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Use of the Simplified Diet Method to Improve Metabolic Control Among Teens and Adults with Phenylketonuria: a Mixed Methods Approach

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USE OF THE SIMPLIFIED DIET METHOD TO IMPROVE METABOLIC CONTROL AMONG TEENS AND ADULTS WITH PHENYLKETOURIA: A MIXED METHODS APPROACH

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Phenylalanine hydroxylase (PAH) deficiency, commonly known as phenylketonuria (PKU), is an inborn error of metabolism that causes the accumulation of phenylalanine and results in intellectual disability if left untreated. The primary treatment for PKU is a lifelong diet that selectively restricts phenylalanine intake and is aimed at keeping blood phenylalanine in the therapeutic range of 120-360 μmol/L. Qualitative research in PKU is limited, but research has stressed the challenges individuals face in following a diet so different than their peers Previous attempts in Europe and Australia have been successful in allowing free use of certain fruits and vegetables, as well as protein counting. In Nebraska, metabolic control remains optimal among children birth to 12 years; however, suboptimal control attenuates for individuals, ages 13 through adulthood. This study was designed to investigate whether the Simplified Diet would improve metabolic control of PKU in older children and adults.

Thirty patients, ranging in age from 13 to 50 years, participated in a study at the University of Nebraska Medical Center and Children's Hospital and Medical Center in Omaha, Nebraska utilizing the Simplified Diet method, which included dietary protein counting, as well as free use of fruits and vegetable containing 50 mg/100 grams food or less per serving. After being educated on the use of the Simplified Diet method using a

written educational tool, participants followed the intervention for an average of 10.5 months. At baseline, phenylalanine levels from the previous year averaged 666 μmol/L; while at follow-up, mean phenylalanine levels significantly decreased to 562 μmol/L ($p=0.003$). There was no significant difference in mean tyrosine level, body weight, or nutrient intake using the Simplified Diet method. There was a significant increase ($p=0.004$) in the number of participants who utilized PHE/protein counting compared with only avoiding high protein foods. Several positive attitudes towards the PKU diet were observed after using the Simplified Diet method. Primary themes included “awareness”, “easier” and “realistic.” The results of this study have important implications for clinical management of PKU, as well as individuals living with PKU.

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LIST OF ABBREVIATIONS

ACMG	American College of Medical Genetics and Genomics
AHRQ	Agency for Healthcare Research and Quality
BH_4	Tetrahydrobiopterin
BMI	Body Mass Index
CHO	Carbohydrate
DRI	Dietary Reference Intake
FDA	Food and Drug Administration
GA-1	Glutaric Acidemia Type 1
GMDI	Genetic Metabolic Dietitians International
g	Grams
IEM	Inborn Errors of Metabolism
IQR	Interquartile Range
mg	Milligrams
MMA	Methylmalonic Acidemia
MSUD	Maple Syrup Urine Disease
n	Number
NBS	Newborn screening

NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
ND	Not determinable
PA	Propionic Acidemia
PAL	Phenylalanine ammonia lyase
PHE	Phenylalanine
PKU	Phenylketonuria
PAH	Phenylalanine hydroxylase
SD	Standard Deviation
SERC	Southeast Regional Newborn Screening and Genetic Collaborative
BH4	Tetrahydrobiopterin
TYR	Tyrosine
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

History of PKU

Phenylalanine hydroxylase (PAH) deficiency, traditionally known as phenylketonuria, (PKU) due to the characteristic phenylketones accumulating in the urine of affected individuals, has a significant place in history as the first inborn error of metabolism identified through population-based screening, thereby initiating a new era in the diagnosis and treatment of genetic disorders.[1] In the 1920's, a 3-year old girl named Carol Buck went from doctors to psychologists to clinics without an answer. Her mother described her as a perfect infant whose development stopped along with an unusual odor to her daughter's urine.[2] PKU was first described as "imbeccillitas phenylpyruvica"[3] Ivar Ashjørn Følling reported the identification of phenylpyruvic acid in the urine of mentally retarded children of similar phenotype and clinical history[4] In 1934 he met six-year old Liv,Egeland, who could only say a few words and walked with assistance, along with her four-year old brother Dag, who couldn't walk, talk, or eat on his own.[2] Følling suggested that the defect might relate to phenylalanine metabolism, but there was uncertainty regarding the normal metabolism of this amino acid.

In 1944, Mary and Frederick Bernheim showed that in normal liver, phenylalanine is predominantly hydroxylated to form tyrosine.[5]. Three years later, Jervis established that the metabolic flaw in PKU was the inability to perform this hydroxylation step, which provided evidence that an excess of phenylalanine in serum, not a deficiency of tyrosine was responsible for PKU [6]

In 1949, Louis Woolf began to suggest that production of carbon-treated casein hydrolysate with restricted quantities of certain amino acids (including sufficient

phenylalanine to avoid phenylalanine deficiency) would produce a desired low-phenylalanine-food.[7] Full details of the diet were published in 1955.[8]. Woolf not only described in considerable detail how to prepare the diet, but also stressed both the need for careful monitoring and the possible need for the diet to be adhered to throughout life.. Woolf's ideas as a chemist were not immediately adopted by the physicians at the Great Ormond Street Hospital in London. Woolf was then approached by Horst Bickel and Evelyn Hickmans of the Birmingham University Children's Hospital,[9] In 1950, Bickel had moved to Birmingham to further his research into congenital metabolic disorders and it was at his suggestion that Følling's ferric chloride test for PKU began to be applied at the Birmingham Children's Hospital to children with mental retardation.[10] On March 13, 1951, Sheila, a 17-month-old girl of Irish descent, became the third patient with PKU, that was confirmed by quantitation of phenylpyruvic acid in urine and phenylalanine in urine and plasma[11]

Bickel was not convinced that the proposed diet had much chance of success; however, he continued with the laborious task of preparing sufficient quantities of low-phenylalanine hydrolysate.[11,12] Sheila's diet was modified and she began using this formula in December 1951, when she was 26 months old. Within a few months, she learned to crawl, stand, her eyes became brighter and her hair became darker, and she no longer hung her head and cried continuously.[11]

