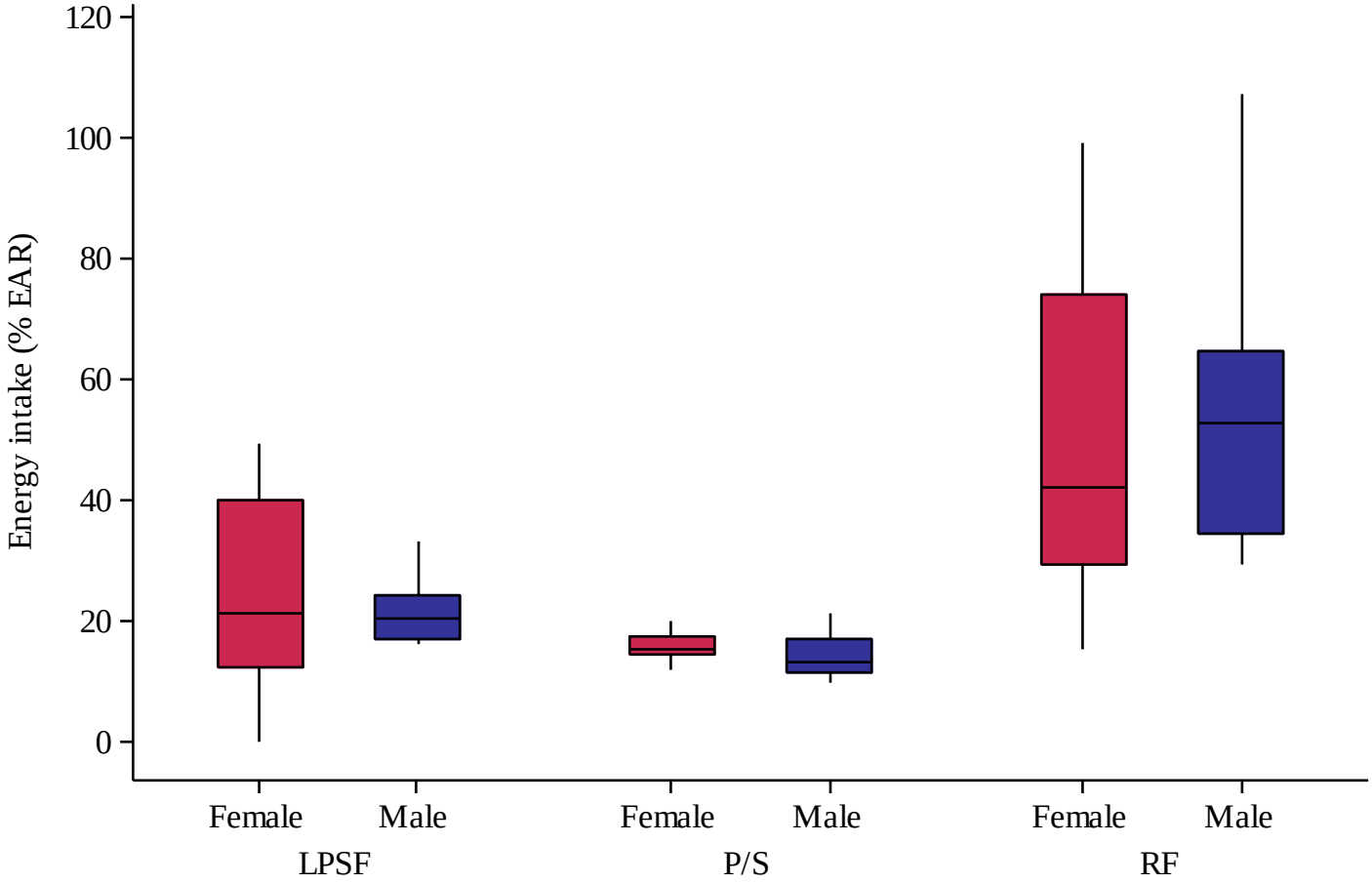
Protein intake - % RNI (% of total energy intake)	**N**	**Median**	**IQR**	**Minimum**	**Maximum**
Total protein intake	30	198 (14.1)	104.5 (3.8)	116 (8.9)	345 (18.9)
Total protein intake from LPSF	30	3 (0.2)	3.0 (0.2)	0 (0.0)	8 (0.6)
Total protein intake from protein substitute	30	137 (10.5)	55.3 (3.5)	93 (5.3)	276 (15.6)
Total protein intake from regular foods	30	41 (2.7)	37.1 (1.2)	11 (1.0)	153 (8.1)

Comparison of total energy intake between gender

No significant difference between genders was seen regarding the total energy from regular foods [boys vs. girls: median (IQR): 29 (30); 42 (45) (p=0.589)] and LPSF [boys vs. girls: median (IQR): 20 (7); 21 (27) (p=0.662)]. Energy intake from the protein substitute tended to differ between the genders [males vs. females: median (IQR): 13 (5); 15 (3); (p=0.056)] (Table 11; Figure 6) although the amount of protein substitute prescribed is determined by weight, not age or gender.

The amount of energy provided by snack foods (i.e. foods which were both LPSF and regular foods such as cakes, biscuits, potato crisps and sweets) did not differ between genders (Table 11). When the energy from carbohydrate and fat was compared between the genders, no difference was found.

Figure 6: Comparison of dietary constituents with gender



P/S – protein substitute

RF – regular foods

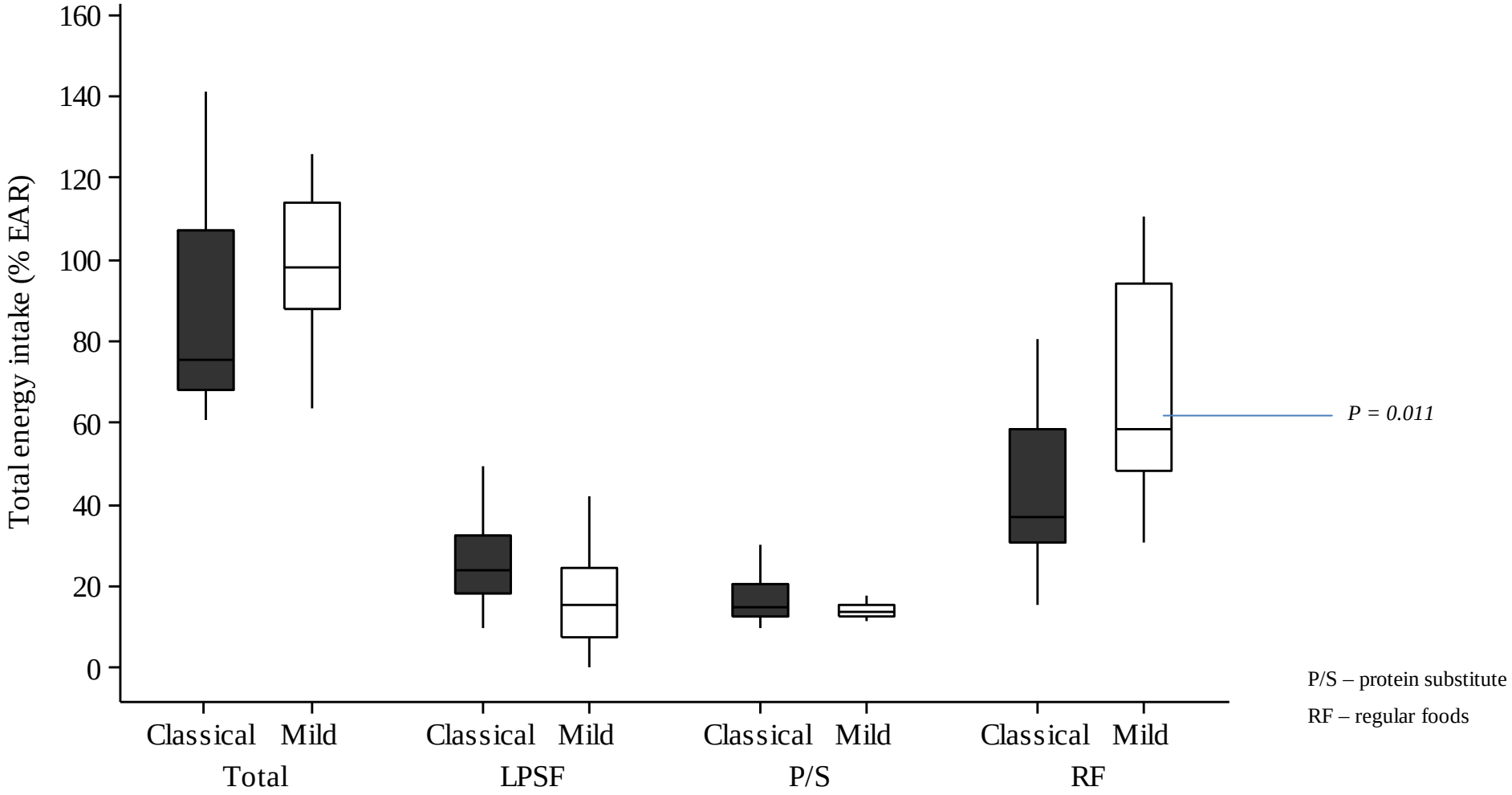
Table 11: Comparison of total energy intake from macronutrients between genders (% EAR)

Characteristic	N	Boys				N	Girls				
	16	Median	IQR	Min	Max	14	Median	IQR	Min	max	*p*
Energy intake (% EAR)	16	88	39	60	126	14	84	40	64	141	*0.803*
Energy intake from LPSF foods (% EAR)	16	20	7	0	42	14	21	27	0	49	*0.662*
Energy intake from Regular Foods (% EAR)	16	29	30	29	110	14	42	45	15	99	*0.589*
Energy intake from Snack Foods (% EAR)	16	17	21	6	45	14	14	22	3	51	*0.506*
Energy intake from Protein Supplement (% EAR)	16	13	5	10	30	14	15	3	12	25	*0.056*
Energy intake from CHO (% EAR)	16	57	23	34	85	14	55	22	38	91	*0.739*
Energy intake from Fat (% EAR	16	19	11	13	37	14	18	13	11	46	*0.589*

Disease severity and dietary intake

The subjects were also classified according to disease severity using the number of prescribed phenylalanine exchanges required to keep their phenylalanine levels within the target range. This was used to determine if there was any association between disease severity and dietary intake (Figure 7). A significant difference was found between the severity of PKU and the percentage energy intake from regular foods [median (IQR): classical; mild: 37 (28); 58 (45)] ($p=0.011$) with the children with mild PKU receiving a greater amount of energy (% EAR) as would be expected, from the regular foods than the classical or mild subjects with PKU. In addition, the energy (% EAR) from the LPSF had a tendency towards, but did not achieve significance ($p=0.062$), with an intake higher from the classical PKU [median (IQR): classical; mild: 24 (14); 15 (17)] (Figure 7).

Figure 7: Energy distribution vs. disease severity



Age and stage of schooling on energy distribution

When the energy intake was compared with the stage of schooling it was noted that, although not significant, the pre-schoolers tended to have a higher energy intake (%EAR) than either the primary school or secondary school groups [pre-school vs. primary school vs. secondary school: median (IQR): 111 (47); 83 (32); 88 (34)] (p=0.098) (Figure 8).

From the comparison of the energy intake (% EAR) from the individual constituents of the PKU diet, i.e. protein substitute; LPSF and regular foods (Figure 8), as was expected, the secondary school age group took significantly less energy than the pre-school and primary school groups from the protein substitute [pre-school vs. primary school vs. secondary school: median (IQR): 20 (8); 14 (3.8); 13 (1.9), p=0.007). The intake of energy (%EAR) from the LPSF was not significantly different between the age groups, although was slightly higher in the pre-school group: [pre-school vs. primary school vs. secondary school: median (IQR): 25.7 (27.2); 22.4 (18.6); 17.6 (6.3) (p=0.103)]. No difference between groups was found regarding the intake of energy from regular foods.

The energy (%EAR) provided by fat and snack foods was no different when the stage of schooling was considered (Figure 9). The intake of carbohydrate was slightly higher in the pre-school group [pre-school vs. primary age vs. secondary median (IQR): 71(30); 54(19); 54 (22) (p=0.090), but did not reach significance.

Figure 8: Comparison of age grouping with intake of energy (%EAR) from main dietary constituents.

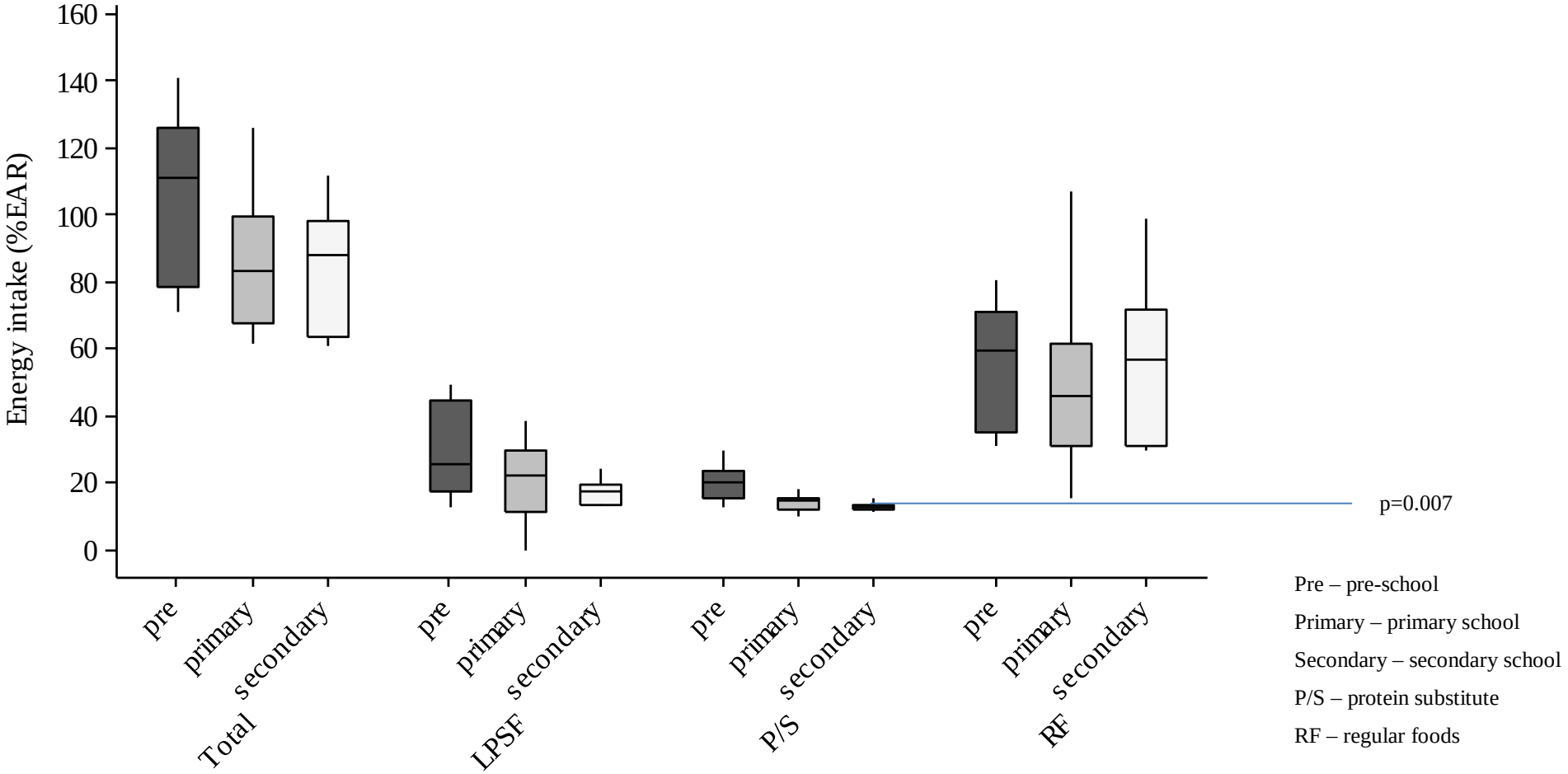
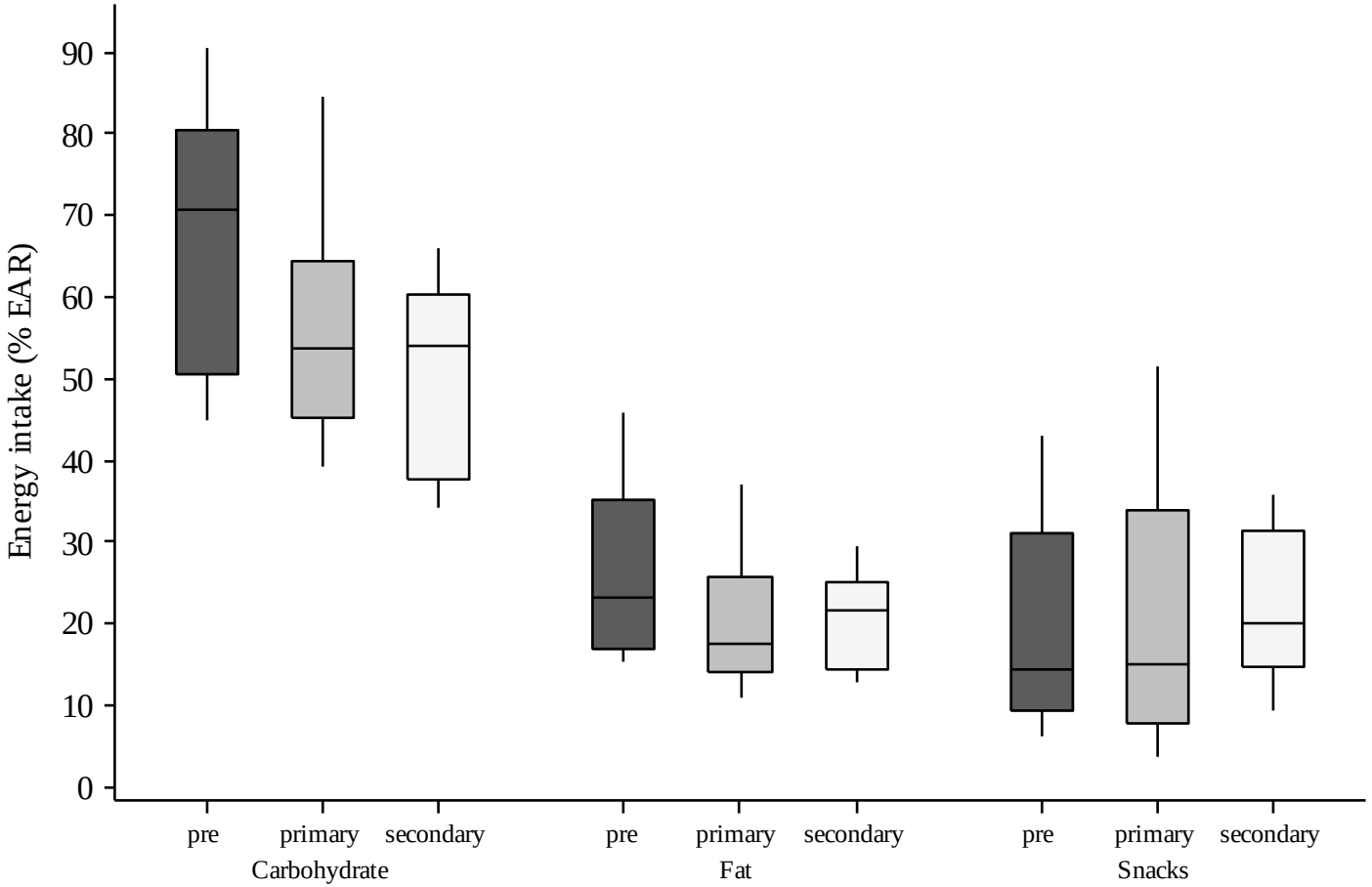


Figure 9: Comparison between age grouping and energy intake (% EAR) from CHO, fat and snack foods

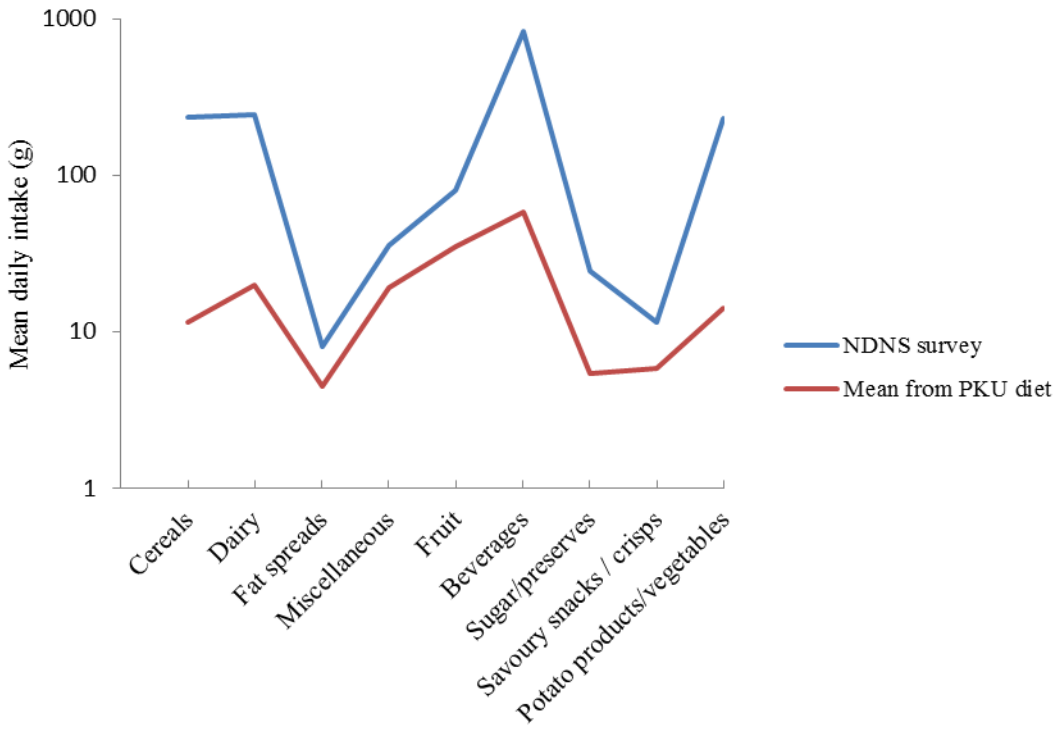


Comparison of food intake and eating habits between the patients with PKU and the general population

The diet for children with PKU and the general population differs in that foods containing phenylalanine are replaced by a protein substitute tailored to the protein requirements of the child. In addition there is a need to replace dietary "filler" foods and a reliance on foods low in protein such as fruit and vegetables. Therefore, for children with PKU there is control of protein intake and an emphasis on the use of LPSF, fruit and vegetables by the healthcare professionals managing their diet. In order to explore what difference this makes to the diet of children with PKU, a comparison was made to that of the general population using data from the NDNS survey.