The main barriers to the institution of dietary treatment for all children with high serum levels of phenylalanine was uncertain about whether they would necessarily become retarded if left untreated. No studies had been carried out to determine directly how many PKU individuals of normal intelligence might be in the general population. By the mid-1960's, a number of children with normal intelligence and high serum phenylalanine had been identified, and the discrepancy between the prevalence of the

high serum phenylalanine estimated from extensive testing in asylums and the prevalence estimated from the results of mass screening of newborns suggested that as many as 50% of persons with high serum phenylalanine might be of normal intelligence.[13] In 1968, Woolf pointed out that treatment of the phenylketonuric infant at birth was the only ethical course to take since he or she stood at least a 50 percent chance of being mentally retarded if untreated.[14] Today, dietary restriction of phenylalanine is tailored to the needs of each patient, is lifelong, and is accompanied by continual monitoring and adjustment, just as Woolf suggested might be necessary over 60 years ago.[12]

Newborn Screening for PKU

The natural history of PKU clearly indicated that in the absence of treatment, developmental retardation began within the first few weeks of life; and early experience of low-phenylalanine dietary treatment suggested that the earlier the treatment began, the more likely it was to be effective.[15]

In 1957, Robert Guthrie met Robert Warner, director of the Children's Rehabilitation Center of Buffalo. Dr. Warner was treating children with PKU by restricting dietary phenylalanine but was having trouble measuring blood phenylalanine levels because he had to send the samples to a firm in California. Dr. Warner proposed that Dr. Guthrie devise a simple method of measuring blood phenylalanine. After only three days, Dr. Guthrie developed a simple test using only a few drops of blood. Dr. Guthrie obtained permission to test 3,000 residents of a state school for the retarded near Rochester, NY. The administrators at the school maintained that all of their residents had been tested with a urine test alone; however, Dr. Guthrie found 23 cases of PKU, four more than had been diagnosed by urine testing. By the fall of 1961,

Guthrie was convinced that all infants should be tested before they left the hospital and his determination was almost unstoppable.[16]

Newborn screening (NBS) for PKU became widespread in North America and in the United Kingdom by the mid-to-late 1960's and in most of the developed world by the early 1970s. Since the initiation of NBS, almost all cases of PKU have been diagnosed following a positive newborn screening test, resulting in significant economic savings to society in addition to unquestioned benefits to affected individuals.[1,17]

History of Dietary Treatment for PKU

A report in the Journal of Clinical Investigation in 1955 by Armstrong and Tyler was one of the first clinical studies describing the use of phenylalanine-free formula demonstrating a decrease in phenylalanine levels in both plasma and its metabolites The authors concluded that all of the observations made on the patients with PKU were consistent with the hypothesis that the effects of some detrimental substances on the central nervous system have been overcome by the use of a phenylalanine-restricted diet in the form of a synthetic formula. Armstrong and Tyler concluded that it seemed probable that such diets should be initiated at a very early age in order to prevent irreversible damage to the central nervous system.[1,18]

During 1967-1983, the Maternal and Child Health Division of the Public Health Service funded a collaborative study of 211 newborn infants identified on newborn screening as having PKU. The infants were treated with a phenylalanine-restricted diet to age six years, and then randomized either to continue the diet or to discontinue dietary treatment altogether. One hundred and twenty-five of the 211 children were then followed until 10 years of age. In 1998, the National Institute of Child Health and Human Development (NICHD) scheduled a Consensus Development Conference and initiated a

study to follow up on the participants from the original Collaborative Study. Based on this follow-up, it was determined that early discontinuation for patients with PKU was associated with poorer outcomes, not only in intellectual ability, but also in achievement test scores and increased rates of medical and behavioral problems.[19]

Additional risks of diet discontinuation arose when women with PKU whose blood phenylalanine levels were not controlled became pregnant. More than 90% of infants born of such pregnancies had mental retardation, and a high incidence of microcephaly, low birth weight, and congenital heart disease also occurred. [20]

Subsequent evidence reviews for PKU guidelines relied upon two independent review processes. The first was a NIH consensus conference held in 2000.[18] The second review was performed by the Agency for Healthcare Research and Quality (AHRQ) as a precursor to a more recent NIH conference held in September 2012.[1, 21]

During the first review, the National Institute of Health (NIH) Consensus Development Conference (US Department of Health and Human Services, Public Health Service, NIH, NICHD, 2001) recommended that patients maintain blood PHE levels between 120 and 360 μmol/L up to age 12 and below 900 μmol/L for nonpregnant adults and adolescents. It was clear, at that time, that excess PHE was highly detrimental to brain development prior to 10 years of age[18].

The second set of management guidelines, launched in 2013, recommended that blood levels in all patients should be maintained in the range of 120-360 μmol/L. At present, there is no evidence to suggest that normalization of blood PHE levels is required, but levels in the 60-120 μmol/L should not be regarded as “too low” particularly in the patient whose PHE intake is not severely restricted. Blood PHE levels should be monitored at least weekly until age one with increased surveillance during periods of

rapid growth and transition of diet, such as with the introduction of solid foods. After one year of age and until 12 years of age, biweekly to monthly sampling is often adequate. In adolescents and adults who are stable and well controlled, monthly testing may be adequate.[1]

Currently, widespread consensus exists regarding the importance of blood PHE control and dietary treatment. [1],[22] In response to the 2013 recommendations by the NIH, AHRQ, the American College of Medical Genetics (ACMG) guidelines for diagnosis and medical management of PKU (ACMG Guidelines) were published in 2014 in conjunction with the Genetic Metabolic Dietitians International (GMDI) and Southeast Regional Newborn Screening and Genetics Collaborative (SERC) evidence and consensus-based recommendations for nutrition management of PAH deficiency.[1,21] These guidelines advanced the 2014 GMDI/SERC recommendations for nutritional management of PAH deficiency by incorporating a rigorous and expanded review of the latest research, graded the body of evidence, and utilized a web-based technology that supported global open-access.[23]

Genetics of PKU

PKU is an autosomal recessive disease. The human PAH gene is located at chromosome 12q23.1 It spans approximately 100 kb and is composed of 13 exons. Most patients are compound heterozygotes and only about 25% of the human PAH genotypes are homoallelic, which makes genotype/phenotype correlations difficult. To date, over 600 mutations have been described.[1]

PAH deficiency, or PKU is a multifactorial disorder requiring both exposure to dietary PHE and genetic deficiency of PAH activity.[1]

The incidence of PKU varies among different nations and ethnic groups.[20] The incidence is 1 per 4,500 in Ireland, 1 per 16,000 in Switzerland, and overall, it is 1 per 15,000 in the United States (1 per 8,000 in Caucasians and 1 per 50,000 in Blacks).[20] Individuals with similar mutant PAH genotypes may have disparate phenotypes. That said, an individual's specific PAH genotype is still the major determinant of metabolic phenotype.[24,25] It is recommended that mutation analysis be obtained for all infants with elevated PHE to provide information that may affect the extent of dietary PHE restriction and the likelihood of response to cofactor supplementation.[26,27]

Nutritional Issues in PKU

Nutritional treatment for patients with PKU is based on a low PHE diet, natural foods containing some PHE in combination with a protein substitute (a mixture of amino acids that are free from or low in PHE), and special low-protein foods to meet the patient's energy requirements.[28]

The characteristics of the diet for patients with PKU resemble those of a vegan diet with respect to the composition of permitted natural foods. Although PKU patients do follow a vegan-like diet, some of the components of usual vegan diets, including cereal and nuts, are restricted in these patients, because of their high protein contents. This type of dietary regimen, provides lower saturated and polyunsaturated fat, cholesterol, carnitine, taurine, iron, zinc, selenium, calcium, folate, A, C, D, E, and B2, B6 and B12 vitamin intakes, because of the very low assumption of PHE-containing animal foods, as well as higher carbohydrate intakes compared with other healthy children and adults.[29]

Improvements in the palatability, presentation, convenience and nutritional composition of both low-protein foods and formulas have helped to improve long-term

adherence with the PKU diet. Long-term dietary guidance and monitoring of the nutritional status of patients with PKU should be part of a follow-up program that continues for life.[29]

Pharmacotherapy in PKU

In 2007, the first pharmacologic agent for the treatment of PAH deficiency, sapropterin dihydrochloride, was approved by the US Food and Drug Administration. Sapropterin (Kuvan, BioMarin Pharmaceutical, Novato, CA) is a synthetic form of the naturally occurring cofactor tetrahydrobiopterin(BH_4).[30,31] BH_4 a cofactor of PAH, can lower elevated PHE levels in a subset of patients with PKU.[31] Kuvan has been available as a non-dietary therapy option for patients with BH_4-responsive PKU since 2007 in the United States, 2008 in European Union, and 2010 in Canada.[25]

Although not deficient in endogenous tetrahydrobiopterin, some patients with PKU who have some residual enzyme activity respond to administration of sapropterin with an increase in the metabolism of PHE to TYR. The mechanism by which residual PAH activity is enhanced is unclear, but Kuvan may act as a pharmacologic chaperone leading to improved folding and increased stability of the mutant protein.[1] Approximately 25-50% of those with PKU are saproterin-responsive.[23,27-29] Patients with mild PAH deficiency are most likely to respond because some stable protein is required for sapropterin to function; however, responsive patients are identified even among those with complete PAH deficiency.[1] The most common dose used for initiation and maintenance is 20 mg/kg/day.[32,36] During clinical trials, no serious side effects of sapropterin therapy were identified.[30,31] Recommendations for determining response to sapropterin and its use in PKU were published by Levy et al, in 2007.[25] In the following

year, Singh and colleagues published recommendations for the dietary management of sapropterin-responsive patients PKU patients.[23]

A second drug, phenylalanine ammonia lyase (PAL), is currently being used in clinical trials and is awaiting Food and Drug Administration (FDA) approval. PAL is a bacterial enzyme that converts phenylalanine to ammonia and trans-cinnamic acid. As a potential enzyme substitution therapy in patients with PKU, PAL can be given orally to decrease PHE content in the gut or injected to reduce blood phenylalanine levels.[37] PAL is effective in lowering PHE levels in patients who do not respond to Kuvan, and potentially all PKU patients.

PKU Diet Adherence Issues

Dietary treatment of phenylketonuria is multifactorial, challenging, and lifelong.[38] Key dietary behaviors associated with optimal control of blood phenylalanine concentrations include avoidance of high protein foods, consumption of PKU formula (protein substitute) throughout the day, and adequate energy intake.[39,40] Suboptimal compliance is generally coupled with higher blood PHE concentrations,[41] which in turn, is associated with less positive neurocognitive outcomes.[42] Dietary compliance is influenced by cognitive, emotional, physiological and cultural factors, and studies examining interventions to improve dietary compliance in PKU are limited. Patients and caregivers vary in their willingness and capacity to adhere to dietary treatment and may comply only with selective aspects.[41]

Dietary management of PKU is a delicate balance; a patient must consume adequate nutrition to support growth and development, while significantly restricting intact protein to prevent an elevation in blood phenylalanine. Dietary success in part depends on the level of underlying phenylalanine hydroxylase activity and factors such

as age, gender, and growth.[43] Patients with classic PKU must reduce dietary phenylalanine to 200-500 mg/day dietary phenylalanine.[44] By comparison, the mean dietary intake of phenylalanine in the US pediatric population is approximately 3,400 mg per day.[45] To maintain a diet low in phenylalanine, patients with PKU must eliminate foods from their diet that are high in protein, including all sources of meat, nuts, dairy and eggs. Allowed foods include fruits, vegetables, fats and oils, sugars, and modified low-protein foods (e.g., low protein bread, pasta). Exact portions of foods containing moderate amounts of protein, including grains, and starchy vegetables (corn, potatoes) are recommended. The majority of an individual's protein requirements are met by medical formulas designed for PKU that contain amino acids other than phenylalanine, as well as vitamins and minerals. Such dietary limitations are effective, but impose a significant burden on patients and their families, which compromise dietary compliance.[41,46]

Traditionally, the diet has been managed by weighing and measuring all foods eaten, looking up the PHE content. For individuals who have high blood PHE concentrations, following the diet is even more difficult, due to the neurocognitive problems associated with high blood PHE concentrations, including executive function deficits, anxiety, and slow processing speed[47] which can interfere with choosing and measuring foods appropriately.[38]