There was a similarity in the pattern of the diet profile, but not in the absolute intake, of the PKU diet and the diet from general UK population (Figure 10). However, the use of cereals, potato products and beverages was much higher in the general population, even though the cereal group included LPSF for children with PKU [mean from PKU diet vs. mean from NDNS survey: median (IQR): 14.2 (21.65; 79.5 (221.5)].

Figure 10: Profile of the diet for PKU and the general population (age 4-18)



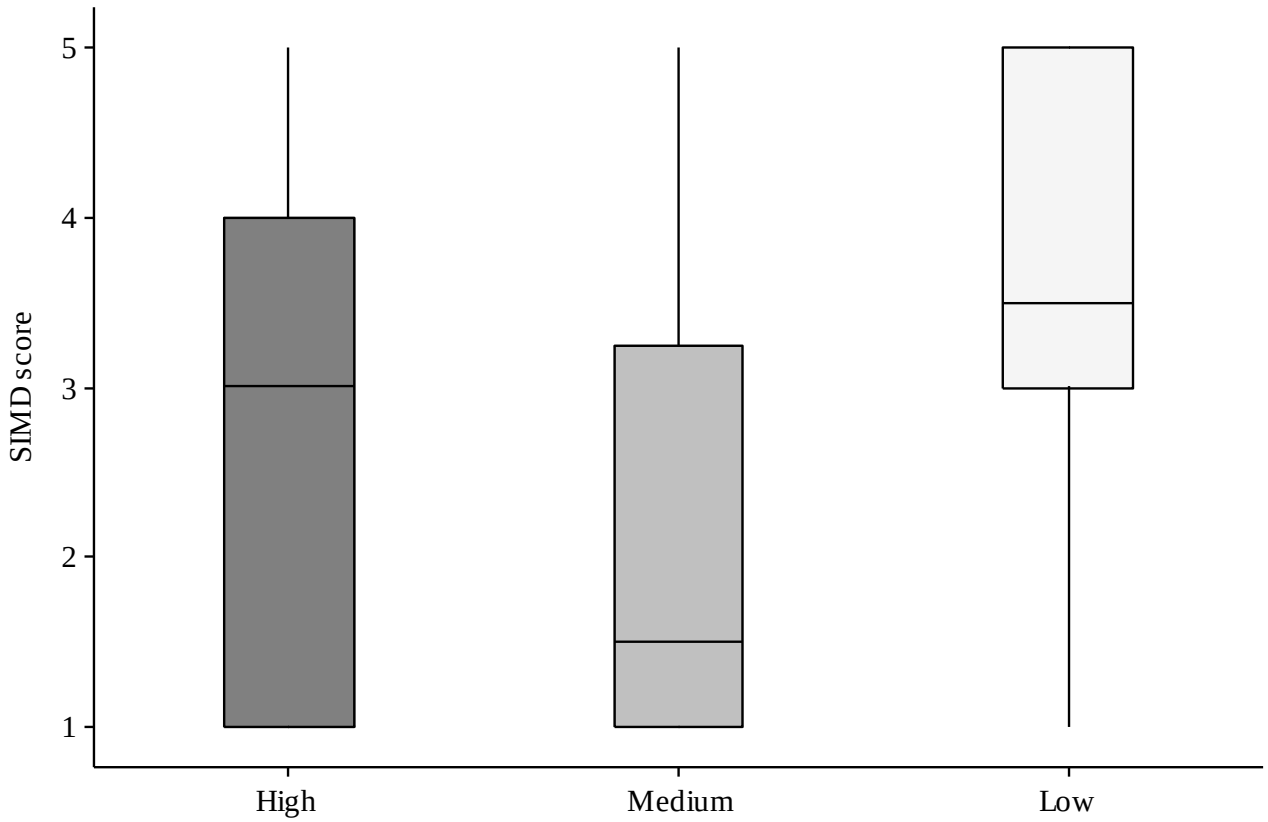
Association between dietary management and the use of LPSF

In order to explore whether there was any relationship between the use of LPSF and the management of PKU, the subjects were allocated into low, medium and high user groups based on their reported use of the LPSF from the analysis of the 7 day weighed food diaries (Table 12).

Subject demographics and use of LPSF

The SIMD score was compared between the three groups LPSF user groups (Figure 11) and there was no difference noted between the three groups [high vs. medium vs. low; median (IQR): 3(3); 1.5 (2.2); 3.5 (2.0)].

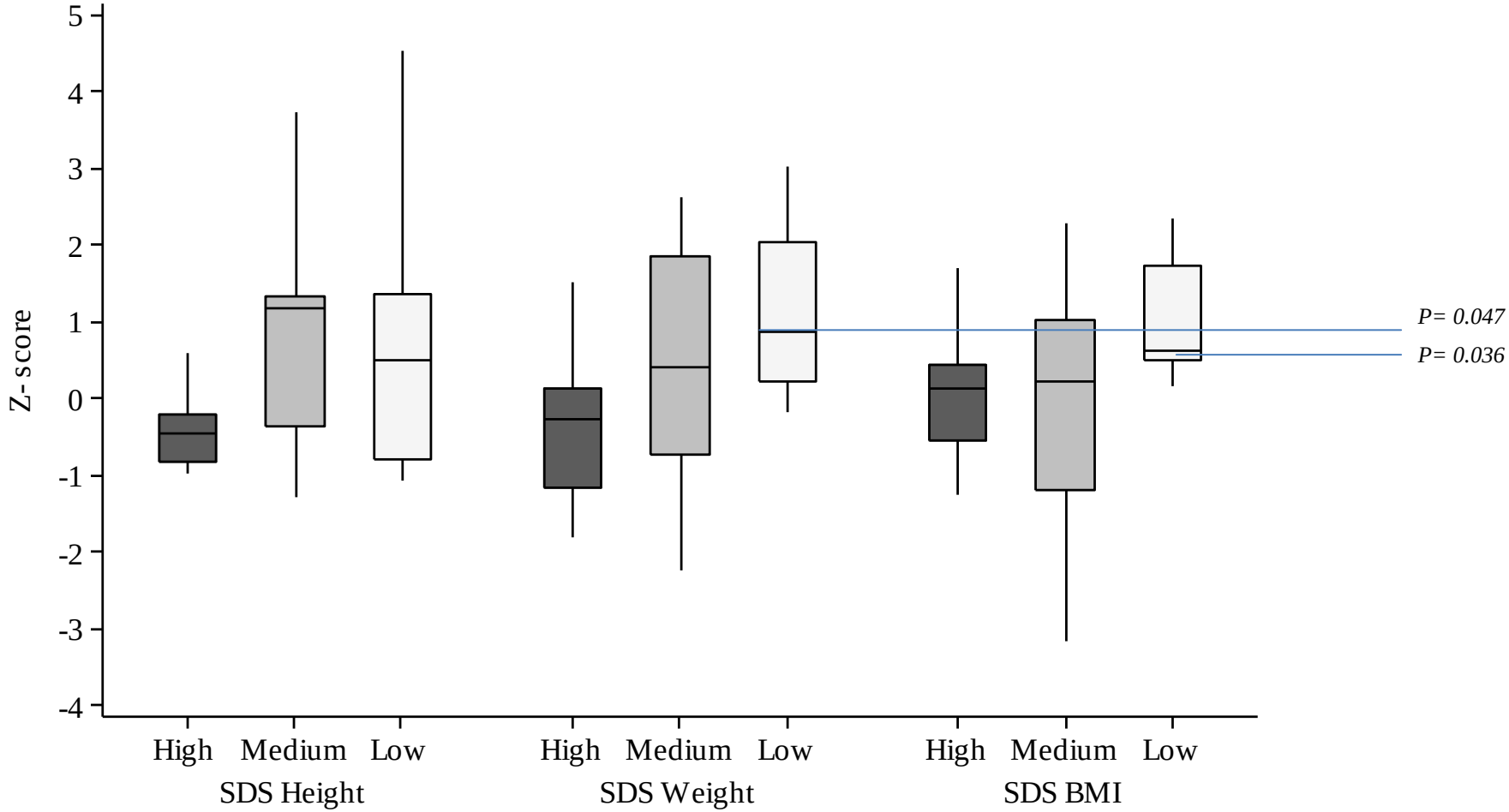
Figure 11: Comparison between use of LPSF and SIMD score



Associations between LPSF use, growth and SDS BMI

When the association between use of LPSF and anthropometric data was explored (Figure 12), a significant difference was found between the low users and high users of LPSF [high vs. medium vs. low: median (IQR): -0.3 (1.3); 0.4 (2.6); 0.8 (1.8); (p=0.047)] for SDS weight, with the low users having a higher weight SDS (Table 12) and SDS BMI [high vs. medium vs. low: median (IQR): 0.1 (1.0); 0.2 (2.2); 0.6 (1.2); (p=0.036)] than the high user group. There was no significant difference for height SDS between the three groups (p=0.072).

Figure 12: Comparison between use of LPSF and anthropometric data



Severity of PKU and use of LPSF

Disease severity in our patient group was expressed using the number of prescribed phenylalanine exchanges required to keep the plasma phenylalanine levels within the recommended target range (120-360 umol/l – 0-5 years; 120-480 umol/l; 5-10 years; 120-700 umol/l; >11 years). When the difference in the number of prescribed exchanges was explored (Table 12) between the three groups, the high users of the LPSF tended to be prescribed fewer phenylalanine exchanges than the low users who were prescribed the greatest number of exchanges [median (IQR) high vs. medium vs. low: 4 (6); 5 (3.2) 9 (4.7) (p=0.056)].

Metabolic control between the LPSF user groups

Clinical control of the patient's phenylalanine levels in our group of subjects was determined by comparing the median of 5 phenylalanine prior to and post completion of the seven day food diary (Table 12). No significant differences between the three user groups were observed. In addition, the percentage of raised phenylalanine results was compared between the three user groups of LPSF, both before and after the diaries were completed (Table 12), again no significant differences were found between each of the groups, [prior to food diary completion/post food diary completion (p=0.347/p=0.630)].

Table 12: Association between anthropometry, disease control and use of LPSF

Food Diary Characteristic	**N**	**High users**				**N**	**Medium users**				**N**	**Low users**				
Boys/girls	11 4/7	Median	IQR	Min	max	10 8/2	Median	IQR	Min	max	9 4/5	Median	IQR	Min	max	*p*
Age, years	11	4.9	3.2	2.7	9.2	10	9.3	8.7	1.7	17.6	9	7.7	7.3	2.5	13.8	*0.131*
Scottish Deprivation score	11	3.0	3.0	1.0	5.0	10	1.5	2.2	1.0	5.0	8	3.5	2.0	1.0	5.0	*0.106*
SDS weight (SD)	11	-0.3†	1.3	-1.8	1.5	10	0.4	2.6	-2.2	2.6	9	0.8	1.8	-0.2	3.0	*0.047*
SDS Height (SD)	11	-0.4	0.6	-2.8	0.6	10	1.2	1.7	-1.3	3.7	9	0.4	2.2	-1.1	4.5	*0.072*
SDS BMI (SD)	11	0.1†	1.0	-1.2	1.7	10	0.2†	2.2	-3.2	2.3	9	0.6	1.2	0.2	2.3	*0.036*
Prescribed no. of protein exchanges (g protein/24 hr.)‡	11	4.0†	6.0	2.0	13.0	10	5.0	3.2	3.0	9.0	9	9.0	4.7	5.0	11.0	*0.056*
Median plasma Phe, umol/l; pre 7 day food diary	11	234	130	111	481	10	253	212	119	842	9	208	169	146	584	*0.909*
Median plasma Phe umol/l; post 7 day food diary	11	257	136	166	750	10	417	350	87	648	8	263	102	190	392	*0.546*
% raised plasma Phe umol/l;* pre 7 day food diary	11	0	0	0	80	10	0	25	0	60	9	0	0	0	20	*0.347*
% raised plasma Phe* umol/l post 7 day food diary	11	0	40	0	80	10	30	40	0	80	8	0	35	0	60	*0.630*

**target range for Phenylalanine levels: 120-360 umol/l – 0-5 years; 120-480 umol/l; 5-10 years; 120-700 umol/l; >11 years:*
‡- based on phenylalanine tolerance: † Significantly different from low users LPSF:

Energy intake and distribution between LPSF user groups

There was no difference in energy intake (% of EAR) between the three LPSF user groups (Figure 13), although the low users of LPSF tended to have the highest energy intake, but this did not reach significance [median (IQR): high vs. medium vs. low: 88% (44); 80% (26); 106% (35) (p=0.076)] (Table 13).

There was no difference in the energy intake (% EAR) from protein substitutes between the groups (p=0.306) (Table 13). However, when the energy intake (% EAR) from regular foods was compared, there was a significant difference between the three groups with the high and medium users having a lower energy intake (%EAR) than the low users [high vs. medium vs. low: median (IQR): (37% (34.6); 40% (24.5); 71% (51.3) (p=0.012)] (Figure 14).

Figure 13: Total energy intake between users of LPSF

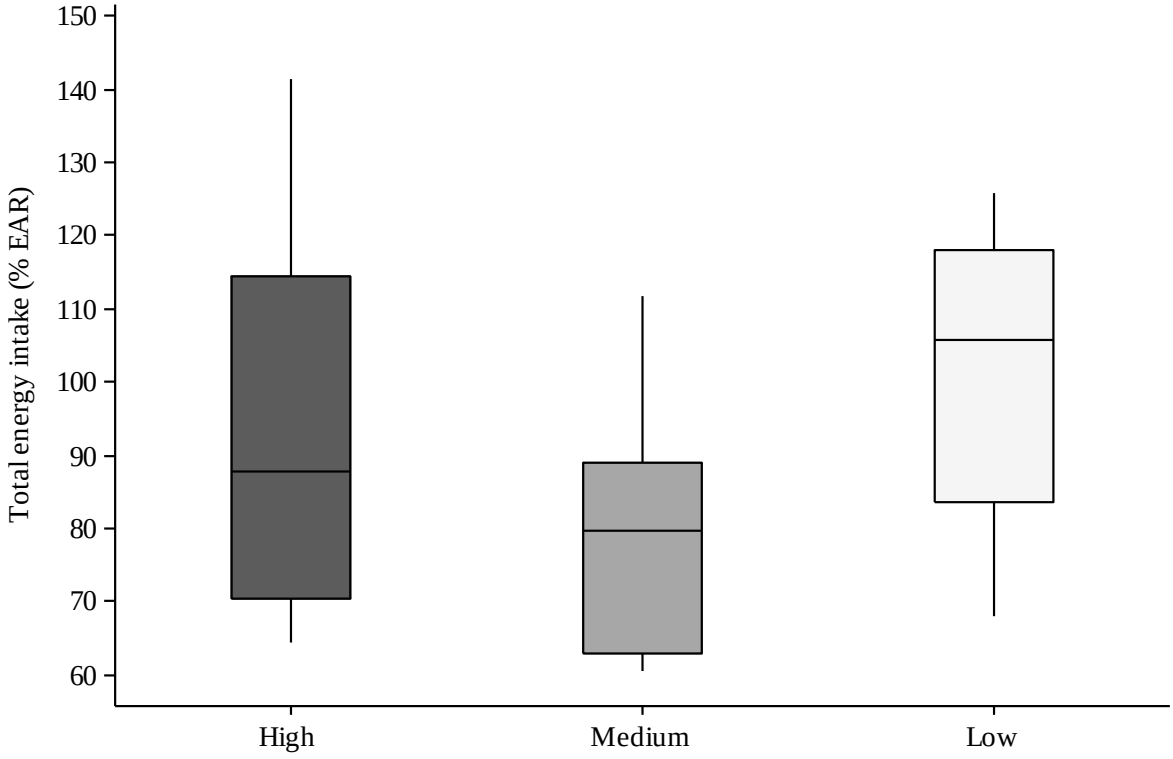
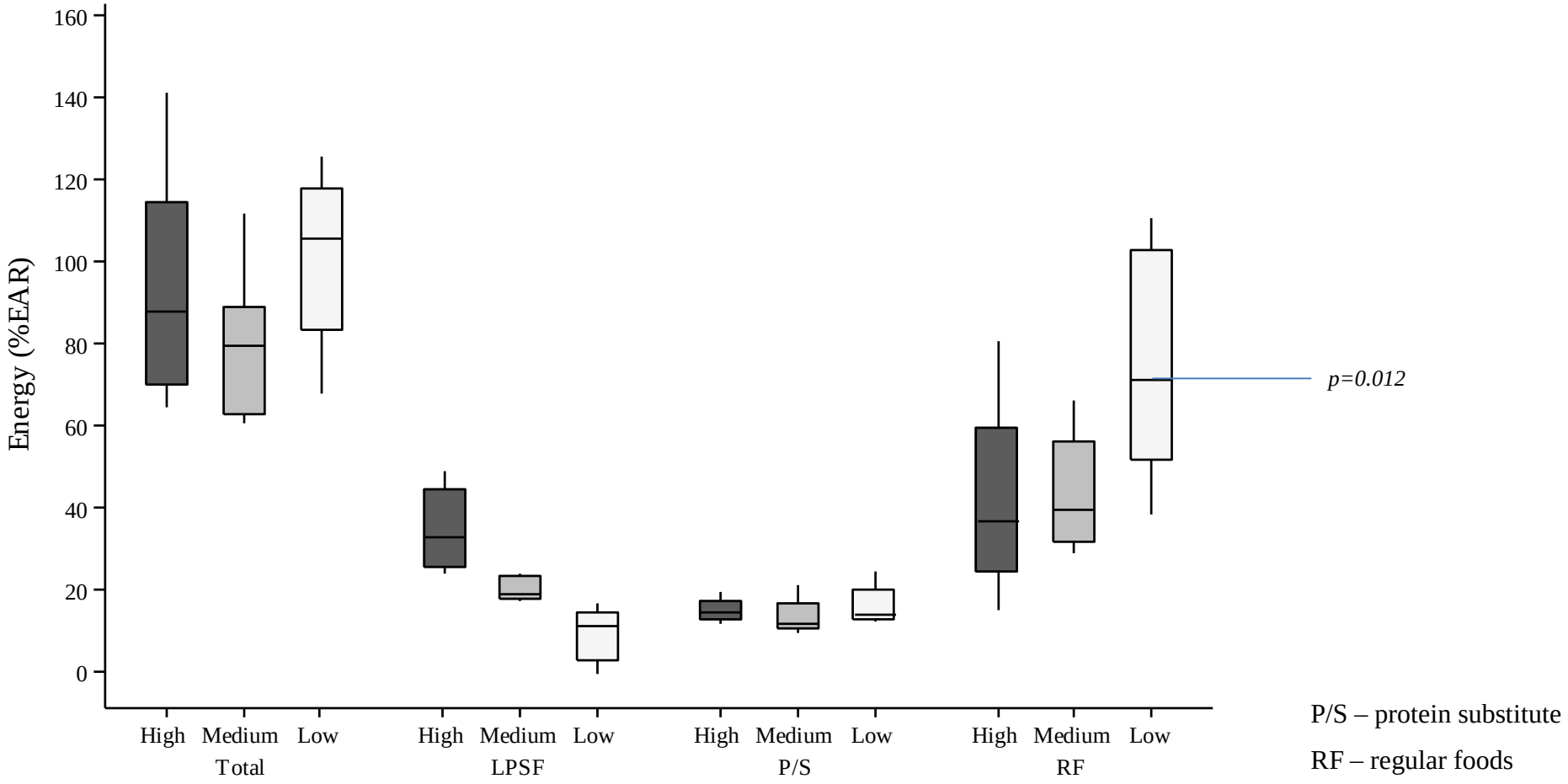


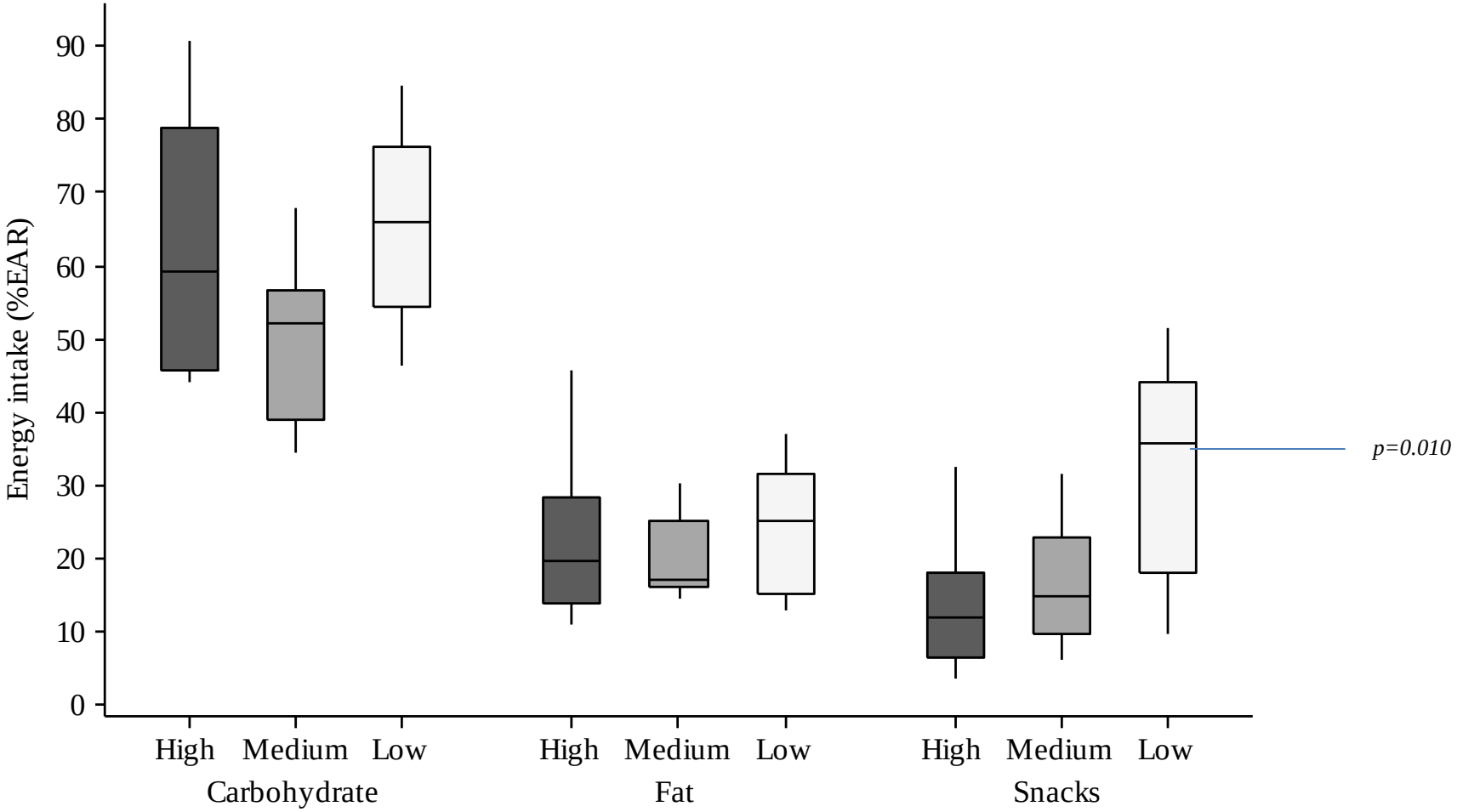
Figure 14: LPSF user groups and energy distribution from diet constituents



When the users of the LPSF were compared for intake of energy (%EAR) from carbohydrate, a tendency towards the low user group taking more energy from carbohydrate than the medium and high users was noted (Table 13) [high vs. medium vs. low: median (IQR): 59% (33); 52% (17.6); 66% (21.7)] (p=0.064). No variation was found in the energy (%EAR) from fat (p=0.773) between the three groups (Figure 15),

The energy (%EAR) from the snack foods which included both LPSF and regular foods was then compared between the three user groups (Table 13) and the low users of LPSF had a significantly higher intake from these foods compared to high and medium LPSF users [high vs. medium vs. low: median (IQR): 12% (11); 14% (13); 36% (26) (p=0.010)] (Figure 15).

Figure 15: Use of LPSF and energy distribution from CHO, fat and snack foods



Dietary protein and phenylalanine intake and use of LPSF

Low users of LPSF had a significantly higher total protein intake (%RNI) than medium users (p=0.036). Low users of LPSF also had a significantly higher intake of protein (% RNI) from regular foods [high vs. medium vs. low: median (IQR): 41.4 (28.0); 31.6 (17.7); 62.4 (54.2); (p=0.033)] compared with both the high and medium users of LPSF (Table13).

No significant difference was seen in the phenylalanine intake (mg Phe) from regular foods between the three groups, although low users of LPSF tended to have a higher median intake than the other two user groups [high vs. medium vs. low (mg Phe): median (IQR): 524 (286); 483(202; 860 (1226)] p=0.081)] (Table 13).

Table 13: Dietary intake and association with use of LPSF

Characteristic	N	High users				N	Medium users				N	Low users				
Food diary	**11**	**Median**	**IQR**	**Min**	**max**	**10**	**Median**	**IQR**	**Min**	**max**	**9**	**Median**	**IQR**	**Min**	**max**	***p***
Boys/girls	**4/7**					**8/2**					**4/5**					
Energy intake (% EAR)	11	87.9	44.2	64.6	141.3	10	79.7	26.0	60.6	111.7	9	105.8	34.6	68.2	125.8	*0.076*
Energy intake from LPSF foods (%/EAR)	11	33.3	19.0	24.1	49.3	10	19.1	5.8	17.6	24.0	9	11.4	11.6	0.0	16.8	*0.000*
Energy intake from Regular Foods (% EAR)	11	37.1*	34.6	15.1	80.6	10	39.8*	24.5	29.4	66.5	9	71.4***	51.3	39.0	110.5	*0.012*
Energy intake from Protein substitute (% EAR)	11	14.6	4.3	12.0	20.0	10	12.0	6.2	9.8	30.0	9	14.0	7.5	12.6	25.0	*0.306*
Energy intake from CHO (% EAR)	11	59.2	33.1	44.0	90.7	10	52.1	17.6	34.3	68.0	9	66.0	21.7	46.1	84.7	*0.064*
Energy intake from Fat (% EAR)	11	19.5	14.4	10.7	45.7	10	17.0	9.2	14.3	30.1	9	25.0	16.4	12.6	37.0	*0.773*
Energy intake from snack foods (%/EAR)	11	12.0	11.3	3.5	32.5	10	14.6	13.1	6.0	31.4	9	35.8	26.0	9.4	51.5	*0.010*
Protein intake (% RNI)	11	201.5	87.7	133.6	273.8	10	142.3*	78.5	116.3	338.0	9	234.5*	130.0	146.3	345.0	*0.036*
Protein intake from LPSF (%RNI)	11	3.4	1.5	1.6	8.2	10	3.2	4.0	1.1	6.9	9	2.4	3.2	0.0	6.7	*0.107*
Protein intake from Protein Substitute (% RNI)	11	145.2	34.3	101.4	197.2	10	111.1	56.3	93.2	244.6	9	159.6	93.1	115.8	276.0	*0.108*
Protein intake from Regular Foods (% RNI)	11	41.4	28.0	10.7	83.8	10	31.6§	17.7	15.2	98.4	9	62.4**	54.2	19.0	153.4	*0.033*
Phenylalanine intake from Regular Foods (mg Phe)	11	524	286	151	826	10	483	202	322	714	9	860	1226	400	2171	*0.081*

**Significantly different from low users LPSF: **significantly different from medium and low users LPSF; ***significantly different from medium and high users LPSF*

Effect of LPSF use and disease severity on quality of life

The association between quality of life and use of LPSF was compared using the PedsQL® questionnaire to determine if there was any relationship between the amount of LPSF used and physical or psychosocial functioning (Table 14). Both for the parent and child reports, no association could be found between the LPSF user groups and total quality of life score [high vs. medium vs. low users: median (IQR): parents: 80 (26); 92 (9); 87 (20);(p=0.493)] and children with PKU: [89(25); 91 (4); 89 (17); (p=0.691)] (Table 14). When the total score for parent and child response was averaged, again there were no comparable differences between the groups of LPSF users [high vs. medium vs. low: median (IQR): 8 (15); 7 (9); 7.0 (12): (p=0.987) (Table 14).

In addition to the use of LPSF on quality of life, a comparison was also made between quality of life and disease severity, no association was found for either parents or subjects for the physical or psychosocial functioning between classical, moderate or mild disease.

Table 14: Association between quality of life and use of LPSF

Characteristic	N	High users				N	Medium users				N	Low users				
Food Diary	**11**	**Median**	**IQR**	**Min**	**max**	**9**	**Median**	**IQR**	**Min**	**max**	**9**	**Median**	**IQR**	**Min**	**max**	***p***
Boys/girls	**4/7**					**7/2**					**5/4**					
Parent report – physical functioning*	11	84	34	50	100	9	97	14	65	100	9	94	9	72	100	*0.519*
Parent report – psychoso-cial functioning**	11	78	22	50	100	9	88	11	35	100	9	83	24	35	96	*0.494*
Total parent report**	11	80	26	52	100	9	92	9	45	100	9	87	20	53	98	*0.493*
Boys / girls	5 2/3					6/2					3/4					
Child report – physical functioning*	5	90	21	66	96	8	96	8	79	100	7	94	13	78	100	*0.351*
Child report – psychoso-cial functioning**	5	70	28	62	98	8	89	10	59	100	7	83	20	50	95	*0.590*
Total child report***	5	89	25	68	97	8	91	4	66	99	7	89	17	63	95	*0.691*

‡: Peds QL® – Paediatric quality of Life Inventory – version 4.0
**mean of score for all items in problems with physical health summary score; ** mean of score for all items in the domains: problems with emotional functioning, social functioning, and school functioning; ***total score for mean of all items for physical and psychosocial domains.*

Diet variety and use of LPSF

The information obtained from the FFQ was mainly used as a means to explore differences in the number of items of LPSF and regular foods used by the subjects (Table 15) which provides an indication of the variety of food use between the three groups. A significantly greater proportion of LPSF items were taken by the high than low users of LPSF [(% of total LPSF items) high vs. medium vs. low: median (IQR): 44% (14.4); 40% (8.6); 27% (38.4) (p=0.048)]. However, when the number of regular foods was assessed, the percentage number of regular food items selected by the three groups was very similar and not significant (p=0.839) (Table 15).

Comparison of use of LPSF and the general population

When the patients with PKU were split into LPSF user groups and compared with the children from the NDNS survey (Figure 16). All users of LPSF showed a similar trend with the eating habits of the NDNS group. Although the low users followed a similar trend to the high and medium users, their intake (g/day) had a greater discrepancy for cereals and potato products by being lower than the other two groups [high vs. medium vs. low; median (IQR): 16.5 (19.9); 18.3 (25.3); 7.8 (19.9); p=0.000].

The LPSF were then allocated into the daily weight (g/day) of intake of the "staple foods" e.g. bread, pasta and milk and "convenience" type foods e.g. pasta in sauce, burger mix and ready-made cakes and reassessed. The low users of the LPSF were shown to have a significantly lower intake of the staple LPSF (g / day) [high vs. medium vs. low: median (IQR): 49.5(32.9); 40.9 (17.9); 11.2 (36.3)] (p=0.015) and also had a significantly lower intake (g/day) of the "convenience" type LPSF than the high or medium user groups [high vs. medium vs. low: median (IQR): 3.7 (5.6); 3.4(4.4); 1.8 (2.4) (p=0.074)] (Table 16).

Similarly, when the LPSF cereal foods and regular cereal foods were combined and reallocated into the same groups as defined in the NDNS (Table 16), along with the remainder of the LPSF which were allocated into the appropriate food groups; such as low protein milk into the dairy group and then re-analysed, the high user group consumed significantly more grams of cereals products, both LPSF and regular foods, than the low user group, with the medium users being comparable to the high user group [high vs. medium vs. low: median (IQR): 10.2 (18.7); 8.9 (12.2); 4.5 (5.) (p=0.030)].

Figure 16: Comparison of users of LPSF and the general population (age 4-18)

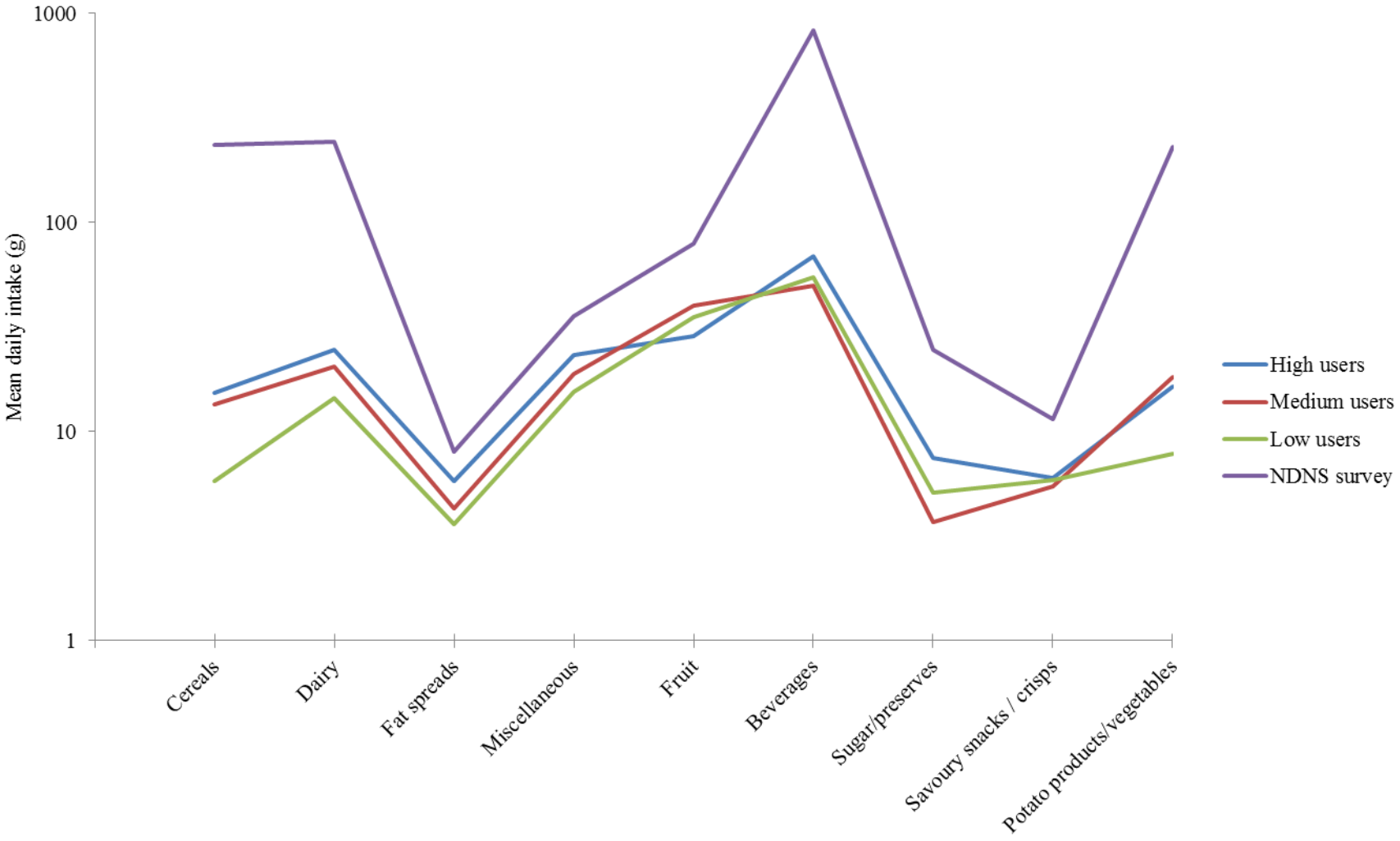


Table 15: FFQ analysis and users of LPSF

Characteristic	N	High users				N	Medium users				N	Low users				
FFQ	**12**	**Median**	**IQR**	**Min**	**max**	**10**	**Median**	**IQR**	**Min**	**max**	**9**	**Median**	**IQR**	**Min**	**Max**	***P***
Boys / Girls	**6/6**					**6/4**					**6/3**					
Energy intake (%EAR)	12	130	45	107	184	10	98	20	62	136	9	71	19	40	108	*0.000*
Energy intake from LPSF (%EAR)	12	47	15	39	87	10	28	6	24	34	9	18	18	0	22	*0.000*
Energy intake from RF (% EAR)	12	68	38	36	96	10	54	26	26	90	9	37	33	26	69	*0.039*
Energy intake from P/S (%EAR)	12	15	6	11	25	10	15	6	4	25	9	14	6	12	20	*0.915*
Energy intake from CHO (%EAR)	12	95	22	71	148	10	64	18	43	100	9	49	12	21	71	*0.000*
Energy intake from Fat (%EAR)	12	26	9	17	42	10	18	6	7	25	9	12	7	6	22	*0.000*
Total Protein intake (% RNI)	12	233	64	164	411	10	181	121	74	337	9	201	103	123	293	*0.274*
Protein from LP (%RNI)	12	10	4	5	15	10	3	4	2	12	9	3	5	0	7	*0.001*
Protein intake from P/S (% RNI)	12	152	11	106	276	10	142	84	36	276	9	141	46	109	203	*0.573*
Protein from RF (% RNI)	12	69	47	26	123	10	40	27	24	97	9	47	92	13	139	*0.123*
Total food items from LPSF foods (%)*	12	44	14	31	61	10	40	8	31	50	9	27	38	0	55	*0.048*
Total food items from RF (%)**	12	42	15	7	57	10	38	12	8	49	9	44	22	26	62	*0.839*
Total food items (%)***	12	42	11	14	55	10	38	12	14	48	9	38	14	25	54	*0.890*

* %Total number of Low protein food items(n = 23); ** %total number of regular foods(n = 109); ***%LPSF + regular foods (n=132)

Table 16: Daily intake of foods (g) from food groups between LPSF users

Characteristic*		**High users**					**Medium users**					**Low users**				
FFQ	**N =**	**Median Mean**	**IQR**	**Min**	**max**	**N =**	**Median Mean**	**IQR**	**Min**	**max**	**N =**	**Median Mean**	**IQR**	**Min**	**Max**	***P***
LPSF – staple foods -	12	49 55	33	32	85	9	41 47	18	10	126	8	11 22	36	0	81	*0.015*
LPSF – "convenience foods"	12	4 6	5	1	24	9	3 4	4	1	12	8	2 1	2	0	3	*0.074*
FFQ - Daily combined food groups (g of food)																
Cereals (Combined natural cereals and LPSF cereal food)	12	10 15	19	6	38	9	9 13	12	5	42	8	4 6	6	2	10	*0.030*
Dairy	12	24 25	13	13	36	9	18 20	17	3	50	8	6 14	19	1	54	*0.079*
Fat spreads	12	5 6	8	0	15	9	5 4	3	0	10	8	4 3	5	0	10	*0.708*
Potato products	12	8 24	7	1	204	9	8 28	7	1	205	8	6 8	6	3	16	*0.669*
Miscellaneous	12	23 23	32	6	47	9	17 19	24	6	43	8	18 15	17	0	35	*0.353*
Vegetables	12	10 8	8	2	14	9	5 8	8	1	19	8	6 7	12	0	14	*0.373*
Fruit	12	25 29	21	0	91	9	40 40	30	0	101	8	15 35	57	1	141	*0.995*
Beverages	12	65 69	38	37	152	9	38 50	43	15	122	8	45 54	64	19	103	*0.249*
Sugar/preserves	12	7 7	7	0	20	9	3 4	5	0	13	8	4 5	4	3	9	*0.116*
Savoury snacks / crisps	12	4 6	9	0	12	9	4 5	10	0	12	8	3 6	11	0	14	*0.949*
Meat / fish	12	0 0	0	0	0	9	0 0	0	0	0	8	1 1	1	0	1	*0.122*

* food groups as defined by National Diet and Nutrition Survey (NDNS)

Discussion

Within the dietary management of PKU, the area least studied is the use of LPSF. These foods have been anecdotally associated with providing diet variety, being used as "filler" foods to satisfy the appetite and also deemed necessary for providing sufficient energy. This second study attempted to evaluate the intake of children following a low phenylalanine diet to characterise the diet of people with PKU and explore associations with the use of LPSF.

Patient characteristics

Association between gender, SIMD and BMI

Of the children with PKU in the study, 68% were of normal weight whereas 28.7% were overweight or obese. This was only slightly less than the Scottish figure of 29.9% (Gray, Leyland 2011), but as the size of our sample population was small, it would suggest that children with PKU have a similar risk in becoming overweight as the general population. In the 2011 report by Gray and colleagues, boys from the households with lower income were more likely to be classed as obese, whereas with our study boys who were overweight or obese came from households with a higher income (groups 3-5).

It might be argued that children with PKU should be more likely to have a normal BMI than their peers without PKU, due to the scrutiny their diet comes under and the regular anthropometric monitoring at PKU clinic. They and their families also have the opportunity for a consistent healthy eating message from a specialised group of health care professionals. It may be that health professionals who manage children with PKU give priority to the management of the disease during the short time allowed during clinic consultations rather than other aspects of overall health and well-being which may be given a lower priority in the day to day support given to families. Health professionals may also be reluctant to broach the subject of yet more restrictions on dietary intake, given that the children are already following a restrictive and potentially socially isolating diet.

In addition, no data was collected on activity levels or sedentary behaviours as part of this study and would be a useful area to study. In turn, this may well give more information on why children with PKU follow similar trends to the general population with rates of overweight and obesity and is a question which warrants further investigation.

Overall energy and protein intake of the subjects

Energy intake

From the diary analysis, it was noted that only one quarter of the subjects took more than 100% of their EAR for energy. A higher proportion of children having less than 100% EAR came from the primary and secondary age groups. These results may be a consequence of under-reporting by the subjects. It is known that food diaries are not an accurate way to record intake as subjects will either alter what they eat or omit to record all they ingest (Rennie, Coward & Jebb 2007, Trabulsi, Schoeller 2001, Klesges, Eck & Ray 1995).

The diaries of the pre-school children in our study were more likely to be completed by a parent or carer e.g. nursery teacher and therefore may be a more accurate recording of intake. In order to obtain greater accuracy of results in future studies, a checklist of foods taken over the week, such as the list in the FFQ, could be compared with the foods recorded in the diary for the same recording period.

Our subjects were asked to record their intake for 7 days and this may have increased the daily burden on both subject and carer and perhaps account for the difference between the age groups. Older children with PKU may not wish to acknowledge when they are deviating from their dietary prescription. In addition, anecdotal reports suggest that parents and carers of older children are less likely to weigh all their exchange foods as they learn to judge portion size and may affect the accuracy of the food diaries (MacDonald, A 2008). Gokmen-Ozel (Gokmen-Ozel et al. 2012) demonstrated that even when weighing exchanges, inaccuracies were noted, therefore leading to an under or overestimate of intake, particularly the calories consumed. However, it appears that the calculated daily energy intake of our participants was within their age recommendations.