In PKU, a high percentage of patients have blood PHE concentrations that are above target ranges[41], particularly in teenagers and adults, indicating inadequate compliance. Patients tend to be described as either compliant or noncompliant, but there is commonly a spectrum of behaviors with a varying degree of partial compliance with one or more aspects of treatment.[38]

A World Health Organization (WHO) study estimated that only 50% of patients with PKU in developed countries follow treatment recommendations.[48]

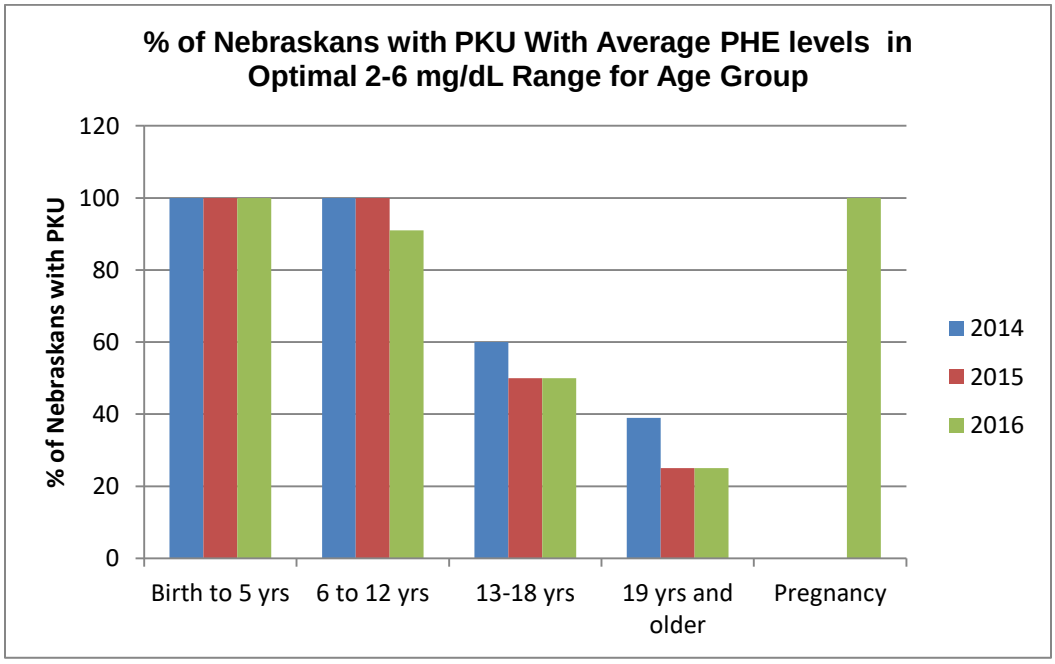
PKU, as well as other inborn errors of metabolism (IEM), have adherence issues similar to many other chronic non-IEM diet-treated conditions such as Type 1 diabetes and cystic fibrosis.[49] It is well established that these children are about twice as likely as other children to have behavioral and emotional problems.[50] At the very least, these conditions are likely to have disruptive effects on the family lifestyle, and participation in normal activities such as holidays or sleepovers may be avoided.[51] Some of the barriers observed in IEM, including PKU, are particularly taxing, not only because of the intricacies of dietary treatment and other treatment demands, but also, traditionally, the lack of treatment consensus and guidelines, and the individual's physical condition and neuropsychological profile.[51]

In Nebraska, adherence and metabolic control are reported by the Newborn Screening Program Annual Report to evaluate the effectiveness of the newborn screening system. This monitoring is also conducted to ensure eligible patients, including those with PKU, have access to the Metabolic Formula Program, as well as the Metabolic Foods Program, which are essential elements to the success of Nebraskans affected with inborn errors of metabolism.[52]

Figure 1 illustrates adherence to the PKU diet for years 2014-2016.[52-54] This table indicated that adherence in birth to 12-year range remains optimal, at almost 100% over all three years. This adherence greatly diminished once patients became teenagers, and the responsibility for the PKU management shifted from parents to the patient. In the 13 to 18-year age range, the optimal PHE level was achieved in only 50-

60% of the patients, and only 25-39% of adults achieved an optimal PHE level, except during pregnancy, when this PHE goal was reached 100% of the time.

Figure 1: Percent of Nebraskans with PKU whose average PHE levels were in optimal 2-6 mg/dl range for their age *group*[50-52]



Research involving Use of Free Fruits and Vegetables and the Simplified Diet Approach

The Simplified Diet method of managing phenylalanine intake in patients with PKU has been studied in Europe and Australia.[55]

In 2003, MacDonald et al., reported from Birmingham, UK that "free use of fruits and vegetables containing 51-75 mg/100 g posed no problem for children with PKU"[56] This was a 15-week study, looking at fifteen subjects with PKU, with a median age of 6 years (range 1-24 years). In a three-part prospective study, subjects sequentially ate fruits and vegetables containing phenylalanine 0-50 mg/100 g for weeks 1 to 3, 51-75

mg/100 for weeks 4 to 8, and 76-100 g/100 g for weeks 9 to 15. Plasma phenylalanine levels were measured twice daily for three consecutive days in weeks 1, 3, 6, 8, 11, and 15. A standard menu was followed on the blood sampling days. Daily diet records of fruits and vegetables were kept throughout the trial. Results of the study showed that control of phenylalanine levels was not adversely affected by the free use of fruits and vegetables containing 51-100 mg/100 g.

Sweeney et al. (2011) reported from North Adelaide, Australia, that "Protein exchanges (foods containing less than 50 mg PHE per uncounted) were an alternative method of measuring PHE intake in the dietary management of phenylketonuria."[57] In Phase 1 of this trial, participants were randomized to continue counting PHE unit exchanges (n=8) or changed to counting gram protein exchanges (n=10), using a new diet chart developed in-house. Foods containing less than 20 mg PHE per serving were now considered "free". In Phase 2, 18 participants were educated to use an updated version of the in-house diet chart, and in this version, foods containing less than 50 mg PHE per serving were considered "free". Results from Phase 1 indicated that PHE levels over 6 months were comparable to pre-study levels, with a mean PHE pre-diet change of 388 ± 169 μmol/L and a mean PHE post change of 388 ± 160 μmol/L. In Phase 2, four participants had a significant improvement in blood phenylalanine levels, nine showed no significant change and one participant's levels were significantly higher. All subjects preferred the new diet guide used in Phase 2 over their previous method used in Phase 1.