The energy obtained from the use of the protein substitute was higher in the females in the study group and also the pre-school age group. The greater amount of energy provided by the protein substitute for the younger children would be expected as the amount of protein substitute (g/kg) is calculated based on age, with the younger children both males and female patients, being given a greater amount of protein per kg than older children to account for a more rapid growth rate (Anonymous1993).

In a regular diet, the percentage energy intake from protein, fat and carbohydrate is based on the FAO/WHO recommendations, with fat providing an average of 35% of the energy; protein 15% and carbohydrate 50% http://www.sacn.gov.uk/pdfs/sacn_02_26.pdf. Rec-

ommendations for the Scottish population are similar with a maximum of 35% energy from fat (http:// www.scotland.gov.uk/Publications/ 2005/01/20577/50672).

The findings from the analysis of the 7 day weighed food diaries, indicated that although the diet for PKU had similar energy intake from protein to that recommended for the general population, of 12-14.6% of EAR (88.5% of the recommended energy intake from protein) with the majority of energy from protein coming, as expected, from the protein substitute, however, the fat intake was much lower at 17-25% of EAR (60% of recommended energy intake from fat). This may be because many of the foods having a higher fat content are restricted in the diet or avoided completely e.g. cheese, meats, although there is no restriction on spreading fats and oils. Those foods commonly providing a lot of fat in the diet of many children are either severely restricted e.g. chips, potato shapes and crisps, or avoided completely such as chocolate and replaced by lower fat alternatives such as chewy sweets. When Thiele and colleagues (Thiele, AG 2012) studied the dietary intake of children before a trial of the co-factor tetrahydrobiopterin (BH_4), they noted similar results with the fat intake (81 ± 25% below the recommendations), found that the carbohydrate intake was lower than the recommendations at 93 ± 22% with 11% of the total food consumption being provided by sweets and snacks.

In addition, it is not as easy to eat out or consume takeaways with a high fat content such as fish and chips or burgers and chips or curries, as these meals also contain high amounts of protein, this may also affect the fat intake as there are no suitable low phenylalanine alternatives. There was no difference noted in energy intake from fat between genders, which is also reflected in the fat intake in the general population (NDNS survey) where girls had a median energy intake from fat of 34% and boys 33%.

The intake of carbohydrate was greater than the recommendations at 52-66% of EAR (118% of recommended energy intake from carbohydrate). This can be explained by the energy coming from fat contributing less to the total energy intake and the energy from protein being the same as that recommended.

Based on the results of the FFQ (Table 16), it is likely that the majority of the carbohydrate intake was from the LPSF staple foods and fruit. However, as it was not possible to get a complete breakdown of the sugar and starch content of the diet of the subjects, it is difficult to say how much the carbohydrate content of any sugary beverages e.g. fizzy drinks or foods in the miscellaneous section, which would include sweets, contributed to the daily intake of carbohydrate. In addition, from the analysis of the FFQ, no particular food group was

taken more by low users of the LPSF than the other two groups. This might indicate that using the FFQ is not a good reflection of intake, or that the completion requires more guidance from the metabolic dietitian to the parent or carer in how to record daily intake.

LPSF such as bread, pasta and rice have comparable calorie content to their regular equivalents (Table 5), therefore energy intake from carbohydrate between the subject group and general population should be comparable. When the age groups were compared, the highest intake of carbohydrate was from the pre-school group with this group a higher intake of LPSF (%EAR) (Figure 8). With this age group, the parents or carers may be more likely to use the LPSF as they are still in the early stages of learning about the diet and may be more experimental with the LPSF. When the children get older and are more conscious of the differences between their diet and that of their peers, children with PKU are less likely to adhere to their dietary restrictions which include use of the LPSF (MacDonald et al. 2012; MacDonald et al. 2010, Walter, White 2004) .

As expected, the patients who had a milder form of PKU had a higher intake of energy from regular foods (including the phenylalanine exchange foods), than in the classical and moderately affected groups in part because low users were prescribed a greater number of phenylalanine exchanges. Also this increased energy intake from carbohydrate containing foods, but particularly not LPSF, or fat and protein in this group, may help to explain the increase in overweight and obesity in the PKU population.

If the study is repeated in the adult PKU population assessing the intake of LPSF and rate of obesity and overweight, it would be interesting to see if it is a low intake of LPSF rather than disease severity that was more prevalent in the overweight group. Adherence to diet starts to deteriorate during adolescence (Sharman, Mulgrew & Katsikitis 2013) and therefore adults are less likely to be on strict diet and adhere to prescribed exchanges than the paediatric population, therefore an association between use of LPSF and obesity would be relevant.

These results illustrate that the overall distribution of energy from macronutrients in the patient population studied are noticeably different from that recommended for the general population, due to restrictions placed on the patient's dietary intake. In the PKU diet, the protein intake especially, is carefully tailored to the requirements of the child and carefully monitored and calculated providing between 3g / kg for very young children up to 1g / kg for the older children. This is less than that normally consumed by the general population (NDNS survey).

When the subjects were allocated into a group dependant on their use of the LPSF, the patients with severe or classical PKU consumed a greater amount of energy from the LPSF than either of the other two groups. Patients with classical PKU have a lower tolerance to phenylalanine and therefore are unable to cope with natural dietary protein i.e. have fewer prescribed exchanges. Therefore in order to satisfy the appetite they would be encouraged fill up with low protein foods which include the LPSF. However, in the milder forms of PKU, patients are given more freedom of choice as where to obtain their daily phenylalanine intake and it is seen that use of the LPSF decreases.

Furthermore, when the energy intake from units of LPSF as recommended by the NSPKU was compared with the energy intake supplied by the LPSF from analysis of the food diaries, the low users of LPSF barely achieved one third of the non-protein energy advised. Even among the high users of the LPSF, the maximum intake of 49% was the highest achieved. The NSPKU recommendations do not distinguish between or give alternative suggestions for patients with different disease severity, merely give broad recommendations for all patients following the PKU diet. This generic approach may not be appropriate and warrants further consideration.

Sufficient energy is needed to allow adequate growth, weight gain and prevent catabolism and a deficit will cause phenylalanine levels to rise, weight gain to falter and growth to be poor. There was no significant difference between the groups for height which would indicate that all subjects did achieve an adequate overall energy intake. However those who were low users of the LPSF had a greater SDS BMI and SDS weight therefore consuming calories in excess of their requirements.

While the phenylalanine levels in all groups, both before and after the 7 day food diary records, were not significantly different and within the target levels required by the metabolic team, this would suggest that low users of LPSF did not take excessive amounts of exchange foods which contributed towards their energy intake, i.e. they were not choosing high protein foods to satisfy the appetite; rather they were relying on low protein, high carbohydrate foods., In addition, although the low users of LPSF had a slightly higher energy intake from carbohydrate than both the medium and high user groups, this did not come from LPSF, but from snack type foods. The snack foods included some use of LPSF snack foods such as low protein cakes, biscuits and energy bars and also included regular cakes, biscuits, crisps, fizzy drinks and sweets. Therefore, the low user LPSF group tended to use more foods which had a low nutrient value, commonly referred to as “junk foods”.

The range of mutations that result in raised phenylalanine levels requiring phenylalanine intake to be restricted and monitored are considerable and some patients will not have any need for the LPSF at all. In order to clarify the level of restriction most benefitting from use of LPSF, and control of BMI, future repeat studies should perhaps band patients into a broader range of groups using degrees of prescribed phenylalanine restriction. This would help to determine the severity of hyperphenylalaninaemia most likely to lead to obesity and overweight.

It was not possible in this study to differentiate between carbohydrate from starch or sugar because of lack of information for the LPSF from specialist food companies, but in future studies, if this information was available, it would help to demonstrate if the increase in overweight and obesity in the low user groups was due to excessive intake of sugary foods such as sweets and fizzy drinks.

The high users of LPSF were shown to have a lower energy intake from regular foods (Table 11) which also included foods used as phenylalanine exchanges such as potatoes and breakfast cereals. The high users of LPSF also took fewer portions of fruit, but similar amounts of vegetables than the other two groups (Table 16). Although foods allowed without restriction in the diet for PKU include fruit and vegetables and Rohde et al (2012) demonstrated that unrestricted intake of fruit and vegetables with phenylalanine content less than 75mg/100g did not affect blood phenylalanine levels, it would be expected that the higher users of LPSF would take more of these unrestricted foods. However, there was no significant difference in number of portions of these foods between the LPSF user groups, suggesting that the low users did not use more fruit and vegetables instead of the LPSF to help satisfy the appetite.

When compared to the general population (Figures 10 and 16), although the children with PKU had a similar trend in their food consumption, they took fewer portions of unrestricted foods. It was not clear why patients with PKU do not consume a greater amount of these foods along with the LPSF to provide diet variety or to satisfy the appetite. It may be that the family do not use a wide variety of fruit and vegetables or that there is difficulty in access to a supply of fresh fruit and vegetables. One of the aims of the study was to compare the diet of children with PKU to the diet of their siblings and the healthy unrelated controls. If this had been achieved, it might have given some insight into the eating habits of a family where there is a child with PKU and allowed comparison with the general population.

Protein intake

Effective dietary management of PKU also relies on an adequate intake of protein from phenylalanine free amino acids and an appropriate intake of phenylalanine from regular foods according to tolerance. Insufficient protein and energy will result in the breakdown of body tissue releasing phenylalanine and consequently raising the blood phenylalanine levels also leading to growth impairment (Rocha et al. 2013). Early recommendations (Cockburn and Clark 1993) for protein suggested that children should receive the RNI for protein with an additional 50% added, due to the diet's artificial nature, which would take bioavailability into account. MacDonald et al in 2011, however, suggested a figure of the RNI with an additional 20% protein added would be adequate and this is similar to the recommendations from the USA and Holland.

The protein substitute is the main source of protein in the form of amino acids in the low phenylalanine diet and the results from the study (Table 13) show that all subjects achieved an adequate intake of protein when compared to the RNI with a median protein intake of 142-234%. When the intake of protein was considered, around 10% of the subjects took greater than 300% of the RNI and no subject took less than 100% RNI. Our results also show that the younger patients are more likely to consume more protein / kg than older children as the recommendation for total protein intake is greater for younger patients (Anonymous1993). This would suggest that the guidelines from these early studies are still providing our patients with an adequate protein intake which can also be confirmed by all patients growing satisfactorily.

The MRC guidelines have not been updated since 1993, however the evidence has been reviewed from studies published over the past decades and new guidelines for patients in the UK will be published in late 2014. This is in addition to the findings of the scientific review published in 2012 which looked at evidence for management of PKU and gave recommendations for future research (Camp et al. 2012).

The impact that phenylalanine from regular foods have in the overall protein intake obtained from the food diaries, show that although the intake of regular foods was greater in the low users, there was no effect on overall phenylalanine control as this was also the group that had milder type of PKU. Therefore as the low users had a greater number of exchanges and no significant difference in maintaining their target phenylalanine levels, because they had mild PKU, this increased intake of protein from regular foods would suggest that low LPSF users made food choices within their dietary recommendations by selecting foods of a

lower phenylalanine content to use as “filler foods”. As the low users did not use the LPSF as dietary “fillers”, it may be suggested that as they were prescribed more exchanges, they did not perceive the same requirement for using the LPSF.

Influence of food choice and PKU diet

Parents or carers exert most influence over food choices in children before they reach teenage years and can therefore restrict a particular food group such as snacks, if wanted. However, as children gain more independence and start to make their own food choices, especially as they reach secondary school, parental influence is less effective. It is also at this age that phenylalanine control starts to deteriorate and adherence to diet becomes an issue (MacDonald et al. 2010, Walter, White 2004). From the results of the study, it was noted that although gender did not affect the intake of energy from snacks, the age of the child had most effect, with the secondary school age obtaining more energy from snack foods than those of primary or pre-school age (Figure 9). However, it was notable that it was not LPSF snack type foods which these children gained most energy, but from regular low phenylalanine foods (Figure 8).

Adherence to diet is a problem not unique to PKU (Borus, J.S. 2010; Bregnballe,V. 2011; Fredericks,E.M. 2010). Young people with diabetes and cystic fibrosis also have difficulty adhering to their treatment regime. So it is not unusual for young people with PKU to want to avoid taking the LPSF which look and taste different to their regular counterparts. Although the survey of adults and carers in Scotland (Cochrane et al. 2014) showed that taste and appearance of the LPSF was acceptable, teenagers have a need to fit in with their peers and using the LPSF out-with the home can make them feel different and therefore they will tend to use low phenylalanine foods to fill them up rather than using the LPSF.

The analysis of the food diaries showed that it was not use of high protein foods such as cheese, meat, ordinary bread or pasta by the teenagers (the pre and post blood spot results bear this out), that they were using to satisfy their appetite rather it was low phenylalanine snack foods. However the number of teenagers completing the food diaries was low, which means drawing any conclusion is difficult. When requested, the teenagers willingly consented to take part in the study, however when given the food diaries and FFQ to fill in and return, it was within this group that the greatest numbers did not return the information for analysis. Therefore there is a need to find a way of engaging teenagers to understand their thoughts and perception on the use of the LPSF. Engaging children with PKU as young as possible in the

preparation of their diet and learning how to cook with the LPSF may encourage use of the products for a longer time than at present. It was shown from the survey results in the first study (Cochrane. et al 2014) that the use of cookery classes benefitted the adults in the preparation and use of the LPSF, if the classes were tailored towards the child or teenager, this might help to raise awareness of the benefits and use of the LPSF.

Association between physical and mental wellbeing and use of LPSF

In this study, no difference between the parent reports and child reports of the LPSF user groups in terms of physical or psychosocial functioning was discovered, suggesting that the use of LPSF does not seem to be associated with how people with PKU or their carers feel about their quality of life and do not view that the use of these products as something that has a negative impact on their life. In 2011, ten Hoedt and colleagues (ten Hoedt, et. al 2011) survey of parents of children with PKU and Galactosaemia found that parents of children with conditions such as PKU, have a poorer quality of life than parents of children without a chronic condition. This may indicate that the overall restrictions placed on the parents and families of the children with PKU negatively affect their day to day wellbeing. However, it was not stated which aspect of the condition contributed most to the poorer quality of life. It may be that a larger sample size in our patient group could demonstrate that greater use of regular foods, for children with milder disease, may place fewer restrictions on the family and therefore result in a better quality of life.

In the 2011 report on Scottish health (Gray, Leyland 2011), it was stated that children's mental wellbeing was shown to be negatively affected by being overweight or obese. This was not shown to be the case in our cohort studied as the results from the Peds QL® bear out. Given that our patient group had the same incidence of overweight and obesity as the general population, measures should be taken to reduce this additional risk factor to mental wellbeing (Rocha, MacDonald & Trefz 2013).

Conclusion

No association between the use of LPSF and socioeconomic status, age or gender or metabolic control was identified. In addition use of LPSF did not have a negative impact on physical or psychosocial well-being. Children with classical PKU consumed more LPSF than those with milder disease. However, low use of LPSF was associated with increasing BMI with food choices being inclined towards snacks of regular foods and using a greater amount of ” junk” foods to satisfy the appetite and provide energy especially in the older children. When the number of portions of foods, both regular and LPSF were compared there was no difference between the groups. No subject met the recommendations for non-protein energy from LPSF suggested by the NSPKU society.

Practical implications and suggestions for future research

The use of LPSF in the diet for PKU has been the mainstay of treatment now for around fifty years, making it unethical to conduct a randomised controlled trial to provide evidence for the role they play in the control of phenylalanine levels and the contribution they make towards nutritional status and other aspects of health and wellbeing.

The first study provided information on the patient's perception of the need for these foods and the results show that patients use them to aid control of phenylalanine levels, help to satisfy the appetite and provide variety. However, this survey did not take into account disease severity and use of the LPSF. Therefore, we do not know if people with milder disease severity have a different perception of their value than people with classical or moderate disease severity. Results also identified many families had a problem with regard to access of these products, but again it is not known if this is associated with other factors such as disease severity which was not explored. In addition, it may be that a lack of awareness and understanding by the primary care staff on the use of the LPSF in management of PKU results in patients and carers receiving repeated problems accessing product. A survey targeting primary care staff in both the patient's G.P practice and local pharmacy might help to identify what level of support and education the metabolic team should provide.

Those with milder disease severity are generally thought of as having greater freedom of choice in use of regular foods to provide phenylalanine requirements and therefore a less restricted diet. However, the results from the second study suggest that this group chose less LPSF and also used more of the snack type foods. This trend is reflected in a higher BMI and incidence of overweight and obesity in the low LPSF user group. It may be that patients who are prescribed a greater number of phenylalanine exchanges do not perceive the LPSF as a valuable contribution to dietary management and prefer to consume energy dense snacks.

It was not possible in this study to obtain a breakdown of the components of the source of carbohydrate in the LPSF and regular foods to determine the proportion of sugar, starch, fibre or non-milk extrinsic sugars using the manufacturer's product information and therefore only total carbohydrate was assessed. It would be useful in a future study to try to obtain more information about these components, especially the sugar content, to determine the role sugary foods play in the energy content of the diet of people with PKU and therefore whether low users consume more sugar than starch in their diet.

This information would give an insight into some of the various factors that play a part in the growing incidence of obesity in patients with PKU (Burrage et al. 2012). As our

results have shown, it is not the energy from fat of the subject's diet that contributes to excess energy intake, but carbohydrate.

The results from Study1 show the value patients and their carers place on practical support from the metabolic dietitian in helping them use the LPSF effectively by the provision of cookery classes. Again, it is not known if it is the patients with the more severe disease who attend these classes or indeed are supported by having foods delivered by the home delivery company to help them overcome difficulties with their primary care practitioner.

Patients with a milder form of PKU may be helped to lower their overall calorie intake by being advised to use foods of a higher protein content to provide their phenylalanine prescription, such as yoghurt, spreading cheese and pulses, therefore requiring the use of a greater amount of LPSF to provide bulk and variety and satisfy the appetite rather than snack foods. This may help to reduce the risk of becoming overweight or obese. Over time, the LPSF have greatly improved in taste, texture and variety and these improvements should appeal to most groups of disease severity. In addition dietitians should encourage patients to use more LPSF to free exchanges, thereby encouraging the use of more nutritious regular foods

The exchange list produced by the NSPKU avoids foods which are slightly higher in natural protein e.g. yoghurt, spreading cheese or nut based products such as peanut butter, therefore patients tend to steer away from these foods and choose foods with a lower phenylalanine content to make up their phenylalanine allowance. The results from study 2 show that patients therefore tend to use snack foods for this purpose.

The NSPKU list could be adapted and expanded to take into account more protein dense foods thereby aiding patients to make suitable choices and necessitating greater use of LPSF. This would make the diet for PKU more liberal and consistent with all patients having the same emphasis being put on using the wide range of LPSF available. However, it would also then necessitate guidance being given on what percentage of the staple LPSF compared to "convenience" LPSF should make up the diet. In the increasing scrutiny of primary care budgets, this guidance by metabolic specialists would give more confidence to the G.P's in that the prescriptions they are issuing are suitable for their patients and perhaps lead to fewer problems in access to the LPSF by families.

As food labels become more informative, we should use this information to give patients greater freedom in use of more protein dense foods, be less wary of them using previously "forbidden foods", but trust that with good dietary education, patients and their carers

will still control their diet effectively. In this way, a wide range of LPSF could be encouraged, therefore steering patients away from using high energy snack foods.

The advice that there should be "diet for life" (Levy, Waisbren 1994, Smith, Beasley & Ades 1991) has brought with it many challenges for adults to face as they grow older. There is an increasing tendency towards obesity, heart disease and diabetes in the general population and tackling this in the general population is difficult (Ortner Hadžiabdic, 2014) and the adult with PKU may not be at any lower risk of developing these conditions. This is an area not yet studied in PKU, as early treated adults are only now reaching their 50's and are just at an age at which these conditions are widespread in the general population.

Another area for study should be the attitude of health professionals managing these patients towards discussing yet further restrictions on an already constrained diet, if we are to tackle the increase in obesity amongst our patient population. We owe it to our early treated adults to ensure that they are not at increased risk of obesity related disorders because they have made unsuitable food choices.

Overall Conclusion

Although, patients with PKU and their carers perceived that the LPSF were important in helping control phenylalanine levels and they did not have any concern with the sensory properties, not all subjects used these foods up to the level recommended by NSPKU. Patients and carers often felt anxious about asking for the LPSF because of the attitude of primary care staff and it was not clear from the survey if disease severity was associated with access to LPSF. A programme of education by the metabolic dietitian for primary care staff in both the G.P practice and pharmacy upon the diagnosis of PKU will ensure new parents receive adequate support at all health care levels.

Health professionals should be aware of the link with low use of the LPSF and the trend towards obesity and overweight amongst their patients. Advice should be given to reduce the use of foods commonly considered to be "junk foods with patients and their carers given directed information on which suitable foods could be used for phenylalanine exchanges, especially for those with milder disease, to ensure staple LPSF are used to satisfy appetite. rather than the "convenience" LPSF or snack foods. Health professionals should not be reticent when advising their patients and patient's carers about the risks of obesity and overweight and assist them in making suitable food choices.

Future studies should be undertaken to explore any link between disease severity and access to LPSF along with attitude of those with less severe disease on the value of the LPSF in the management of their disease.

Issues, limitations and reflection on the study

Recruitment

The PKU clinic at the Royal Hospital for Sick Children in Glasgow, Glasgow Royal Infirmary and Southern Genera Hospital, have the largest cohort of patients on a restricted phenylalanine diet in Scotland. There are around 70 children and 160 adult patients in total. It was therefore envisaged that recruitment to the study would enable the designated number of 60 therefore easy to obtain. Subjects were to be contacted by letter 3 months prior to their annual review appointment. The decision to contact the families three months before the clinic visit was thought to enable patients to be aware of the study in a timely fashion and not have to wait up to a year before they would consent to take part. It also allowed a constant recruitment of subjects.