In 2012, Rohde et al., reported from Germany that "although total PHE intake increased by an average of 58 mg per day during the 2 weeks of free fruit and vegetable consumption, blood PHE concentrations were unchanged."[58] This study was a cross-over study, with a two-week period of conventional treatment (accounting for protein

from fruits and vegetables) and a two-week period with free fruit and vegetable consumption involving fourteen children ages 2-10 years. The instruction was to follow liberal fruit and vegetable consumption in the randomized first or second study period Detailed daily dietary records were obtained throughout the study. PHE and nutrient content were calculated and blood PHE concentrations were monitored daily. Results showed that fruits and vegetables containing less than 75 mg/100g did not adversely affect metabolic control in children with PKU.

A Dutch study, published in 2008, investigated which methods patients and parents used to determine PHE intake and the relationship between the methods applied, subject age, and blood PHE concentrations.[59] In this study, a questionnaire was received from 188 patients. Of these patients, 75 used exact measurement, 75 used estimation, and 38 used both methods. The number of patients who estimated PHE intake clearly increased with age. Whatever method was used, an increase in blood PHE concentrations was seen with age. During childhood, exact measurement was used more frequently, and from adolescence on, PHE estimation was used more frequently. Whether patients estimated or used exact measurements, there was not a statistically significant difference in PHE concentrations in the three age groups (<10 years, 10-15 years, and 16-29 years) although blood PHE concentrations tended to be lower in adolescence using exact measurements. Data suggested that estimation and exact measurement of PHE intake were both reliable methods. It was concluded, that, in addition to exact measurement, patients should be instructed in both methods at an early age, so that both methods can be used adequately.

Rohde et al (2015) enrolled 149 patients in a multicenter study investigating the effect of the dietary regime on metabolic control open to German-speaking, metabolic centers.[60] Patients were separated according to age and dietary regime, revealed by a

questionnaire on dietary habits. Dietary regimes varied and were separated into 5 groups, from daily strict calculations of all PHE intake (Group 1) to a regime based on estimation of PHE intake and including high protein food (Group 5). Median PHE concentrations in children did not differ significantly among diet groups; however, exact PHE calculations (Group 1) had significantly lower percentages of blood PHE levels above the upper recommended limit. Patients in Group 5 with the use of high-protein foods showed the poorest metabolic control. Median PHE concentrations of all other regime groups were within recommended ranges, including groups not calculating special low-protein foods, fruit and vegetables, and using a simplified diet of recording PHE intake.

Bernstein et al described the experiences of five dietitians at three metabolic centers in the United States; one that began using the Simplified Diet in 2015, one that had been using this approach since 1965, and one that had used this method with adults returning to diet since 1983, but only recently for all patients with PKU.[60] While the foods counted or allowed as free varied slightly from clinic to clinic, the concept of only counting certain foods while allowing others to be consumed freely was the same. Observations from the three clinics were made in the following areas of the use of the Simplified Diet: transitioning to the Simplified Diet, implementing the Simplified Diet with infants, and using the Simplified Diet with adults returning to treatment. Challenges were reported as minimal. While the simplified method had been well accepted, implementing the Simplified Diet was more of a challenge with parents who had followed the diet carefully for many years, and at first, had been resistant to change. No statistical testing was conducted. It was noted that research on the long-term nutrient intake and metabolic control of patients on the Simplified Diet was needed.

To date, no clinical trials have been conducted or published in the United States to demonstrate efficacy of either “free” use of fruits and vegetables, or use of the simplified diet approach, including counting grams of protein instead of phenylalanine.

Qualitative Research in PKU

Science is the collection of grand explorations of things; physical, biological, and sociological, all historically involving quantitative research. But each of the divisions of science also has a qualitative side, in which personal experience, intuition, and skepticism work alongside each other to help refine the theories and expectations.[61]

Qualitative research in the area of inborn errors of metabolism, including PKU, is limited. The only qualitative research involving the PKU diet was conducted by Sharman et al. This study was conducted to investigate factors affecting adherence to the PKU diet in adolescence and was conducted as part of a Metabolic Disorders Association conference in Australia. Eight adolescents with PKU were part of a focus group to gather information about factors that encourage and discouraged dietary adherence. Thematic analysis revealed that the adolescents encountered problems explaining the nature and food requirements of their condition to other people. Friends, family and wanting to maintain “normal” cognitive abilities were identified as factors that encouraged dietary adherence.[62]

Another qualitative study’s aim was to explore young and early treated Norwegian adults’ experiences by conducting in-depth interviews in 2011 with 11 adults with PKU, aged 20-30 years.[63] This was the first qualitative study on people with PKU in Norway, and the process was inspired by grounded theory. All participants reflected on their own health and existence by expressing positive counterfactual thoughts. They considered themselves lucky to have had parents who had managed the diet, they were

grateful for the time and place they were born and for information and treatment availability, although the results also showed some ambiguous attitudes towards the hospital which provided the treatment.

In 2008, a qualitative component was devised in a mixed methods research study at the Bambino Gesu Children's Hospital in Italy, to understand the patients' perspectives of their PKU. Results were from a cohort of 44 patients at least 7 years of age. Themes from the study included disease definition, knowledge of disease, impact on daily life, and impact on daily life. It was acknowledged that knowledge about PKU evolved with the age of the patients. Dietary restriction was not the only problem perceived by patients; consuming food not like other people's led to a highly challenging feeling of "difference". Despite the restrictions and feelings of "difference", the patients showed a good level of autonomy in adhering to the diet.[64]

Definition of Mixed Methods Research

Several definitions for mixed methods research have emerged over the years that incorporate various elements of methods, research processes, philosophy, and research design.[65] An early definition of mixed methods came from writers in the field of evaluation. Greene, Caracelli and Graham emphasized the mixing of methods and the disentanglement of methods and philosophy.[66] Tashakkori and Teddlie also defined mixed methods as the combination of "qualitative and quantitative approaches in the methodology of a study.[67] A current definition of mixed methods comes from Creswell and Plano Clark, who, defined mixed methods research as "a research design with philosophical assumptions as well as methods of inquiry.[68] As a method, it focuses on collecting, analyzing and mixing both quantitative and qualitative data in a single study or series of studies. Its central premise is the use of quantitative and qualitative

approaches, in combination, provides a better understanding of research problems that neither approach can achieve alone.[68]

Statement of Study Hypotheses and Specific Aims

The primary hypothesis for this study is that the use of the Simplified Diet (protein counting plus the free use of fruits and vegetables containing ≤50 mg PHE per 100 g food) improves metabolic control in patients with PKU compared to the standard dietary therapy.