However, not all patients or carers replied to the letter and were therefore approached to take part in the study at their clinic appointment. The majority of patients and parents when approached in this way agreed to take part and gave consent for extra blood tests and anthropometric measurements to be taken. However, although they were given the FFQ and the 7 day food diary along with instructions on how to complete it and a stamped addressed envelope for return of documents, many patients did not return the study documents. Patients and parents were contacted by phone and email, but some documents did not arrive and contributed to the missing data and small numbers in the study. In any future studies, it is envisaged that letters would be sent out to all families at the start of the study and a reminder sent in the week before the clinic visit to aid recruitment.

Recruitment of adults

In the study proposal, adults were to be recruited into the study from the adult clinics at Glasgow Royal Infirmary and the Southern General Hospital. A decision was taken to concentrate recruitment on children first and then recruit adults. However, at the time that adults were to start recruitment, a separate study into the psychosocial effects of PKU in the adult commenced and it was not deemed suitable to ask these patients to take part in the LPSF study. This meant that no adults were recruited and also had an impact on the overall numbers of subjects able to be recruited. Future studies investigating issues distinct to adults than paediatric subjects would enable the investigator to concentrate on one group at a time and perhaps give information valuable for management of the adult PKU population.

Recruitment of healthy controls

The low numbers of healthy controls recruited was due in part to the lack of available time and an unexpected increase in workload experienced by the investigator. Although posters were displayed around the hospital and the invitation extended to family members of the children attending clinic, insufficient numbers were able to be recruited. This led to the decision not to include the data obtained from completed diaries and FFQ. However, information obtained will be used in the future if the study time can be extended. It was disappointing not to be able to compare the diets of siblings with other controls to examine the impact of having a family member on special diet on the eating habits of the family, but this will be explored in future studies. In addition, the majority of the healthy child controls were recruited from groups 4 and 5 of the SIMD and would lead to bias with the results, so in any future studies, greater effort would be made to increase numbers from the groups 1-3.

Difficulties with the postal service

Patients and carers were asked to return the 7 day food diary and PedsQL® questionnaire by post after completing it within two weeks of their annual review appointment. This proved to be optimistic on the part of the investigator. Some documents were completed only after several contacts with the family. Some participants claimed to have completed the questionnaires and posted them which did not then arrive. During the study, there was some difficulty with the postal service and at least one document had to be collected from the main postal sorting office when not delivered. In the future, it would be better to arrange collection of the documents personally or return at the next clinic appointment so that fewer documents would be lost with a reminder just before the visit.

Investigator's relationship to subjects

As the principal investigator was also the clinical supervisor of their dietary management, it was thought that this would aid recruitment as the subjects might feel more at ease and able to ask questions or decline to take part if they felt unable to complete all the study requirements. However, the opposite seemed to be true. Many participants agreed to take part, have the necessary anthropometric assessments, blood tests but did not complete the food diaries or FFQ, which was the main requirement of the study. It was felt that perhaps they felt unable to refuse, or did want to take part, but were unable to complete the necessary tasks involved and then felt uncomfortable when questioned regarding returning the documents.

Bias from food recording

Using the results from foods diary analysis, the overall contribution that the LPSF, protein substitute and phenylalanine exchanges play in the management of PKU was evaluated. The results from the week to week monitoring of the phenylalanine levels pre and post diary completion were compared to determine any bias on phenylalanine levels leading to patients being more careful in what they consumed. No difference was found in the percentage of results outside the target range which might indicate that dietary habits did not change significantly during the week because of the length of time the subjects were asked to record their intake.

Many of the teenage subjects who gave consent to take part in the study did not return the questionnaires and some of the questionnaires were not as accurately completed as would be desired. This may then have led to a distortion in the overall results of the study. The 7 day weighed food diary is one of the most accurate methods of recording intake, but has its drawbacks as participants are known to alter intake when completing diaries. It is also time consuming and laborious to the subjects and prone to inaccurate recording because foods were not weighed. Using one method only, may have improved the number of complete food diaries or FFQ and thereby increased the study numbers. The weighed, daily food diary and FFQ are often used in the management of any dietary treated disorder. A useful study would be to compare results from both methods of recording and thereby accuracy of recording, which would give a useful tool in the day to day clinical management of PKU.

Investigator's clinical duties

The amount of time needed to complete the necessary investigations at the time of recruitment was underestimated by the investigator. No additional clinical time was allocated for recruitment at the annual review or follow-up. This did not impact on the clinical management of the patient, but did impact on the time available to chase up subjects and carers to enable the return of documents. This in turn led to a lower than expected number of patients completing the study. As mentioned earlier, the investigator's workload increased with an additional clinical area and led to the reduction in time available to chase up missing documentation.

Subject's clinic attendance

Patients with PKU under the care of the metabolic service are given 3 monthly clinic appointments and each year has blood taken for a nutritional screen. This meant that at each

PKU clinic it was variable the number of patients who might be eligible to take part in the study. Some weeks there might be 4 or 5 patients suitable and the next week there would be none. The greatest impact of this was the time available for follow-up of patients and getting the necessary documentation out and then returned to the patient before the review.

The number of subjects estimated which would give a suitable sample size was based on the recruitment of subjects prior to their annual review clinic visit. Due to the poor response to the invitation letter and subsequent enrolment at clinic, a letter to the whole population group at the start of the study period, followed up by a second letter, just prior to the clinic appointment would perhaps have improved recruitment of subjects and sibling controls.

Micronutrients

The design of the study included evaluating micronutrient status associated with nutrient intake as assessed from a 7 day food diary and FFQ. The aim was to compare accuracy of recording using the two recording methods with micronutrient status.

Ethical approval was given to evaluate all features of the management of PKU, but this then led to too many aspects being considered. Sufficient data was collected so that it would be useful for a future study to consider the intake of micronutrients compared with results from the annual nutritional screening.

Appendix

Evidence table 1: Micronutrient status in PKU

Author	Aim	Subjects	Micronutrient studied	Intervention	Outcome measures	Results	Comments
Barretto et al 2008 Brazil	To investigate erythrocyte Zinc, serum selenium and copper levels in children with PKU	32 1/12 – 19 yrs.	Zinc Copper Selenium	3 day dietary food record Same protein substitute – age appropriate – Se free Venous blood: 7-10mls	Cu – all normal Zn – N – 62% Se – n – 9.4% Lowest Se in oldest pts., Highest Zn in youngest	a. Diet def. in Se, b. Bioavailability of Zn may be affected by excess fibre and other minerals. c.P/S source needs to contain micronutrients. d.P/S needed to supply nut demands of children in Brazil	No mention of Phe levels – compliance with diet No separation of diet and P/S intake
Sievers, E et al 2000		3 PKU, 10 controls breast-fed	Iron Copper Manganese	2-5ml milk, Faeces Usual diet	Retention of Fe, Cu, Mn exceeded controls		No mention of how many infants breast or formula fed
Sievers E; Arpe T; Schleryerbach U; Schaub J 2000 Germany	To determine if retention of Molybdenum and plasma conc in infants with PKU is equivalent to healthy breastfed infants	4 PKU – Low Phe formula + breast or normal formula milk 17 healthy controls	Mb	Collection of pooled breast milk, test weighing of breast fed infants, bottles weighed Urine collection Faeces, plasma samples	Intake and retention of Mb	Intake and retention of Mb 18 times greater in infants with PKU than Intake and retention of Mb 18 times greater in infants with PKU than healthy breast fed infants	No comparison with bottle fed infants,
Fisberg RA et al 1998 Brazil	To study the effects of the Phe restricted diet.	42 children 1-12 years 31 controls	Zinc Copper	3 day dietary record P/S given according to manufacturer's instructions Weight Height 5ml venous blood	Children with PKU < 7, Height / age lower than controls Plasma Zn higher in controls Plasma copper similar in controls		Low protein foods not readily available therefore affecting calorie intake

Author	Aim	Subjects	Micronutrient studied	Intervention	Outcome measures	Results	Comments
Sievers, E; Oldigs, H-D; Dorner, K; Schaub, J 1990 Germany	Evaluation of balance data for Fe, Cu, Mn	3 infants with PKU 10 healthy controls – previously studied- breastfed	Fe, Cu, Mn	Wt. before feeding 2-5ml sample of prepared diet. Faeces	Fe, Cu, Mn concentrations in breast milk and PKU diet	Median concentrations and retention of Fe, Cu, Mn of PKU diet exceeded mean concentrations in breast milk	Not clear what type of normal feeds infant received to provide Phe allowance – Ordinary formula or breast milk. Small sample size No indication of amount of "normal" feed infant received.
Acosta PB Et al 2004 USA	To determine the incidence of iron def.	37 children with classical PKU 2-13 years For 1 year Children grouped according to P/S prescribed	Iron	Hb, haematocrit, ferritin, TFR concentrations 3 monthly diet diaries		a.Fe within reference ranges no child had Hb concentrations suggesting anaemia b.7 elevated TFR/ferritin ratio c.4 elevated transferrin receptors	No mention of Phe levels although compliance with P/S mentioned as a cause of low ferritin in 1 pt.
Tondo et al 2009 Spain	Study a range of elements in pts. with IEM with and without dietary treatment Co, Mg, Mn, Se, Zn Mo, Cu assessed	72 pts. 2 months – 44 years 92 controls 2 groups On dietary restriction and group 2 not on diet	Copper Selenium Zinc	Data from manufacturers for P/S + 3 day questionnaire P/S contained vitamins and minerals		25 Pts. on dietary treatment had decreased Se levels 14 pts. had decreased Co levels All other elements normal Se and Co dietary intake higher than RDI	
Jochum F et al 1999 Germany	Monitor effects of low selenium intake	87 children with PKU 5-15 years 34 controls	Selenium	Wt., Hot, neurological, dermal examination. Hair, urine and blood taken to measure Se		a.Se in plasma, whole blood and hair lower than in controls b.Urinary Se excretion lower	No mention of dietary intake, Phe level apart from stating that children well controlled. No mention of P/S

Author	Aim	Subjects	Micronutrient studied	Intervention	Outcome measures	Results	Comments
Robinson, M; et al 1999	To determine if relaxed or discontinued diet predisposes to B12 deficiency	N=83 (11-38 years) Patients divided into 3 groups: Strict: diet + aa supplement Relaxed: diet + aa supplement – 1g Protein /kg Unrestricted: no protein restriction and no aa supplement	B12	Neurological examination Vitamin B12 assay Red cell folate Dietetic Assessment	Blood Phe levels Vitamin B12 levels Erythrocyte folate levels	Vitamin B12 < normal range: 6 in unrestricted group 3 relaxed group 1 in strict diet	1 patient in strict diet group with low B12 had not been taking vitamin/mineral supplements
Hvas,A.M; Nexo, E; Nielson J.B Denmark 2005	To examine vitamin B status in adult PKU patients. Query need for supplementation	Patients with PKU > 17yrs Unrestricted diet n=31 (18-43yrs)	B12	FFQ 7 day food diary Serum cobalamins Erythrocyte folate Information on neurological symptoms	Neurological symptom score Serum total transcobalamin Cobalamin saturated transcobalamin	75% had biochemical B12 deficiency ↓B12 intake ↓B6 intake <RDA intake of Protein Normal folate status	Small numbers of participants 11/31 took vitamin preparation 7/31 took no supplement 24/31 took LNAA No distinction between B12 deficiency and severity of PKU
Taylor et al 1984 Liverpool	Effect of diet on calcium and trace element status of children with PKU	25 children 6 mths. – 17.5 yrs. Sibling controls	Calcium Trace elements	Pts. received P/S without added vits and minerals, Given mineral, multivitamins and additional Vit D Whole blood and hair samples analysed		a.Hair Zn and Ca lower than controls b.Hair cu higher than controls c.Blood Zn and Cu within normal ranges d.1 Pt. blood Cu above normal range	No mention of Phe levels, dietary intake not measured, no mention of compliance with P/S

Author	Aim	Subjects	Micronutrient studied	Intervention	Outcome measures	Results	Comments
Bohles 1991	To investigate carnitine conc in patients with PKU in relation to iron availability	3 groups N=169 - <2 yr.(n=32) - >2 yr.(n=75) - Off diet(n=62) Control N=57	Serum ferritin Serum carnitine	Daily iron intake Blood test		Some patients may have low carnitine even on strict diet. Low Hb	Correlation between Fe and carnitine (Fe co-factor for carnitine synthesis)
Acosta PB 1996	Review of studies of intakes of Fe, Zn, Vitamin A of children treated for PKU	Studies with between 8-21 children	Zinc Iron Vit A	3 day food intake records, plasma analysis of ferritin, Zn and retinol analysed		Differences seen in the plasma concentrations between casein hydrolysate and L amino acid mixes	
Arnold, G et all 2001	Mean Hb low < control group for all ages	41	Iron	Parent /patient report – 3 day recall prescriptions reviewed 145% USA RDA for Fe in P/S for ages 1-4 175% Fe for ages 7-11 111-114% Fe for ages >12 years		No significant diff in mean Hb, haematocrit Mean red cell count ↓ Mean Hb low < control for all ages	Compliance with p/s not known
Vugteveen, I; et al 2011 Netherlands Retrospective study	Functional B12 deficiency should be considered rather than serum B12 concentrations	Diet treated Patients with PKU N=91 investigated n=16 – insufficient data or pregnancy M=36; F=39 (1-37 years)	B12 MMa Hcy	Data on Serum B12, serum MMA and plasma Hcy collected at the same time. From laboratory database.	Functional B12 deficiency defined as MMA concentration above P 97.5 Metabolic control – average Phe conc in year preceding measurements of B12, MMA and Hcy	18 patients with abnormal concentrations of B12, MMA and / or Hcy No correlation with blood Phe concentration	Folate and B6 not measured. No measurement of compliance with protein substitute or vitamin/mineral supplementation Population treated as one group

Author	Aim	Subjects	Micronutrient studied	Intervention	Outcome measures	Results	Comments
Acosta PB, Yannicelli S 1998 Company supported study	Evaluation of Phenex – 1 amino acid mix for selected micronutrients, biochemical indices of trace mineral status while on treatment for PKU	27 infants with PKU	Iron	Indices of iron status, plasma trace and ultra trace minerals retinol and α-tocopherol concentrations assessed at 3 and 6 months. Monthly 3 day diet diaries. Data compared with similar formula from another company in two studies		Mean intakes met / exceeded RDA's. Individually 2 low for Ca, 4 for Fe, 2 for Mg and Mn, 1 for Zn and 1 for Vit A	Data not complete. Study concluded that the formula supported normal iron status and trace minerals when fed in recommended amounts.
Acosta PB 1996	Review of studies of intakes of Fe, Zn, Vitamin A of children treated for PKU	Studies with between 8-21 children	Zinc Iron Vit A	3 day food intake records, plasma analysis of ferritin, Zn and retinol analysed		Differences seen in the plasma concentrations between casein hydrolysate and L amino acid mixes	
Bodley 1993	Investigate serum Fe conc and dietary intake in infants and children with PKU	N=53 < 1 yr. (n=6) 1-3 yr. (n=22) 4-12 yr.(n=25)	Serum ferritin Hb conc	Review of dietetic information + blood results	Serum ferritin Dietary iron intake	All patients had normal Hb 17% (<1 yr.); 73%(1-3yr) and 44%(4-12yr) had subnormal serum ferritin	May need greater than RNI iron due to low bioavailability Age 1-3yr most at risk
Darling 1992	Investigate Se status in PKU patients	N=73 10-27 yrs. 66 on diet from diagnosis 7 on diet >9yrs Controls n=25 (13 were family members)	Se	1. Supplemented protein substitute 2. Unsupplemented Protein substitute	Blood test Serum Se Se content of soil	Serum Se in subjects and controls significantly different Se in supplemented group significantly ↑than unsupplemented group	No clinical signs of Se deficiency seen in subjects
Reilly C; Barrett J et al 1990	Assessment of treated children with PKU	20 children <1-14 years. 20 siblings as controls	Selenium	3 day weighed dietary record, food samples taken for analysis. 3. Blood and urine samples collected 2-4 hours prior to clinic visit		No difference between the groups for plasma or urine Zn, Cu, Fe or Mn Plasma Se significantly lower	Mineral mixture given to children with PKU did not contain Se

Author	Aim	Subjects	Micronutrient studied	Intervention	Outcome measures	Results	Comments
Longhi 1987	To study TE status and serum ferritin levels	N=19 n-=8 treatment started at diag-nosis after birth (gp A) n=11 treatment started late alt-hough diag-nosed at birth (gp B) Divided into 3 groups: 1-5months 6-12 months 13-24 months	Fe, Zn, Cu, Se, Mn	3 day dietary recall Blood test	Trace element intake as a proportion of RDA for the 3 age groups	Se, Fe intake ↓RDA in the group A of all ages. In Gp B – Zn, Mn, & Se intake ↓ RDA Mn intake ↑ in age gp 6-12 months	Patients with PKU are at risk of Fe, Cu, Zn, Se due to insuf-ficient intake Require Se sup-plementation

Evidence Table 2: Management of PKU and reimbursement of medical foods in Europe and USA

Date	Author	Title	Aim	Findings	Comments
2012	Giovannini et al Italy	Phenylketonuria: nutritional advances and challenges	Review of treatment advances and challenges in PKU management	-compliance in infancy and childhood adequate, but difficult in adolescence and adulthood.	- Other factors than degree of control of Phe influence cognitive outcome
			- goals of dietary treatment	-dietary regime provides higher CHO and lower saturated fat intake.	-Children with PKU have lower plasma cholesterol than healthy children
			- nutritional characteristics	-restricted diet contributes to low DHA intake. Subtle neurological deficits seem with early treated children	-DHA is essential and should be supplied beyond infancy. Studies are needed to evaluate optimal dosage of DHA supplementation.
			-LCPUFA supplementation	-progress has been made with palatability, composition, compliance, formulation, age related formula	-protein substitutes should be continued to be monitored for efficacy
			-Protein substitutes	-GMP natural protein in purified form is free from phe. May provide acceptable, alternative protein source.	-GMP may help compliance
			-Glycomacropeptide	-alternative to conventional PKU treatment, many treatment rationales	-studies needed into role, dose and composition of LNAA
			-New strategies • LNAA • BH4 • PAL	-BH4 responsive patients have lower plasma phe >30% reduction. PAH mutation dependant -mouse models show promising lowering of plasma phe	-BH4 will be in conjunction with dietary treatment -may be useful in conjunction with dietary therapy, independent from PAH mutation

Date	Author	Title	Aim	Findings	Comments
2013	Fidika et al Germany	Quality of life among parents of children with PKU	Evaluate parental QOL Identify predictors of PQOL	Cross-sectional study in Germany. Inclusion: -parents with one or more children with PKU -children and adolescents <20 years, living with parents -children diagnosed through NS. Method: -self reported questionnaire Subjects: -13 fathers(14.6%); 76 mothers(85.6%) n=89	Low numbers of fathers meant no comparison between them drawn. Parents of Pre-school children had lowest total PQoL scores (m=61.3; SD=12.9), significant difference with school age children (p=0.011) Parents perceive QoL positively, but need child age appropriate support.
2012	Belanger-Quintana et al Multi-centre study	Diet in Phenylke-tonuria: A snap-shot of special dietary costs and reimbursement systems in 10 in-ternational centres	Exploratory data gathered from 10 international specialist PKU centres	All centres members of European nutrition Expert panel on PKU, 1 centre from each country, not representative of other centres in each country. -retail price per package of 3 commonly used protein substitutes representing 4 phases of patient ages -retail price for 3 commonly used types of low protein foods - state benefits available to alleviate cost of dietary management	- costs varied widely between countries - all counties reimbursed to a degree through subsidy or insurance. - Protein substitute most expensive component of dietary management and funded in full - low protein food reimbursement and availability varied widely - cost of low protein foods influenced by local reimbursement system – lower cost for patients self-financing -Study highlights inequality in healthcare policies and access to products in Europe
2012	Camp K et al USA	Nutritional treat-ment for inborn errors of metabo-lism: Indications, regulations, and availability of medical foods and dietary supple-ments using phe-nylketonuria as an example	Review of the indications for use, regulation, availability and categorisation of specific nutri-tional products for the treat-ment of IEM.	Nutritional products divided into 2 categories by pur-pose: -medical foods containing protein without the affected amino acid. Provided 85-90& protein needs of patient, may contain vitamins and minerals - foods modified to be low in protein which are an alter-native to foods which are excluded or limited in diet. - IEM infant formulas regulated by FDA as infant for-mulas - medical foods for >1 yr. exempt from nutritional labelling and health claims Act(1990)	- Newborn screening programme identifies individuals with IEM where nutritional treatment is standard of care, but lack of evidence –based research documenting effectiveness of treatment means inconsistent access to treatment. - Inconsistencies remain in medical food health insurance cover by states.

Date	Author	Title	Aim	Findings	Comments
2012	Macdonald et al UK	Adherence issues in Inherited Metabolic Disorders treated by low natural protein diets.	-study highlights issues in gaining dietary adherence in amino acid disorders	-adherence issues similar to other chronic disorders such as CF, DM -burden on family of day to day management -some children identified as having behavioural problems -family with poor parenting strategies associated with less adherent children -lack of available information for parents -cultural barriers affect adherence -limited availability of special medical foods	-study needed to evaluate effects of diet and adherence leading to standardised educational tools in order to support patients and carers
2011	Macdonald et al UK	Retrospective, observational data collection of the treatment of phenylketonuria in the UK, and associated clinical and health outcomes	-retrospective, observational chart review at 3 specialist centres. - 125 subjects included: 20 adults and 105 children -study explored number and consistency of returned blood samples - study explored how often subjects out of recommended range - phe concentrations in range if >70% within target	Inclusion criteria: -diagnosis of PKU ->4 phenylalanine measurements for adults and >6 phenylalanine measurements/year -data collected on 141 subjects	-discrepancy in classification of "adult" – aged 16 or 18 -small sample size -only 3 centres contributed -self- selected population - 16 subjects did not meet inclusion criteria - clinical contacts analysed but no mention of this in primary aim of study
2010	MacLeod E , Ney D USA	Nutritional management of Phenylketonuria	Review of development of dietary prescription, outcomes of nutritional management, compliance with the diet across age ranges and new options for management	-recommendations for protein exceed RNI for protein by 30% (UK) - evidence suggests no increased requirements -phe requirements for adults-9.1mg/day -phe requirements for children – 14mg/day, but must be assessed individually -natural protein <25% in diet - monitoring – needed to adjust phe prescription -new therapies such as BH4, LNAA may be effective for some with PKU -GMP – may result in reduction of phe conc as taste better than conventional protein substitutes	-unclear if those with PKU have a lower risk for cardiovascular disease -mouse models suggest increased plasma phe adversely affects bone development -compliance decreases with age – 50% 10-14 and 79% 15-19 year olds have blood phe conc >target range -only 19% adults able to achieve compliance after 9 months back on diet -Generally used in conjunction with diet restriction may improve control - only very small studies evaluating GMP -more studies needed regarding optimising health of patients as they age.

Date	Author	Title	Aim	Findings	Comments
2011	Macdonald et al UK	Nutrition in Phenylketonuria	Review present knowledge concerning protein, AA, vitamins and trace element status, in addition to growth and body composition	-no data to support higher doses of phe free aa, but 20% extra given in line with guidelines to compensate for losses / reduced digestibility as per vegetarian diets -few reports documenting phe intake of adolescents or difference between prescribed and actual intake. No recent reports of phe deficiency -growth data from Germany, Netherlands and France reported poor growth, only protein intake not energy intake recorded - studies reporting tendency to overweight from late 1970's -restricted diet requires supplementation of micronutrients, many factors affect micronutrient status	-if phe levels within target ranges, dietary phe not measured -phe allocation varies from country to country-but no method shown to be better than other and comparison of phe control shown to be similar. - data from UK, with higher protein intakes did not show any growth retardation -no mention of general population changes -Selenium deficiency most commonly reported problem
2009	Ahring K et al Multi-centre	Dietary management practices in phenylketonuria across European centres	Summary of dietary practices in individual treatment centres within 10 European countries. Questionnaire obtaining information on: numbers of patients; management guidelines; training of dietitian's; roles and responsibilities; monitoring; reimbursement of dietary products; challenges for management 2nd Questionnaire on: Policy on protein substitution; allocation of phe; phe exchange system; infant feeding practices; tyr supplementation and monitoring	-Newborn screening varied between centres (4-10 days) and threshold conc triggering intervention varied (300 – 600 umol/l) -Routine anthropometric assessments similar - Biochemical and Haematological measurements varied -IQ not routinely assessed -Dietitian's training and status varied between centres	

Date	Author	Title	Aim	Findings	Comments
2013	Guest J.F et al UK	Costs and outcomes over 36 years of patients with phenylketonuria who do and do not remain on a phenylalanine restricted diet.	Retrospective study to quantify the costs and consequences of managing PKU in the UK. Estimating the cost of diet for life. Patient data used from 390 G.P practices in UK THIN database (The Health Improvement Network) – information entered by G.P's – Study population: – all patients in the database who had at least one PKU read code n=94 ->15 years Mean age 36; 68% female; 85% (n=80)on diet from <1 yr. of age Pts. divided into 4 groups: Group1-patients who had remained on diet 45% (n=36) Group 2-Patients who had discontinued diet and remained off (n=32) Group 3-discontinued diet and restarted (n=12) Group 4-untreated PKU – never on diet (n=14) Data included information on prescriptions, tests, G.P consultations, demographics	-Incidence of neurological symptoms for Gp 2 double that of Gp 1. -11% patients on diet (Gp 1)received >74% of prescribed amount of aa supplement; 55%recieved <25% 9 (based on expected amount used) -number of prescriptions ↑60% between ages 5-10 in Gp1 -neurological/psychiatric symptoms accounted for 22/29% visits to GP for Gp4 compared to ≤14% in other groups -prescriptions for AED –n=290 per pt. for Gp4 compared to n=30 in other groups -mean cost of managing pt. for first 36yrs - £149.374 – Gp1: £21.367 for Gp4 -mean cost - £89,000 -aa supps- main cost-57-63% -LPSF-9-11% -diet estimated to cost £9,500 per year	Estimated 2500 with PKU in the UK – cost to treat with optimum diet - £24m – <0.1% total NHS budget (2008/2009) -small sample size -reflects low incidence of disease -gaps in patient records -hospital costs not considered, costs incurred by patients/families/time off work -well- being/behaviour/QoL not considered -burden PKU imposes on society not estimated - GP role in patient management demonstrated by study -as number of patients received less than opt aa supplements, a need for clear management guidelines for PC -need for a comparative study between the groups to quantify consequences of stopping diet

Date	Author	Title	Aim	Findings	Comments
2013	Eijgelshoven I et al Netherlands	The time consuming nature of phenylketonuria: a cross-sectional study investigating time burden and costs of phenylketonuria in the Netherlands	Cross sectional study via questionnaire Systematic literature review identified aspects of PKU management that imposed a burden on patients and carers 69 participants invited -22 adult patients(median age 28yrs) 15 mild /6 severe PKU -24 caregivers of paed pts. (median age 11 yrs.)-9 mild/12 severe PKU completed questionnaire (67%) Recruited from 7 metabolic centres Pts. aged 0-4 excluded – study joined to existing registry	Median time burden for managing PKU – 265hrs/yr.: 527 hrs.-caregivers/175 hrs. –adults -46% total time burden – dietary management -11%-monitoring protein/phe intake -severe PKU associated with greater time burden $p<0.05$ -pts. / caregivers of with severe PKU had greater OOPC than pts. / caregivers of children with mild PKU $p<0.05/p<0.05$ -aa supps –reimbursed -LPSF – 99% OOPC	Outcome of study demonstrates time burden and costs resulting on having PKU or caring for child with PKU -time 1hr 24mins/day –caregivers / 30 mins adult -higher for severe disease -main OOPC – LP foods Time burden higher for caregivers than adults with PKU reflects relaxing diet in adulthood. Severe PKU more time consuming than mild due to increased monitoring and stricter control. Costs of dietary products reimbursed by govt or health insurance in Europe. Limitations: Small sample size Pts. unwilling to disclose income Age limit
2013	Berry S.A. et al USA	Insurance coverage of medical foods for the treatment of inherited metabolic disorders	To define limitations of insurance coverage of medical foods and feeding equipment -survey of 350 parents of children with IMD	Majority-99%- of families had some sort of insurance cover 41% had out of pocket expenses: -11% families purchasing medical foods -26% purchasing supplements -33% needing medical supplies -59% using modified LP foods Adults had worse coverage than children with IMD Internet used for purchase of medical foods and incurred greater expense	Conditions are identified through national newborn screening programmes and there is improved outcomes in prompt treatment and effective dietary therapy, but medical foods not consistently covered by states leading to inequalities in health care provision and a burden on families Greater awareness needed by health care providers and policy makers to overcome burden on families.

Date	Author	Title	Aim	Findings	Comments
2010	Alaei M Et al Iran	Family Social Status and Dietary Adherence of Patients with Phenylketonuria	Evaluation of family social status and dietary adherence in Iran. Cross-sectional study 105 patients – 46 male/59 female diagnosed by Newborn screening Social status defined by: -number of children in family - number with PKU -parents education (4 groups), employment and marital status Also recorded: age at diagnosis and duration of treatment	44.8% mean plasma phe >above ref range. No association between parents education and mean phe levels Increase in mean rank phe of patients whose parents divorced Increase in phe in patients whose parents unemployed Positive correlation between age of diagnosis and mean phe, treatment duration and number of affected children in family	Younger pts. (<12yr) had better dietary control No relation between parent's education and control Adherence poorer in divorced parents Control not affected by employment status Social status affects adherence Level of dietary knowledge in carers needing investigating.
2004	Walter J et al UK	Blood phenylalanine control in adolescents with phenylketonuria	Multicentre study of control in PKU to determine compliance of adolescent patients with current recommendations Phe levels in children 10-12 yrs. between 1994-02 analysed Pts. diagnosed via NBS -frequency of blood sampling -% phe conc > recommended -mean phe for pts. -difference between males and females 75 patients data collected (42 male)	phe levels increased with age – at 10 years 20% > recommended -at 19 years -75% >recommended Blood sampling fell from 83% to 51% at age 20 yrs. Mean phe greater in males from age 18 yrs. –not sig	During adolescence phe control declines with age Females did not have better control As females need good control during pregnancy, strategies for improving compliance needed.

Date	Author	Title	Aim	Findings	Comments
2006	MacDonald et al UK	Home delivery of dietary products in inherited metabolic disorders reduces prescription and dispensing errors	Aim: to investigate efficacy of a commercial home delivery of special products used in the management of IMD. To investigate if errors in dispensing are reduced. -62 patients with IMD -4 non-English speaking; 7 on complex feeding regimes -study over 12/12 -50 with PKU -12 with other IMD -aged 6/12 – 30 yrs. (2>18yrs) -50% not randomly allocated to subject group (2 older pts. allocated to this group): 50% to control group (pts. with >2 diff company products in control gp)	Control group: 9 pts. had 12 dispensing errors – inappropriate P/S for age: 1 error in subject group -wrong flavour: 11 errors in control group: 1 in subject group -prescription delays -39 incidents with 16 subjects: 1 in subject group Control group: P/S -55% prescribed >90% P/S in 12/12 -24% had <50% P/S -31%-obtaining prescriptions Subject group: -86% prescribed >90% No difference in biochemical control	-no information on division of non-PKU patients between groups -No information on division of non-English speaking pts. between groups -delivery of products does not imply consumption of products-many factors affecting biochemical control-not just receiving product on time -subjects/controls called monthly by phone which may affect compliance with disease control/prescription request. Better education /communication of primary care may have similar benefits to home delivery of products.

Date	Author	Title	Aim	Findings	Comments
2010	Weaver M et al USA	Medical foods: Inborn errors of metabolism and the reimbursement dilemma	Highlights inequalities in payment and reimbursement with the special products needed f or management of IMD	-survey of states' newborn screening representatives -state policies on reimbursement Definition of Medical foods given from Orphan Drug Act -3 scenarios given highlighting burden placed on families and inequalities between states for reimbursement of same condition/treatment -highlights problems faced by adults with IMD in obtaining medical foods	- 4 options proposed: -require legislation to ensure equal reimbursement of medical foods -state model policy -legislation to require third party payers to offer coverage -work with health insurance companies to improve reimbursement policies
1993	Millner B USA	Insurance coverage of special foods needed in the Treatment of Phenylketonuria	Highlights failure / inequalities of private health insurance companies in the payment of essential dietary products for patients identified through national NBS programmes	3 treatment centres in New York surveyed Information obtained on patients with PKU and other IMD -special foods prescribed -assistance provided by specialist centres to help patients find suppliers -how the foods were paid for - 236 patients identified – 213<21 yrs./23 >21yrs -117 had private health insurance -62 Medicaid	Coverage varied between Medicaid and private insurance at the 3 centres and coverage varied between what was paid for by these between centres -cost of special products a major burden to families -staff at centres were required to negotiate between companies and patients -suppliers of products not an issue -varied schemes developed by the centres to help support patients Need for a uniform policy for reimbursement of essential products required in the treatment of conditions identified through national NBS programmes
2010	Macdonald A. et al UK	The reality of dietary compliance in the management of phenylketonuria	Literature review of the challenges in dietary compliance in PKU -assessment of compliance measures -factors associated with compliance – age, health professionals attitude, -strategies to improve compliance	-Education – pts. understanding of condition -Improved communication between patients and HP -simplifying dietary regimen -involvement of family members -promoting self-management -increased access to HP -behavioural approaches	Some patients will not adhere to treatment regardless of input Need to collect data to monitor trends in treatment Need for agreed standards of care and assessment measures between centres Need for evidence for achieving dietary compliance

Appendix 1: Questionnaire on the use of the low protein foods available on prescription

The use of the low protein foods that are available on prescription is part of the treatment of Phenylketonuria.

We would like to investigate the use of these foods and any on-going issues related to their use.

We are particularly interested in getting some information from you on this.

I would be very grateful if you could take time to answer the following questions. In order to get reliable results, it is important to answer all the questions, however if there is a question you do not want to answer, please miss it out and move onto the next question.

We would be grateful for some information on where you live and who you are:

Your postcode: ……..

Are you:

a) A parent of a child on a low protein diet ☐

If so, what is the age of your child with PKU: …… ☐

b) Following a low protein diet ☐

If so, what is your age: ……. ☐

c) A carer of a person on a low protein diet ☐

d) Male / Female (please circle)

1. In the past month could you tell us how much and what type of low protein foods you ordered:

Low protein food	Amount ordered over past month	Did you receive your full order Yes / No	If not- how much did you receive	If you did not receive the full order, why was this	Was this a typical month for this product Yes / no	Comments
Prescription bread – unsliced loaf						
Prescription bread – sliced loaf						
Prescription rolls						
Pizza bases						
Prescription cake mix						
Prescription cakes						
Flour mix						
Low protein food	**Amount ordered over past month**	**Did you receive your full order Yes / no**	**If not- how much did you receive**	**If you did not receive the full order, why was this**	**Was this a typical month for this product Yes / no**	**Comments**
Pasta – spaghetti / noodles						
Pasta shapes						

Pasta Lasagne						
Cous cous						
Rice						
Pasta in sauce						
Snack pot						
Breakfast cereal – flakes / loops						
Hot breakfast						
Low protein food	**Amount ordered over past month**	**Did you receive full order Yes / no**	**If not- how much did you receive**	**If you did not receive the full order, why was this**	**Was this a typical month for this product Yes / no**	**Comments**
Biscuits / crackers						
Burger mix						
Sausage mix						
Dessert mix						
Energy bars E.g. Duobar / Vitabite						
Milk replacement						
Egg / egg white replacer						
Other products						

2. We would also like to find out about any other issues you may have experienced over the past 12 months.

Have you received any comments from any of the following health professionals regarding your prescription?

What member of staff made the comment (please tick all that apply)

G.P ☐

Receptionist ☐

Practice Manager ☐

☐

Pharmacist ☐

☐

Counter assistant ☐

Other (please state who): …….

If possible could you let us know what was said?

How did this make you feel?
(please circle)

More confident confident No different anxious
very anxious

How do you feel about asking for new foods to be added to your prescription list, or trying other foods on the prescription list?

More confident confident No different anxious
very anxious

3. Over the previous month, Could you tell us what condition the low protein food was in when you received it?

Please rate the following:

	Very rarely	**rarely**	**occasionally**	**often**	**always**
Good condition					
Well within date					
Very close to the use by date					
The food was past its use by date					
The food was damaged					
The packaging was damaged					
The food was unfit for use for other reasons (e.g. mouldy)					

4. Do you use the Home Delivery service for low protein foods: yes / no

If not, can you say why:

If yes, has this helped any problems you have had in the past: yes / no

What problem has it helped with?

5. Do you use the low protein flour mix to make your own foods: yes / no

If yes, could you say what you use in a month:

	Number of packets used / month	**Was this a typical month Yes / no**	**Comments**
Homemade bread			
Homemade rolls			
Homemade shaped bread e.g. pitta bread/wraps			
Homemade pastry			
Homemade cakes			

5. Thinking about trying out new recipes, please rate the following:

	Strongly agree	agree	neither agree or disagree	disagree	strongly disagree
I don't like trying new foods in case I don't like them					
I don't like trying new recipes in case they don't work out					
When I make something new, it is not eaten					
I have difficulty getting the foods on pre-scription when I want to try anything new.					
I lack time to try new recipes					
I don't feel confident using food labels to help me decide what foods are suitable					
I never adapt recipes from magazines and websites					

6. In general how would you rate the following prescription foods:

	Very poor	poor	Neither poor or good	Good	Very good
Smell					
Taste					
Texture					
Appearance					
Ease of use					

7. How useful do you find the low protein foods:

Please circle: Useful Neither useful or not useful Not useful

8. We would like to know why you use the low protein foods.

	most important	important	neither important or unimportant	unimportant	very unimportant
To give variety in the diet					
They taste good					
They can generally be used freely					
I have been told to use them					
Because they help satisfy my appetite					
Because they help me to control my PKU					

9. Have you attended any cookery workshop organised by your dietetic department
Yes / No

If yes, has this helped you increase the variety of the low protein foods you use:
Yes / No
Comments:

10. Any other comments about the low protein foods you would like to make:

Thank you very much for taking the time to answer the questions. We hope that the information you have given us will help us to address many of the issues that occur with the prescription of the low protein foods.

Appendix 2: Parent / Guardian Information Sheet (PKU Child)

13032011 Version 2

Parent / Guardian Information Sheet (PKU Child)

Low protein staple foods, nutritional status and disease management in children and adults with phenylketonuria (PKU).

You and your child are invited to take part in a study which evaluates the current dietary management options of children and adults with PKU. We are happy to answer any questions you may have.

What is the purpose of this research study?

The purpose of the study is to find out how the current dietary management options (e.g. low protein staple foods, protein exchanges and protein substitute) can contribute to the management of PKU and other nutritional aspects of people with PKU

Why has my child been chosen to take part in this study?

Your child has been chosen because they have PKU and is under care of the metabolic specialists at Yorkhill Children's Hospital.

Does my child have to take part?

No. If you agree to participate and then change your mind this is absolutely fine. You do not have to give a reason. Your decisions about this will not affect the standard of care you or your child will receive. If you are happy to take part, please sign the enclosed consent form and send it back to us in the pre-addressed post-paid envelope. You will be given a copy of the signed information sheet and consent form to keep for your records.

What does my child have to do if we agree to take part?

If you are happy to participate and you have returned the signed consent form please fill in the enclosed questionnaire which asks you about the foods which your child take regularly in the diet and how often these foods are taken. **Please bring this questionnaire with you at your regular clinical appointment**. During this appointment, we will also measure your child's body composition using special weighing scales and grip strength with a special instrument. None of these tests cause any discomfort. We will also collect extra blood (around ½ tablespoon) to use to check for some other vitamins and minerals not usually checked by your doctor, but don't worry it will not involve any more needles or jags. We will also ask your permission to use any left-over blood collected during your visit to use for these extra tests. In the week following this appointment, we will also ask you to record accurately how much food is eaten and what drinks are taken over a 7 day period by your child. Scales will be provided for this if you do not already have suitable scales. It is very important that your child does not to change their eating habits during their participation in this study.

We will ask your permission to collect some information about your child's condition from your child's medical records and you will be asked to complete a simple questionnaire which will tell us about how the management of the PKU diet can affect your quality of life. You and your child may be seen 2 times by the researcher during the study. The first will be at the hospital during the annual review and the second when you have completed the dietary records. If this requires additional visits to the hospital any of your travel and other expenses will be reimbursed.

Are there any other disadvantages and risks of taking part?

There are no risks and none of the methods in this study causes pain. There may be some inconvenience due to diet record keeping as they may take some time to fill in.

What are the possible benefits of taking part?

Although there are no direct benefits to you or your child from participating in this study, its findings may allow us to evaluate the role and importance of the current dietary management options of PKU.

What if there is a problem?

It is quite unlikely that you will come across a problem by participating in this study. Any complaint about the way you have been dealt with during the study will be addressed. If you have any complaints please contact either the research team directly (0141 201 0163) or contact the Comments/complaints Officer at 0141 201 0000.

Will my or my child's taking part in this research be kept confidential?

Yes, all data collected will be treated in confidence. Only the researcher of this study will be aware of your participation. When we present the findings of this study, we will remove your name and any personal details.

What will happen to the results of the research study?

The results of this study will be published in scientific journals and presented at scientific conferences. If you wish we can send you a report of your results.

Who is organising and funding the research.

The organiser of the research project is Barbara Cochrane (0141 201 0163), Specialist Dietitian as part of her MSc project. If you have any questions you should contact her by telephone. It is funded from a grant obtained from: The PKU research fund at Yorkhill.

Who has reviewed the study?

Before any research goes ahead it has to be checked by an Ethics Committee. This project has been checked by the West of Scotland Ethics Committee.

Thank you for reading this – please ask any questions if you need to

Appendix 3: Information Sheet for young people (Child with PKU)

13032011 Version 2

Information Sheet for young people (Child with PKU)

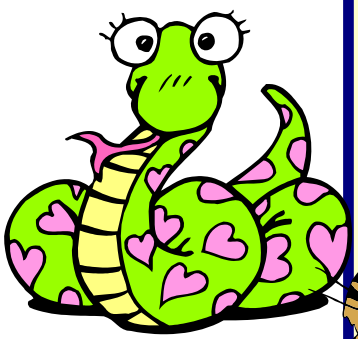
Low protein staple foods, nutritional status and disease management in children and adults with phenylketonuria.

What is it all about?

Hello! We would like you to take part in a research study that will try and find out if the low protein foods that you get from your G.P. help you with your PKU management and diet.

If you and your carer are happy to participate, we would like to look at the following:

- What you eat or drink every day for a week and which are your favourite foods.
- Finding out how much muscle you have in your body and how strong you are by asking you to stand on our special scales and by holding a special instrument. None of these measurements hurt and most of the children find them fun.
- To measure some vitamins in your blood. We will **NOT** want you to have any extra jags. We just ask you for a little extra blood when the doctor needs the blood from the yearly blood test.
- To have a look at your medical notes.
- ask you a few question about the way you with PKU



But first, before the study starts**,** we will ask you if you want to take part and ask you to sign a form with your carer to let us know you are happy to take part.

What if I do not want to take part or change my mind?

You are able to choose not to take part in this study. If you decide not to, we will not change anything about how we look after you at the hospital. This is also the case if you decide to take part, but then change your mind during the study and wish to stop the study.

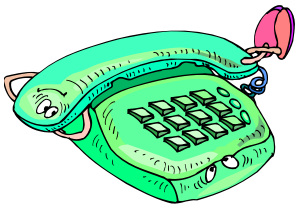
What happens at the end of the study?

The results of this study may help us to look after children with PKU better. We will also present these findings to others. We will remove your name and any of your personal details first.

Will we tell anyone about you taking part in the study?