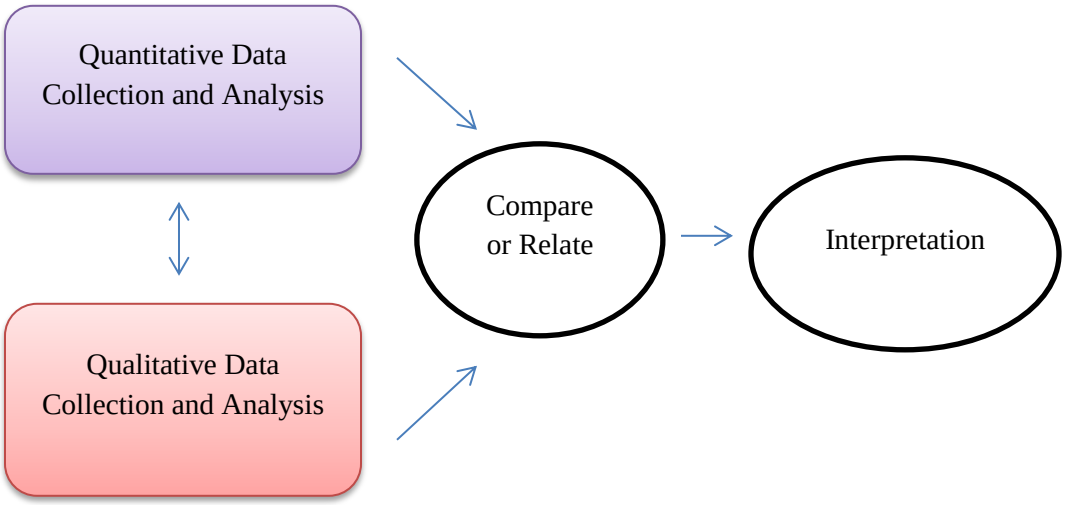
Specific aims include determination of whether the Simplified Diet method improves metabolic control over traditional diet management. Patient education of the Simplified Diet method will be conducted. Participants' weights and nutrient intakes will be monitored. Patient interviews will be conducted to determine attitudes and feelings toward using the Simplified Diet method.

Chapter 2

METHODS

This study utilized a convergent parallel, also known as triangulation design, which was characterized by the collection of both qualitative and quantitative data, with concurrent timing, stressing equal importance of each type of data for the study. Each type of data was analyzed separately and then merged together in a convergence model, which compared and contrasted the separate results. This is the most well-known design across the disciplines.[65]

Figure 2: Convergent Mixed Methods Design[65]



The purpose of the convergent design is "to obtain different but complementary data on the same topic." [69] This design is used when the researcher wants to triangulate the methods by directly comparing and contrasting quantitative statistical results with

qualitative findings for corroboration and validation purposes.[65] Figure 2 illustrates the mixed methods study flow for this study.