No, only the researchers and your family doctors will know about your taking part in this study. It will not be possible for anyone to find out your name.

If you have any questions, or if there is anything you do not understand about the study, you can contact Barbara Cochrane, Dietitian, 0141-201-0163.



Appendix 4: Consent form

Study Number:

CONSENT FORM

Low protein staple foods, nutritional status and disease management in children and adults with Phenylketonuria.

Name of Researcher: Please initial box

1. I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that anonymous data which is collected only as part of this evaluation will be looked at and securely stored by responsible individuals. I give permission for these individuals to have access to my records. ☐

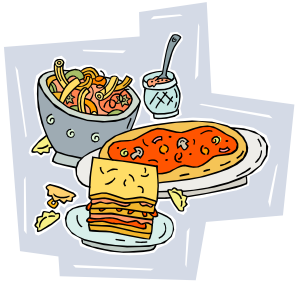
4. I agree to my GP being informed of my participation in the study. ☐

5. I agree to take part in the above study. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

When completed, 1 for carer; 1 for researcher site file; 1 (original) to be kept with case records

Appendix 5: Food Frequency Questionnaire



Food Frequency Questionnaire for People with PKU

We would like to find out how often you are using foods that make up your diet over a typical week. This should include all main meals, snacks and drinks. You should also include any meals such as take – always, in cafes and at relatives' houses. For each food and drink listed there is a portion size offered, if you have more than one of the portions, put extra ticks in the box.

How to complete the questionnaire

For every line on the questionnaire, you should tick one box to say how many times per week you use the food.

- If you do not usually have any of this food, please tick the first box (rarely or never)
- If you have the food or drink **more than once a month**, but less than once per week, please tick the next box (one or two per month)
- If you have the food or drink every **week but not every day**, please tick one of the weekly boxes to tell us how many measures of this food or drink you have in a normal week (1 per week, 2-3 times / week, 4-6 times per week)
- If you have the food or drink **every day**, please tick one of the daily choices (1 per day, 2-3 per day, 4-6 per day)

For dishes that are made up of more than one food you may have to split it up into its separate parts e.g. salad sandwich (2 slices low protein bread, butter, tomatoes, lettuce, mayonnaise)or Lasagne (lasagne sheets, tomatoes, onion, peppers, white sauce)

For a few foods, you may have more than one measure at a time e.g. you may use 2 exchanges of breakfast cereal – use the daily choice to account for this. i.e. 2 exchanges of cereal would be 2-3 per day.

If you have any food or drink that is not listed e.g. Takeaways, or if you are not sure where to put any food or drink, please use the section for "other foods".

Example:

If you have a small bowl of cereal most days, with a carton of milk replacer and the Protein substitute each day – 3 times a day and never eat apples, your form should look like this:

	Portion size	**Rarely or never**	**1-2 per month**	**1 day / week**	**2-3 days / week**	**4-6 days / week**	**Every day**	**… times / day**
Loprofin loops flakes	1 small bowl					X		
Low protein milk replacer	1 carton						X	
Apple	1	X						
PKU Cooler 20	1 pouch						X	3

It is very important that you put a tick on every line.

If you rarely or never have the food, it is very important that you tick the box for rarely or never

Low Protein Foods and Protein Substitutes

Loprofin loops flakes	1 small bowl							
First Play hot breakfast	1 sachet							
Bread								
Low protein sliced loaf / roll	1 slice / 1 roll							
Juvela Pizza base	1 base							
Crackers	1 cracker							
Cookies	1 biscuit							
Wafers	1 wafer							
Rusks	1 rusk							
Pasta								
Shapes – penne, spirals etc.	2 tablespoons -cooked							
Cous cous	2 tablespoons -cooked							
Pasta in sauce	1 sachet							
Biscuits								
Crackers	1 cracker							
Cookies	1 biscuit							

Wafers	1 wafer							
Rusks	1 rusk							
Low protein flour mix	1 x 500g pkt							
Low protein cake mix	1 x 250g pkt							
Low protein rice pudding mix	1 sachet							
Low protein dessert mix	1 sachet							
PK Foods jelly mix	1 sachet							
Low protein milk replace-ment	1 carton							
Low protein burger / sausage mix	1 sachet							
First Play LP Snax	1 pkt							
First Play Chocolate spread	1 teaspoon							
Low protein energy bar	1 bar							
Egg replacer	1 teaspoon							
Protein Substitutes								
PKU Gel	1 sachet							

PKU Anamix Junior	1 sachet							
PKU Cooler 10	1 pouch							
PKU Cooler 15	1 pouch							
PKU Cooler 20	1 pouch							
PKU Lophlex LQ 10	1 pouch							
PKU Lophlex LQ 20	1 pouch							
XP Maxamum	G							
Lophlex Powder	1 sachet							
PKU Express	1 sachet							
Ordinary Foods								
Weetabix	½ biscuit							
Sweetened cereals e.g. Coco pops, Frosties	6 dessert spoons							
Ordinary white or brown bread or rolls	1 slice or 1 roll							
Croissants, garlic bread	1 roll or 2 slices							
Other breads – e.g. Wraps,	1 piece							

tortillas, pitta, Naan								
Low calorie bread e.g. Nimble	1 slice							
Full fat cow's milk	1 small glass or ¼ pint							
Semi-skimmed cow's milk	1 small glass or ¼ pint							
Soya milk	1 small glass or ¼ pint							
Flavoured milk	1 small glass or ¼ pint							
Yoghurt-flavoured	125g pot							
Cheese spread	1 tablespoon							
Cheesley	1 small slice							
Yoghurt drinks	1 small glass or ¼ pint							
Full Fat cream cheese	1 tablespoon							
Cheddar-type cheese	1 small slice or stick							
Cream	1 tablespoon							

Eggs-boiled, fried scrambled or omelette	1 egg							
Yoghurt-plain	1 small pot							
Edam, Brie	1 small slice or stick							
Cheese strings	1 string							
Boiled potatoes	½ medium sized							
Chips	6							
Roast potatoes	1 small							
Baked potato	1 small – size of kiwi							
Potato shapes e.g. smiles	1 shape							
Potato croquette	1							
Chip shop chips	1 small bag							
Fast food chips, e.g. McDonald's, Burger King	1 small portion							
Ordinary boiled rice	3 dessert spoons							
Ordinary pasta	2 tablespoons							
Baked beans	1 dessert spoon							
Tinned spaghetti	1 ½ dessert spoons							

Peas	1 ½ dessert spoons							
Sweetcorn	2 dessert spoons							
Crisps	1 small pkt							
Tinned soup	1 small bowl							
Homemade soup- low protein	1 small bowl							
Mayonnaise	1 teaspoon							
Ketchup / bottled sauces	1 teaspoon							
Jar sauce e.g. for pasta / sweet and sour	½ jar							
Gravy	1 tablespoon							
Carrots – cooked	1 tablespoon							
Onions	1 tablespoon							
Broccoli	2 florets							
Cauliflower	2 florets							
Mushrooms	1 tablespoon							
Sweet potato	1 tablespoon							

Butternut squash	1 tablespoon							
Tomatoes	1 small							
Cucumber	2 slices							
Lettuce	1 leaf							
Swede or turnip	1 tablespoon							
Cabbage	1 tablespoon							
Green beans	1 tablespoon							
Peppers	¼ pepper							
Coleslaw	1 tablespoon							
Apple	1							
Orange	1							
Banana	1							
Grapes, melon, pear	1 small serving							
Strawberries	6							
Dried fruit	1 tablespoon							
Tinned fruit	1 tablespoon							
Pure fruit juice	1 small glass							
Diluting juice – sugar free	1 glass – made up							
Diluting juice – ordinary	1 small glass – made up							

Children's juice – Capri sun/ fruit shoots	1 bottle / pouch							
Fizzy drinks -sugar free	1 can / small bottle							
Fizzy drinks -with sugar	1 can / small bottle							
Sports drinks / Lucozade	1 bottle							
Tea	1 cup							
Coffee	1 cup							
Wine	1 glass							
Spirits	1 measure							
Beer / larger	1 can / bottle							
Alcopops	1 bottle							
Cider	1 glass							
Water	1 glass							
Jam / marmalade	1 teaspoon							
Honey	1 teaspoon							
Chocolate spread	1 teaspoon							
Butter / margarine	1 teaspoon							
Sugar	1 teaspoon							

Popcorn	1 small mug							
Breadsticks	1							
Ready to eat jelly	1 tub							
Chocolate e.g. fudge	1 bar							
Jelly sweets e.g. Haribo	1 bag							
Chewy sweets e.g. chewits / skittles	1 bag							
Ice cream / sorbet	1 scoop							
Ice lolly	1							
Meat burgers	1 small burger							
Mince	1 tablespoon							
Sausages	1 sausage							
Bacon	1 rasher							
Cold meat	1 slice							
Stewed, fried, grilled or roast beef, pork or lamb	1 tablespoon							
Chicken nuggets	1 serving							
Meat or chicken pies, or sausage rolls	1 individual pie or 1 roll							
Casseroled fried or grilled	1 tablespoon or 1							

turkey or chicken	slice							
Fish	1 small fillet or 1 serving							
Fish cakes or fish pie	1 fish cake or 1 serving							
Grilled or poached white fish	1 fillet							
White fish cooked in batter or breadcrumbs	1 serving							
Smoked oily fish – kipper, mackerel or salmon	1 small fillet							
Grilled oily fish (fresh tuna, salmon, mackerel or herring)	1 small fillet							
Tinned tuna	1 tablespoon							
Prawns	1 tablespoon							
Other foods not included, e.g. take-aways – Please specify								

Thank you so much for completing this questionnaire. Please bring it with you at you next clinical appointment and hand it in to Barbara or your metabolic dietitian

Appendix 6: **Information leaflet**

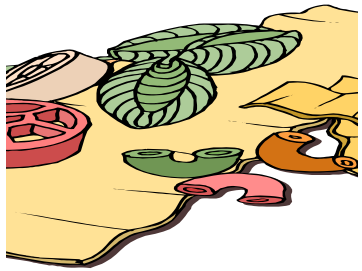
Dear (Parent)

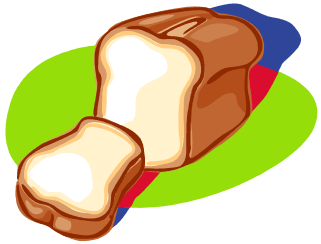
Participation in the study is entirely voluntary. If you do not wish to take part or want to withdraw at a later stage, the standard of care you receive will not be affected.

This study is taking place along with researchers from the University of Glasgow.

Information leaflet

How important are low protein foods to you?





The importance of low protein specialist foods

Phenylketonuria (PKU) is one of the more common inherited disorders diagnosed through the newborn screening programme.

Around 6-8 babies are diagnosed with the disorder in Scotland each year.

The use of the low protein diet in the treatment of Phenylketonuria (PKU) is well recognized as being successful in preventing the harm to the developing brain that may be caused by high phenylalanine levels.

Part of that treatment for both adults and children, with PKU, is the use of specially manufactured low protein foods such as bread, pasta and milk. However, although these foods look different and taste differently from the ordinary products, they are needed to provide energy and help fill people up.

Because the foods have to be provided on prescription from your General Practitioner, sometimes there is difficulty with getting what you need at the time you need them.

Some of the foods also are difficult to use and this can be off putting as they do not always turn out as planned.

This study is to find out how the foods are used, what problems and concerns people have about using them and also try to find out how much of the foods are needed to provide sufficient energy for growth and to prevent excess use of the foods that have to be restricted (exchange) foods.

At present, there have been few, if any studies looking at the contribution the low protein foods make to the diet.

What is involved in the study?

What we would like to find out is what type of foods are eaten, how much and how often they are taken by the use of a weighed food diary, a questionnaire to ask about what type of low protein foods are eaten and the difficulties you may experience and also a food frequency questionnaire which tells us what are the most common foods eaten.

All of these will help us find out the contribution the foods make to the overall nutritional dietary intake.

This will help to tell us the type of foods you like using and what difficulties you may come across when trying to obtain sufficient supplies of the foods or in trying new foods. This in turn will help us to help you manage the diet well.

In addition to the questionnaires and food diary, we would like to compare the results to the regular blood tests you do and the annual blood test taken when you come to the clinic. We will not ask for any extra blood tests.

We are also interested in comparing the diet for PKU with the dietary intake of people without PKU to give us an idea of how the PKU diet compares in energy to the diet of the general population. We might ask if other members of your family would be interested in filling in a food diary at the same time.

During your visit to the out-patient clinic you will be asked if you would like to take part in the study. One of the benefits might enable us to help you and your G.P decide how much of the special foods you need to keep you healthy.

Bibliography

“Treatment of Phenylketonuria. Report to the Medical Research Council of the conference on Phenylketonuria”. *Br Med J. 1963 June 29;1(5347):1691-7.*

"Recommendations on the dietary management of phenylketonuria. Report of Medical Research Council Working Party on Phenylketonuria.", 1993, *Archives of Disease in Childhood*, vol. 68, no. 3, pp. 426-427.

Acosta, P., Fernhoff, P., Warshaw, H., Elsas, L., Hambidge, K., Ernest, A. & McCabe, E. 1982, "Zinc status and growth of children undergoing treatment for phenylketonuria", *Journal of Inherited Metabolic Disease*, vol. 5, no. 2, pp. 107-110.

Acosta, P.B. & Yannicelli, S. 1994, "Protein intake affects phenylalanine requirements and growth of infants with phenylketonuria", *Acta paediatrica (Oslo, Norway : 1992).Supplement*, vol. 407, pp. 66-67.

Acosta, P.B., Yannicelli, S., Singh, R., Mofidi, S., Steiner, R., DeVincentis, E., Jurecki, E., Bernstein, L., Gleason, S., Chetty, M. & Rouse, B. 2003, "Nutrient intakes and physical growth of children with phenylketonuria undergoing nutrition therapy", *Journal of the American Dietetic Association*, vol. 103, no. 9, pp. 1167-1173.

Alaei, M., Asadzadeh-Totonchi, G., Gachkar, L. & Farivar, S. 2011, "Family Social Status and Dietary Adherence of Patients with Phenylketonuria", *Iranian journal of pediatrics*, vol. 21, no. 3.

Allan, J. & Brown, J. 1968, "Maternal phenylketonuria and foetal brain damage. An attempt at prevention by dietary control", *Some Recent Advances in Inborn Errors of Metabolism. Edinburgh, Scotland: E & S Livingstone*, pp. 14-35.

Allen, R.J. 1960, "The detection and diagnosis of phenylketonuria", *American Journal of Public Health and the Nation's Health*, vol. 50, pp. 1662-1666.

Armstrong, M.D. & Tyler, F.H. 1955a, "Studies on phenylketonuria. I. Restricted phenylalanine intake in phenylketonuria", *The Journal of Clinical Investigation*, vol. 34, no. 4, pp. 565-580.

Armstrong, M.D. & Tyler, F.H. 1955b, "Studies on phenylketonuria. I. Restricted phenylalanine intake in phenylketonuria", *The Journal of clinical investigation,* vol. 34, no. 4, pp. 565-580.

Barretto, J.R., Silva, L.R., Leite, M.E., Boa-Sorte, N., Pimentel, H., Purificacao, A.C., Carvalho, G., Fontes, M.I. & Amorim, T. 2008, "Poor zinc and selenium status in phenylketonuric children and adolescents in Brazil", *Nutrition research (New York, N.Y.),* vol. 28, no. 3, pp. 208-211.

Bélanger-Quintana, A., Burlina, A., Harding, C.O. & Muntau, A.C. 2011, "Up to date knowledge on different treatment strategies for phenylketonuria", *Molecular Genetics and Metabolism,* vol. 104, pp. S19-S25.

Bentovim, A., Clayton, B.E., Francis, D.E., Shepherd, J. & Wolff, O.H. 1970, "Use of an amino acid mixture in treatment of phenylketonuria", *Archives of Disease in Childhood,* vol. 45, no. 243, pp. 640-650.

Berry, S.A., Brown, C., Grant, M., Greene, C.L., Jurecki, E., Koch, J., Moseley, K., Suter, R., van Calcar, S.C. & Wiles, J. 2013, "Newborn screening 50 years later: access issues faced by adults with PKU", *GeNetics in mediciNe,* vol. 15, no. 8, pp. 591-599.

Bik-Multanowski, M., Didycz, B., Mozrzymas, R., Nowacka, M., Kaluzny, L., Cichy, W., Schneiberg, B., Amilkiewicz, J., Bilar, A. & Gizewska, M. 2008, "Quality of life in non-compliant adults with phenylketonuria after resumption of the diet", *Journal of Inherited Metabolic Disease,* vol. 31, no. 2, pp. 415-418.

Blainey, J.D. & Gulliford, R. 1956, "Phenylalanine-restricted diets in the treatment of phenylketonuria", *Archives of Disease in Childhood,* vol. 31, no. 160, pp. 452-466.

Blau, N., Hennermann, J.B., Langenbeck, U. & Lichter-Konecki, U. 2011, "Diagnosis, classification, and genetics of phenylketonuria and tetrahydrobiopterin (BH4) deficiencies", *Molecular Genetics and Metabolism,* vol. 104, pp. S2-S9.

Blau, N., van Spronsen, F.J. & Levy, H.L. 2010, "Phenylketonuria", *Lancet,* vol. 376, no. 9750, pp. 1417-1427.

Borus, J.S. & Laffel, L. 2010, "Adherence challenges in the management of type 1 diabetes in adolescents: prevention and intervention", *Current opinion in pediatrics,* vol. 22, no. 4, pp. 405-411.

Bregnballe, V., Schiotz, P.O., Boisen, K.A., Pressler, T. & Thastum, M. 2011, "Barriers to adherence in adolescents and young adults with cystic fibrosis: a questionnaire study in young patients and their parents", *Patient preference and adherence,* vol. 5, pp. 507-515.

Brimblecombe, F.S., Blainey, J.D., Stoneman, M.E. & Wood, B.S. 1961a, "Dietary and Biochemical Control of Phenylketonuria", *British Medical Journal,* vol. 2, no. 5255, pp. 793-798.

Brimblecombe, F.S., Blainey, J.D., Stoneman, M.E. & Wood, B.S. 1961b, "Dietary and Biochemical Control of Phenylketonuria", *British Medical Journal,* vol. 2, no. 5255, pp. 793-798.

Burrage, L.C., McConnell, J., Haesler, R., O'Riordan, M.A., Sutton, V.R., Kerr, D.S. & McCandless, S.E. 2012, "High prevalence of overweight and obesity in females with phenylketonuria", *Molecular Genetics and Metabolism,* vol. 107, no. 1, pp. 43-48.

Camp, K.M., Lloyd-Puryear, M.A. & Huntington, K.L. 2012, "Nutritional treatment for inborn errors of metabolism: indications, regulations, and availability of medical foods and dietary supplements using phenylketonuria as an example", *Molecular Genetics and Metabolism,* vol. 107, no. 1, pp. 3-9.

Camp KM, Parisi MA, Acosta PB, Berry GT, Bilder DA, Blau N, Bodamer OA, Brosco JP, Brown CS, Burlina AB, Burton BK, Chang CS, Coates PM, Cunningham AC, Do browolski SF, Ferguson JH, Franklin TD, Frazier DM, Grange DK, Greene CL, Groft SC, Harding CO, Howell RR, Huntington KL, Hyatt-Knorr HD, Jevaji IP, Levy HL, Lichter-Konecki U, Lindegren ML, Lloyd-Puryear MA, Matalon K, MacDonald A, McPheeters ML, Mitchell JJ, Mofidi S, Moseley KD, Mueller CM, Mulberg AE, Ne rurkar LS, Ogata BN, Pariser AR, Prasad S, Pridjian G, Rasmussen SA, Reddy UM, Rohr FJ, Singh RH, Sirrs SM, Stremer SE, Tagle DA, Thompson SM, Urv TK, Utz JR, van Spronsen F, Vockley J, Waisbren SE, Weglicki LS, White DA, Whitley CB, Wilfond BS, Yannicelli S, Young JM.

"Phenylketonuria Scientific Review Conference: State of the science and future re search needs", *Molecular genetics and metabolism,* Jun;112(2):87-122.

Centerwall, S.A. & Centerwall, W.R. 1969, "Phenylketonuria and phenylalanine diet", *The Journal of Pediatrics,* vol. 74, no. 6, pp. 1008-1009.

Centerwall, W.R., Centerwall, S.A., Acosta, P.B. & Chinnock, R.F. 1961, "Phenylketonuria. I. Dietary management of infants and young children", *The Journal of Pediatrics,* vol. 59, no. 1, pp. 93-101.

Clayton, B.E., Heeley, A. & Heeley, M. 1970, "An investigation of the hyperaminoaciduria in phenylketonuria associated with the feeding of certain commercial low-phenylalanine preparations", *British Journal of Nutrition,* vol. 24, no. 02, pp. 573-580.

Clayton, B., Francis, D. & Moncrieff, A. 1965, "A Method for Feeding the Phenylketonuric Infant", *British MedicalJjournal,* vol. 1, no. 5426, pp. 54.

Clayton, B., Moncrieff, A. & Roberts, G.E. 1967, "Dietetic treatment of phenylketonuria: a follow-up study", *British Medical Journal,* vol. 3, no. 5558, pp. 133-136.

Cochrane, B., Schwahn, B., Galloway, P., Robinson, P. & Gerasimidis, K. 2013, "A questionnaire survey on the usage of low protein staple foods by people with phenylketonuria in Scotland", *Journal of Human Nutrition and Dietetics,.*

Fidika, A., Salewski, C. & Goldbeck, L. 2013, "Quality of life among parents of children with phenylketonuria (PKU)", *Health and quality of life outcomes,* vol. 11, pp. 54-7525-11-54.

Fisberg, R.M., Da Silva-Femandes, M.E., Fisberg, M. & Schmidt, B.J. 1999a, "Plasma zinc, copper, and erythrocyte superoxide dismutase in children with phenylketonuria", *Nutrition (Burbank, Los Angeles County, Calif.),* vol. 15, no. 6, pp. 449-452.

Fisberg, R.M., da Silva-Fernandes, M.E., Schmidt, B.J. & Fisberg, M. 1999b, "Nutritional evaluation of children with phenylketonuria", *Sao Paulo medical journal = Revista paulista de medicina,* vol. 117, no. 5, pp. 185-191.

Fisch, R.O., Walker, W.A. & Anderson, J.A. 1966, "Prenatal and postnatal developmental consequences of maternal phenylketonuria", *Pediatrics,* vol. 37, no. 6, pp. 979-986.

Følling, I. 1994, "The discovery of phenylketonuria", *Acta Paediatrica,* vol. 83, no. s407, pp. 4-10.

Fredericks, E.M. & Dore-Stites, D. 2010, "Adherence to immunosuppressants: how can it be improved in adolescent organ transplant recipients?", *Current opinion in organ transplantation,* vol. 15, no. 5, pp. 614-620.

Gassió, R., Artuch, R., Vilaseca, M.A., Fusté, E., Colome, R. & Campistol, J. 2008, "Cognitive functions and the antioxidant system in phenylketonuric patients.", *Neuropsychology,* vol. 22, no. 4, pp. 426.

Gentile, J., Ten Hoedt, A. & Bosch, A. 2010, "Psychosocial aspects of PKU: hidden disabilities–a review", *Molecular Genetics and Metabolism,* vol. 99, pp. S64-S67.

Gibbs, N.K. & Woolf, L.I. 1959, "Tests for phenylketonuria: results of a one-year programme for its detection in infancy and among mental defectives", *British Medical Journal,* vol. 2, no. 5151, pp. 532-535.

Gokmen-Ozel, H., Daly, A., Davies, P., MacDonald, A., 2012, "The development of non-weighed pilot method for estimating phenylalanine exchanges in non-phenylketonuria volunteers", *Journal of Human Nutrition and Dietetics*, vol 25, pp. 27-32.

Gokmen-Ozel, H., Ferguson, C., Evans, S., Daly, A. & MacDonald, A. 2011, "Does a lower carbohydrate protein substitute impact on blood phenylalanine control, growth and appetite in children with PKU?", *Molecular Genetics and Metabolism,* vol. 104, pp. S64-S67.

Gokmen-Ozel, H., MacDonald, A., Daly, A., Hall, K., Ryder, L. & Chakrapani, A. 2009, "Long-term efficacy of 'ready-to-drink' protein substitute in phenylketonuria", *Journal of Human Nutrition and Dietetics,* vol. 22, no. 5, pp. 422-427.

Gray, L. & Leyland, A. 2011, "Adult and child obesity", *The 2010 Scottish Health Survey – Volume 1: Main Report*. Edinburgh, Scottish Government. www.scotland.gov.uk/Publications/2011/09/27084018/51.

Greeves, L., Carson, D., Craig, B. & McMaster, D. 1990, "Potentially Life-Threatening Cardiac Dysrhythmia in a Child with Selenium Deficiency and Phenylketonuria", *Acta Paediatrica,* vol. 79, no. 12, pp. 1259-1262.

Greeves, L., Thomas, P. & Carson, D. 1995, "Radiological assessment of the hand and wrist in phenylketonuria and hyperphenylalaninaemia", *Pediatric Radiology,* vol. 25, no. 5, pp. 353-355.

Griffiths, P., Campbell, R. & Robinson, P. 1998, "Executive function in treated phenylketonuria as measured by the one-back and two-back versions of the continuous performance test", *Journal of Inherited Metabolic Disease*, vol. 21, no. 2, pp. 125-135.

Guest, J., Bai, J., Taylor, R., Sladkevicius, E., Lee, P. & Lachmann, R. 2013, "Costs and outcomes over 36 years of patients with phenylketonuria who do and do not remain on a phenylalanine-restricted diet", *Journal of Intellectual Disability Research,* vol. 57, no. 6, pp. 567-579.

Guldberg, P., Rey, F., Zschocke, J., Romano, V., François, B., Michiels, L., Ullrich, K., Hoffmann, G.F., Burgard, P. & Schmidt, H. 1998, "A European multicenter study of phenylalanine hydroxylase deficiency: classification of 105 mutations and a general system for genotype-based prediction of metabolic phenotype", *The American Journal of Human Genetics*, vol. 63, no. 1, pp. 71-79.

Guthrie, R. & Susi, A. 1963, "A Simple Phenylalanine Method for Detecting Phenylketonuria in Large Populations of Newborn Infants", *Pediatrics,* vol. 32, pp. 338-343.

Hanley, W.B,. Feigenbaum, A.S.J., Clarke, J.T.R., Schoonheyt, W.E., Austin, V.J,. 1996, "Vitamin B12 deficiency in adolescents and young adults with phenylketonuria", *European Journal of Paediatrics*, vol.155, pp. s145-s147.

Hardelid, P., Cortina-Borja, M., Munro, A., Jones, H., Cleary, M., Champion, M., Foo, Y., Scriver, C. & Dezateux, C. 2008, "The Birth Prevalence of PKU in Populations of European, South Asian and Sub-Saharan African Ancestry Living in South East England", *Annals of Human Genetics*, vol. 72, no. 1, pp. 65-71.

Huemer, M., Huemer, C., Möslinger, D., Huter, D. & Stöckler-Ipsiroglu, S. 2007, "Growth and body composition in children with classical phenylketonuria: results in 34 patients and review of the literature", *Journal of Inherited Metabolic Disease,* vol. 30, no. 5, pp. 694-699.

Klesges, R.C., Eck, L.H. & Ray, J.W. 1995, "Who underreports dietary intake in a dietary recall? Evidence from the Second National Health and Nutrition Examination Survey.", *Journal of Consulting and Clinical Psychology,* vol. 63, no. 3, pp. 438.

Lee, P., Smith, I., Piesowicz, A. & Brenton, D. 1999, "Spastic paraparesis after anaesthesia", *Lancet,* vol. 353, no. 9152, pp. 554.

Lee, P.J., Ridout, D., Walter, J.H. & Cockburn, F. 2005, "Maternal phenylketonuria: report from the United Kingdom Registry 1978-97", *Archives of Disease in Childhood,* vol. 90, no. 2, pp. 143-146.

Lenke, R.R. & Levy, H.L. 1980, "Maternal phenylketonuria and hyperphenylalaninemia. An international survey of the outcome of untreated and treated pregnancies", *The New England Journal of Medicine,* vol. 303, no. 21, pp. 1202-1208.

Leuzzi, V., Pansini, M., Sechi, E., Chiarotti, F., Carducci, C., Levi, G. & Antonozzi, I. 2004, "Executive function impairment in early-treated PKU subjects with normal mental development", *Journal of Inherited Metabolic Disease,* vol. 27, no. 2, pp. 115-125.

Levy, H.L. & Waisbren, S.E. 1994, "PKU in adolescents: rationale and psychosocial factors in diet continuation", *ActaPpaediatrica (Oslo, Norway: 1992).Supplement,* vol. 407, pp. 92-97.

Lord, B., Ungerer, J. & Wastell, C. 2008, "Implications of resolving the diagnosis of PKU for parents and children", *Journal of Pediatric Psychology,* vol. 33, no. 8, pp. 855-866.

Mabry, C.C., Denniston, J.C. & Coldweu, J.G. 1966, "Mental retardation in children of phenylketonuric mothers", *New England Journal of Medicine,* vol. 275, no. 24, pp. 1331-1336.

MacDonald, A., Rylance, G., Asplin, D., Hall, K., Harris, G. & Booth, I. 1994, "Feeding problems in young PKU children", *Acta Paediatrica,* vol. 83, no. s407, pp. 73-74.

MacDonald, A. 2000, "Diet and compliance in phenylketonuria", *European Journal of Pediatrics*, vol. 159 Suppl 2, pp. S136-41.

MacDonald, A., Chakrapani, A., Hendriksz, C., Daly, A., Davies, P., Asplin, D., Hall, K. & Booth, I.W. 2006, "Protein substitute dosage in PKU: how much do young patients need?", *Archives of Disease in Childhood*, vol. 91, no. 7, pp. 588-593.

Macdonald, A., Daly, A., Davies, P., Asplin, D., Hall, S.K., Rylance, G. & Chakrapani, A. 2004, "Protein substitutes for PKU: what's new?", *Journal of Inherited Metabolic Disease*, vol. 27, no. 3, pp. 363-371.

MacDonald, A., Ferguson, C., Rylance, G., Morris, A.A., Asplin, D., Hall, S.K. & Booth, I.W. 2003, "Are tablets a practical source of protein substitute in phenylketonuria?", *Archives of Disease in Childhood*, vol. 88, no. 4, pp. 327-329.

MacDonald, A., Gokmen-Ozel, H., van Rijn, M. & Burgard, P. 2010, "The reality of dietary compliance in the management of phenylketonuria", *Journal of Inherited Metabolic Disease*, vol. 33, no. 6, pp. 665-670.

MacDonald, A., van Rijn,M,. Feillet, F., Lund, A.M., Berstein, L., Bosch, A.M., Gizewska, M., van Spronsen, F.J., 2012, "Adherence issues in inherited metabolic disorders treated by low natural protein diets", *Annuls Nutrition and metabolism*, vol 61, pp. 289-295.

MacDonald, A., Lee, P., Davies, P., Daly, A., Lilburn, M., Gokmen Ozel, H., Preece, M.A., Hendriksz, C. & Chakrapani, A. 2008, "Long-term compliance with a novel vitamin and mineral supplement in older people with PKU", *Journal of Inherited Metabolic Disease*, vol. 31, no. 6, pp. 718-723.

MacDonald, A., Lilburn, M., Cochrane, B., Davies, P., Daly, A., Asplin, D., Hall, S.K., Cousins, A., Chakrapani, A., Robinson, P. & Lee, P. 2004, "A new, low-volume protein substitute for teenagers and adults with phenylketonuria", *Journal of Inherited Metabolic Disease*, vol. 27, no. 2, pp. 127-135.

MacDonald, A., Lilburn, M., Davies, P., Evans, S., Daly, A., Hall, S.K., Hendriksz, C., Chakrapani, A. & Lee, P. 2006, "'Ready to drink' protein substitute is easier is for people

with phenylketonuria", *Journal of Inherited Metabolic Disease,* vol. 29, no. 4, pp. 526-531.

MacDonald, A., Rylance, G., Davies, P., Asplin, D., Hall, S.K. & Booth, I.W. 2003, "Administration of protein substitute and quality of control in phenylketonuria: a randomized study", *Journal of Inherited Metabolic Disease,* vol. 26, no. 4, pp. 319-326.

MacDonald, A., Davies, P., Daly, A., Hopkins, V., Hall, S.K., Asplin, D., Hendriksz, C. & Chakrapani, A. 2008, "Does maternal knowledge and parent education affect blood phenylalanine control in phenylketonuria?", *Journal of Human Nutrition and Dietetics, vol 21, pp 351-358.*

McBean, M.S. & Stephenson, J.B. 1968, "Treatment of classical phenylketonuria", *Archives of Disease in Childhood,* vol. 43, no. 227, pp. 1-7.

Mirás, A., Bóveda, M.D., Leis, M.R., Mera, A., Aldámiz-Echevarría, L., Fernández-Lorenzo, J.R., Fraga, J.M. & Couce, M.L. 2013, "Risk factors for developing mineral bone disease in phenylketonuric patients", *Molecular Genetics and Metabolism,* vol. 108, no. 3, pp. 149-154.

Mitchell, J.J., Trakadis, Y.J. & Scriver, C.R. 2011, "Phenylalanine hydroxylase deficiency", *Genetics in Medicine,* vol. 13, no. 8, pp. 697-707.

Moncrieff, A. & Wilkinson, R.H. 1961, "Further experiences in the treatment of phenylketonuria", *British Medical Journal,* vol. 1, no. 5228, pp. 763-767.

National Institutes of Health Consensus Development Panel, 2000, " National Institutes of Health Consensus Development Conference Statement: Phenylketonuria Screening and Management October 16-18", *Paediatrics,* vol. 108, no 4. pp. 972-982.

Ortner Hadžiabdic, M., Mucalo, L., Hrabac, P., Matic, T., Rahelic, D., Božikov, V., 2014, "*Journal of Human Nutrition and Dietetics", Sept 13. [Epub ahead of print].*

Pollitt, R. 2012, "Commentary: What degree of hyperphenylalaninaemia requires treatment?", *Journal of Inherited Metabolic Disease,* vol. 35, no. 5, pp. 927-930.

Rennie, K.L., Coward, A. & Jebb, S.A. 2007, "Estimating under-reporting of energy intake in dietary surveys using an individualised method", *British Journal of Nutrition*, vol. 97, no. 06, pp. 1169-1176.

Robert, M., Rocha, J., van Rijn, M., Ahring, K., Bélanger-Quintana, A., MacDonald, A., Dokoupil, K., Gokmen Ozel, H., Lammardo, A. & Goyens, P. 2013, "Micronutrient status in phenylketonuria", *Molecular Genetics and Metabolism*, vol. 110, pp. S6-S17.

Robertson, L., McStravick, N., Ripley, S., Weetch, E., Donald, S., Adam, S., Micciche, A., Boocock, S. & MacDonald, A. 2013, "Body mass index in adult patients with diet-treated phenylketonuria", *Journal of Human Nutrition and Dietetics*, vol. 26, no. s1, pp. 1-6.

Robinson, M., White, F.J., Cleary, M.A., Wraith, E., Lam, W.K. & Walter, J.H. 2000a, "Increased risk of vitamin B12 deficiency in patients with phenylketonuria on an unrestricted or relaxed diet", *The Journal of Pediatrics*, vol. 136, no. 4, pp. 545-547.

Robinson, M., White, F.J., Cleary, M.A., Wraith, E., Lam, W.K. & Walter, J.H. 2000b, "Increased risk of vitamin B12 deficiency in patients with phenylketonuria on an unrestricted or relaxed diet", *The Journal of Pediatrics*, vol. 136, no. 4, pp. 545-547.

Rocha, J.C., van Spronsen, F.J., Almeida, M.F., Ramos, E., Guimarães, J.T., Borges, N., 2013, "Early dietary treated patients with phenylketonuria can achieve normal growth and body composition", *Molecular Genetics and Metabolism*, Vol 110 Suppl: S40 - 3

Rocha, J.C., Macdonald, A., Trefz, F., 2013, "Is overweight an issue in phenylketonuria?", *Molecular Genetics and Metabolism*, Vol. 110, Suppl: S18-24.

Rohr, F., Munier, A. & Levy, H. 2001a, "Acceptability of a new modular protein substitute for the dietary treatment of phenylketonuria", *Journal of Inherited Metabolic Disease*, vol. 24, no. 6, pp. 623-630.

Rohr, F.J., Munier, A.W. & Levy, H.L. 2001b, "Acceptability of a new modular protein substitute for the dietary treatment of phenylketonuria", *Journal of Inherited Metabolic Disease*, vol. 24, no. 6, pp. 623-630.

Schweitzer-Krantz, S. & Burgard, P. 2000, "Survey of national guidelines for the treatment of phenylketonuria", *European Journal of Pediatrics,* vol. 159, no. 2, pp. S70-S73.

Sharman, R., Mulgrew, K., Katsikitis, M., 2013, "Qualitative analysis of factors affecting adherence to the phenylketonuria diet in adolescents", *Clinical Nurse Specialist,* vol 27 pp. 205-210.

Smith, I., Cockburn, F., Barwell, B., Brenton, D., Chapple, J., Clark, B., Curzon, G., Davidson, D., Heeley, A. & Laing, S. 1993, "Recommendations on the dietary management of phenylketonuria", *Archieve of Disease in Childhood,* vol. 68, no. 3, pp. 426-427.

Smith, I., Beasley, M.G. & Ades, A.E. 1991, "Effect on intelligence of relaxing the low phenylalanine diet in phenylketonuria", *Archives of Disease in Childhood,* vol. 66, no. 3, pp. 311-316.

Smith, I., Beasley, M.G. & Ades, A.E. 1990, "Intelligence and quality of dietary treatment in phenylketonuria", *Archives of Disease in Childhood,* vol. 65, no. 5, pp. 472-478.

Smith, I., Francis, D.E., Clayton, B.E. & Wolff, O.H. 1975, "Comparison of an amino acid mixture and protein hydrolysates in treatment of infants with phenylketonuria", *Archives of Disease in Childhood,* vol. 50, no. 11, pp. 864-870.

Stevenson, R.E. & Huntley, C.C. 1967, "Congenital malformations in offspring of phenylketonuric mothers", *Pediatrics,* vol. 40, no. 1, pp. 33-45.

ten Hoedt, A.E., Maurice-Stam, H., Boelen, C.C., Rubio-Gozalbo, M.E., van Spronsen, F.J., Wijburg, F.A., Bosch, A.M., Grootenhuis, M.A., 2011, "Parenting a child with phenylketonuria or galactosaemia: implications for health-related quality of life", *Journal of Inherited Metabolic Disease, vol. 34, pp. 391-398.*

Thiele, A,G., Weigel, J.F., Ziesch, B., Rohde. C., Mutze. U., Ceglarek. U.,Thiery. J., Müller. A.S., Kiess. W., Beblo. S. 2012, "Nutritional changes and micronutrient supply in patients with phenylketonuria under therapy with tetrahydrobiopterin (BH_4)", *Journal of Inherited Metabolic Disease, DOI 10 1007/8904_2012_176*

Trabulsi, J. & Schoeller, D.A. 2001, "Evaluation of dietary assessment instruments against doubly labeled water, a biomarker of habitual energy intake", *American journal of physiology.Endocrinology and Metabolism,* vol. 281, no. 5, pp. E891-9.

Tsiountsioura, M., Wong, J., Upton, J., McIntyre, K., Dimakou, D., Buchanan, E., Cardigan, T., Flynn, D., Bishop, J. & Russell, R. 2014, "Detailed assessment of nutritional status and eating patterns in children with gastrointestinal diseases attending an outpatients clinic and contemporary healthy controls", *European Journal of Clinical Nutrition,* .

van Bakel, M.M., Printzen, G., Wermuth, B. & Wiesmann, U.N. 2000, "Antioxidant and thyroid hormone status in selenium-deficient phenylketonuric and hyperphenylalaninemic patients", *The American Journal of Clinical Nutrition,* vol. 72, no. 4, pp. 976-981.

van Spronsen, F.V., Verkerk, P., Houten, M.V., Smit, G., Meer, S.V.D., Bakker, H. & Sengers, R. 1997, "Does impaired growth of PKU patients correlate with the strictness of dietary treatment?", *Acta Paediatrica,* vol. 86, no. 8, pp. 816-818.

van Spronsen, F., Ahring, K.K. & Gizewska, M. 2009, "PKU—What is daily practice in various centres in Europe?", *Journal of Inherited Metabolic Disease,* vol. 32, no. 1, pp. 58-64.

Vegni, E., Fiori, L., Riva, E., Giovannini, M. & Moja, E. 2010, "How individuals with phenylketonuria experience their illness: an age-related qualitative study", *Child: care, health and development,* vol. 36, no. 4, pp. 539-548.

Vugteveen, I., Hoeksma, M., Monsen, A.L., Fokkema, M.R., Reijngoud, D.J., van Rijn, M. & van Spronsen, F.J. 2011, "Serum vitamin B12 concentrations within reference values do not exclude functional vitamin B12 deficiency in PKU patients of various ages", *Molecular Genetics and Metabolism,* vol. 102, no. 1, pp. 13-17.

Waisbren, S.E., Noel, K., Fahrbach, K., Cella, C., Frame, D., Dorenbaum, A. & Levy, H. 2007, "Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis", *Molecular Genetics and Metabolism,* vol. 92, no. 1-2, pp. 63-70.

Walter, J.H. 2011, "Vitamin B12 deficiency and phenylketonuria", *Molecular Genetics and Metabolism,* vol. 104, pp. S52-S54.

Walter, J.H. & White, F.J. 2004, "Blood phenylalanine control in adolescents with phenylketonuria", *International Journal of Adolescent Medicine and Health,* vol. 16, no. 1, pp. 41-45.

Weglage, J., Flinders, B., Ullrich, K., Rupp, A. & Schmidt, E. 1996, "Psychosocial aspects in phenylketonuria", *European Journal of Pediatrics,* vol. 155, no. 1, pp. S101-S104.

Williams, R.A., Mamotte, C.D. & Burnett, J.R. 2008, "Phenylketonuria: an inborn error of phenylalanine metabolism", *The Clinical biochemist.Reviews / Australian Association of Clinical Biochemists,* vol. 29, no. 1, pp. 31-41.

Wilson, K.M. & Clayton, B.E. 1962, "Importance of choline during growth, with particular reference to synthetic diets in phenylketonuria", *Archives of Disease in Childhood,* vol. 37, pp. 565-577.

Woolf, L., Griffiths, R., Moncrieff, A., Coates, S. & Dillistone, F. 1958, "The dietary treatment of phenylketonuria", *Archives of Disease in Childhood,* vol. 33, no. 167, pp. 31.

Woolf, L. & Vulliamy, D. 1951, "Phenylketonuria with a study of the effect upon it of glutamic acid", *Archives of Diseases in Childhood,* vol. 26, no. 130, pp. 487-494.

Woolf, L.I., Griffiths, R. & Moncrieff, A. 1955, "Treatment of phenylketonuria with a diet low in phenylalanine", *British Medical Journal,* vol. 1, no. 4905, pp. 57-64.

Yi, S. & Singh, R.H. 2008a, "Protein substitute for children and adults with phenylketonuria", *Cochrane Database Syst Rev,* vol. 4.

Yi, S.H. & Singh, R.H. 2008b, "Protein substitute for children and adults with phenylketonuria", *Cochrane database of systematic reviews (Online),* vol. (4), no. 4, pp. CD004731.

Yu, J.S. 1970a, "Phenylketonuria: a review", *Postgraduate Medical Journal,* vol. 46, no. 537, pp. 430-436.

Yu, J.S. 1970b, "Phenylketonuria: a review", *Postgraduate Medical Journal,* vol. 46, no. 537, pp. 430-436.