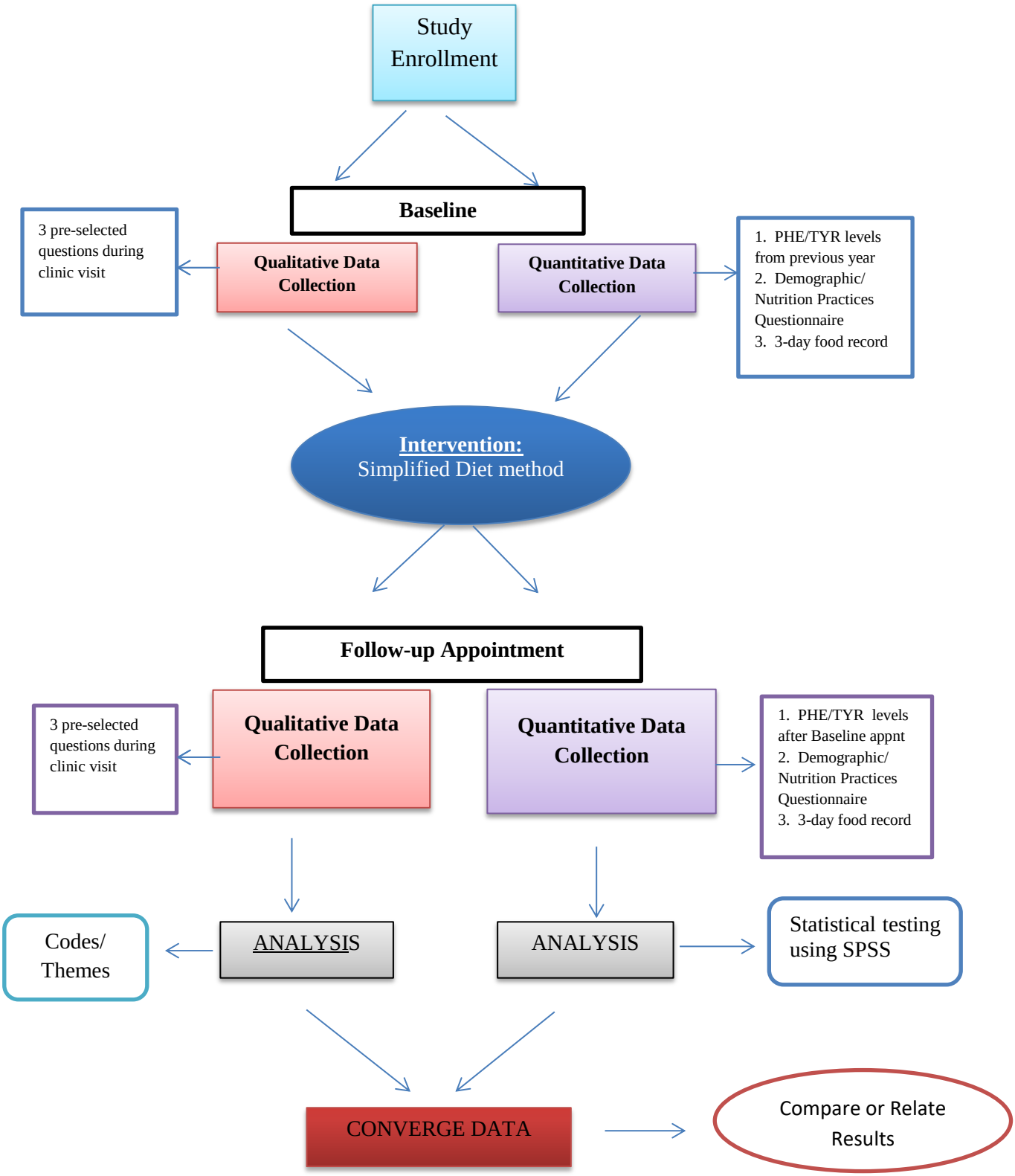
Study Design

The study was a cross-over mixed methods design using matched, historical phenylalanine levels from the previous 2 years for comparison. Patients were also asked about their attitudes towards their current PHE or protein counting system. The intended length of study was 12 months; however; based on each participant's plan of care, this time frame was based on each participant's recommended follow-up time frame.

Study Participation

Inclusion criteria for this study included both males and females ages 13-65 years of age who had a positive diagnosis of PKU and were currently treated at either the Children's Hospital and Medical Center Pediatric Metabolic Management Clinic or the University of Nebraska Medical Center Adult Metabolic Management Clinic. All patients needed to be following a prescribed protein-restricted diet, as well as daily intake of PKU formula. Exclusion criteria for this study included any woman who was planning pregnancy, or became pregnant during the study, anyone who initiated Kuvan for the first time during the study, or any patient currently enrolled in other research studies (BioMarin Prism 301/302 Protocol; IRB Protocol # 376-13-FB) or extensions of this trial. This study received IRB approval from the Pediatric Institutional Review Board in October 2015 and study participants ages 13-18 years and their parents signed a Parental Consent Form and received a Youth Information Sheet. For those ≥19 years of age, an Adult Consent Form was signed (Appendix A-C).

Figure 3: Mixed Methods Design Study Flow



This study was listed at www.clinicaltrials.gov. (Clinicaltrials.gov ID: NCT02555579)

Intervention: Simplified Diet Method Worksheet Development and Education

Upon enrollment into the study, and after demographic and qualitative data were collected, Study participants were educated using a 4-page newly developed simplified diet handout entitled, "PKU for Life—the PKU Way. (Appendix F) Teaching the simplified diet involved a 3-step approach, with the abbreviation "PKU" being used for simplification of diet approach and education. PKU for purposes of this simplified diet education stood for "P"-**P**lenty of free fruits and vegetables, "K"—**K**eep track of your protein intake and "U"—**U**se of PKU formula. Education was provided for each step. For "P"—Plenty of "free" fruits and vegetables, participants were provided a list of all fruits and vegetables that were considered "free" which included any fruit or vegetable that contained 50 mg phenylalanine or less per 100 g food per serving. Participants were instructed they could eat these foods without weighing, measuring, or counting them as they may have previously been instructed. For "K"—Keep track of your protein intake, patients were instructed on the use of protein counting with a food label for illustration and instruction. Participants were given a written pocket guide entitled, "pkufoodlist" and this list could be used to look up protein content of numerous foods if a food label wasn't available to look at for protein content. Based on the patient's previous PHE or protein intake, each participant was given a protein goal to monitor and record. Patients were encouraged to use apps on their smartphone to help keep track of their protein intake. "U"—Use of PKU Formula was the last step of the education. This step reviewed current formula intake and provided list of all available PKU formulas. Discussion was held on the importance of optimal formula intake. Each participant was educated individually and each participant was given a handout to take with them.

Dietary management aimed for blood PHE levels within the guidelines established by the ACMG and GMDI.

Measurement of Blood PHE levels

All blood levels of phenylalanine and tyrosine were collected using a filter paper blood spot, either collected in clinic, or collected at home. Participants were offered an IRB-approved $5 gift card for each level they collected during the study period. Phenylalanine and tyrosine levels were analyzed by tandem mass spectrometry, at PerkinElmer Laboratories (Pittsburgh, Pennsylvania). Phenylalanine/tyrosine levels used as historical controls were obtained from each participant's electronic medical record at the University of Nebraska Medical Center or Children's Hospital and Medical Center.

Assessment of Dietary Intake

A 3-day food record was requested at study enrollment and at the study completion visit. Based on current clinical practice, a letter and blank food records were sent to each clinic patient/parent prior to their regularly scheduled clinic visit. (Appendix D). All clinic patients were asked to provide current formula information including amount of formula used, as well as past 3 days of oral intake, including date/time, food/drink, including brand, if known, amount of food, and amount of PHE/protein for each item listed. Patients were encouraged to bring these completed food records to clinic, or bring copy of any electronic food records they were using. This was collected at initiation and completion. MetabolicPro (GMDI) was used to analyze diet records for macro- and micronutrient intake.

Demographic/Nutrition Practices Information Questionnaire

Patients were asked to provide current age, highest education level, and current marital status. Other demographic information collected included gender, Kuvan usage, and current protein prescription. Patients were given a multiple-choice question about their current method of keeping track of their diet. The options were: a) "I count mg of phenylalanine (example: no more than 300 mg/day), b) I count grams of protein (example-no more than 5 grams of protein/day), c) I don't count-I just avoid high protein foods, or d) other (please explain). The last question was "Do you use any apps to keep track of your phenylalanine (PHE) or protein intake?" If marked yes, patients could write out which apps they use. Height, weight, and BMI were also compiled on this form (Appendix G).

Data Analysis Plan

Descriptive summaries were calculated for demographic and treatment variables, including means, medians, Interquartile range, frequencies, and percentages. The change in pre- and post-intervention variables that are continuous (phenylalanine level, tyrosine level, weight,) were compared using a two-sided paired t-test. Pre- and post-intervention variables that are dichotomous were compared using McNemar's test. Correlations were used to look at the change in PHE compared to time in study. Kruskal-Wallis U Test and Mann-Whitney U Test were used to determine if there were differences between different demographic groups and the change in phenylalanine levels.

Qualitative Data

Sampling

All subjects were interviewed at their initial visit, prior to initiating the simplified diet, as well as during the follow-up visit approximately one year later, after they had utilized the simplified diet approach.

Patient Interview

Participants were interviewed in a private clinic room at either Children's Hospital and Medical Center or the University Medical Center in Omaha, Nebraska. Patients were in clinic for a regular medical check-up for their PKU where they saw members of the healthcare team during that visit.

Interview Protocol

Once consent was obtained, an explanation about the purpose of the study was given. Participants were told that each interview would take approximately 15 minutes, but they were free to take additional time if needed. Audio recordings were not obtained for this study. Each question was read to the participant (Appendix E). Detailed descriptions of answers to questions were written during the interview and participants were told that their answers would be written down during the interview. Open-ended questions were asked, and adequate time was given for participants to answer each question.

Central question:

1. How do you feel about your current PHE/protein counting system?

Sub-questions:

2. How has your role in managing your PKU changed as you have gotten older?

3. If you could design the “perfect” PHE/protein counting system to teach to other patients, describe to me what that would be?

Assurances were given to each patient that their confidentiality was maintained for the entirety of the study. All written answers were then collected and stored in a locked file cabinet in a locked office at the University of Nebraska Medical Center until the data were analyzed into codes and themes. Participants were asked these questions twice during the study; at the beginning of the study, and again at their follow-up appointment, after they had used the simplified diet approach. Data analysis plan included transcription and separation of responses into codes and themes, both at enrollment and after use of the simplified diet.

Chapter 3

RESULTS

Baseline Data

The study enrolled 33 patients with a diagnosis of PKU, and data were collected from October 26, 2015 until October 12, 2017. Three participants withdrew from the study (2 from pregnancy,1 relocated/lost-to-follow-up). All completing participants were on a PHE-restricted diet and medical formula at the time of enrollment and throughout the study Mean age at enrollment was 27.6 years (range 13-50 years). Study participants' characteristics are summarized in Table 1.

The study population was fairly equal among males and females (47% male, 53% female). Mean age was 27.6 years (range 13-50 years). Average Body Mass Index (BMI) was 27.36 (kg/m^2), SD 4.78 kg/m^2. Participants were stratified into categories based on current weight status. Adults were categorized based on their BMI, while children 13-18 years were categorized based on their BMI percentiles for age.[70-71] None of the thirty patients were classified as underweight, nine (30%) were in the normal weight category, eleven (37% were considered overweight, and ten (33%) were classified as obese. One-third (n=10) of study participants were married, while two-thirds were unmarried. Two-thirds (n=20) of the study population had a high school education or less, while one-third had at least a college degree. Twelve (40%) were currently taking the medication Kuvan. The patients' current protein prescription was used to classify them into 3 categories according to the severity of protein restriction: Sixteen participants (53% had a current protein prescription of 10 grams or less per day, while five participants (17%) were prescribed 11-24 grams per day, and nine (30% were prescribed 25-40 grams per day. The median protein prescription was 10 grams,

ranging from 6-40 grams per day. Only 11 participants kept track of their phenylalanine intake with protein or PHE counting; however, the majority of patients (63%) were just avoiding high protein foods at enrollment.

Table 1: Participant Characteristics

Characteristic	n (%)
Gender	
Male	14 47%)
Female	16 (53%)
Age, years	
13 to 18	8 (27%)
19 to 34	14 (46%)
35 to 50	8 (27%)
Body Mass Index, kg/m^2 or %ile	
Underweight (<18.5 or <5thile)	0 (0%)
Normal weight (18.5-24.9 or 5-84th %ile)	9 (30%)
Overweight (25-29.9 or 85-94th %ile)	11 (37%)
Obese (≥30 or ≥95th %ile)	10 (33%)
Marital Status	
Married	10 (33%)
Not married	20 (67%)
Education Level	
High school or less	20 (67%)
College degree or higher	10 (33%)
Kuvan usage	
Currently taking Kuvan	12 (40%)
Not on Kuvan	18 (60%)
Current Protein Prescription	
10 grams or less	16 (53%)

11-24 grams	5 (17%)
25-40 grams	9 (30%)
Current method of tracking diet	
Count PHE/protein	11 (37%)
Avoidance of high protein foods	19 (63%)

Comparison of Metabolic Control and Weight

Table 2 shows the comparison of Baseline Data compared with the Follow-up data (after the anticipated 12-month Simplified Diet method intervention). At enrollment, the mean phenylalanine level was 666 μmol/L; interquartile range (IQR) 411-884 μmol/L, with a mean tyrosine of 51.9 μmol/L; IQR 38.8-91 μmol/L, and a mean BMI of 27.45 kg/m^2 with an IQR of 23.6-31.1 kg/m^2. After using the Simplified Diet method, mean phenylalanine level showed a statistically significant decrease (p=0.003) to 514 μmol/L; IQR 294-807, with no change in mean tyrosine level (mean tyrosine 51.1; IQR 34-63.5, p=0.193) and mean BMI increased to 28.26 kg/m^2; IQR 23.9-31.9 but this increase was not statistically significant (p=0.063)

Table 2: Baseline vs. Follow-up Data

	Baseline		**Follow-up**		
	mean	**IQR**	**mean**	**IQR**	**p value**
Phenylalanine (μmol/L)	666	411-884	562	294-807	0.003*
Tyrosine (μmol/L)	51.9	38.8-91.0	51.1	34-63.5	0.193
BMI (kg/m^2)	27.45	23.6-31.1	28.26	23.9-31.9	0.063

Figure 4 represents the change by plotting baseline baseline and the follow-up PHE level for each participant, with the squares representing a historical PHE level at baseline, and the circles representing the mean PHE level after the intervention

(Simplified Diet method). Twenty-two participants (73%) showed a decrease in their mean PHE levels, with a reduction range of 0.4 µmol/L to 580 µmol/L. Eight participants (27%) showed an increase in mean PHE level, ranging from 5 µmol/L to 271 µmol/L

Figure 4: Comparison of Mean PHE levels at Baseline vs. Follow-up

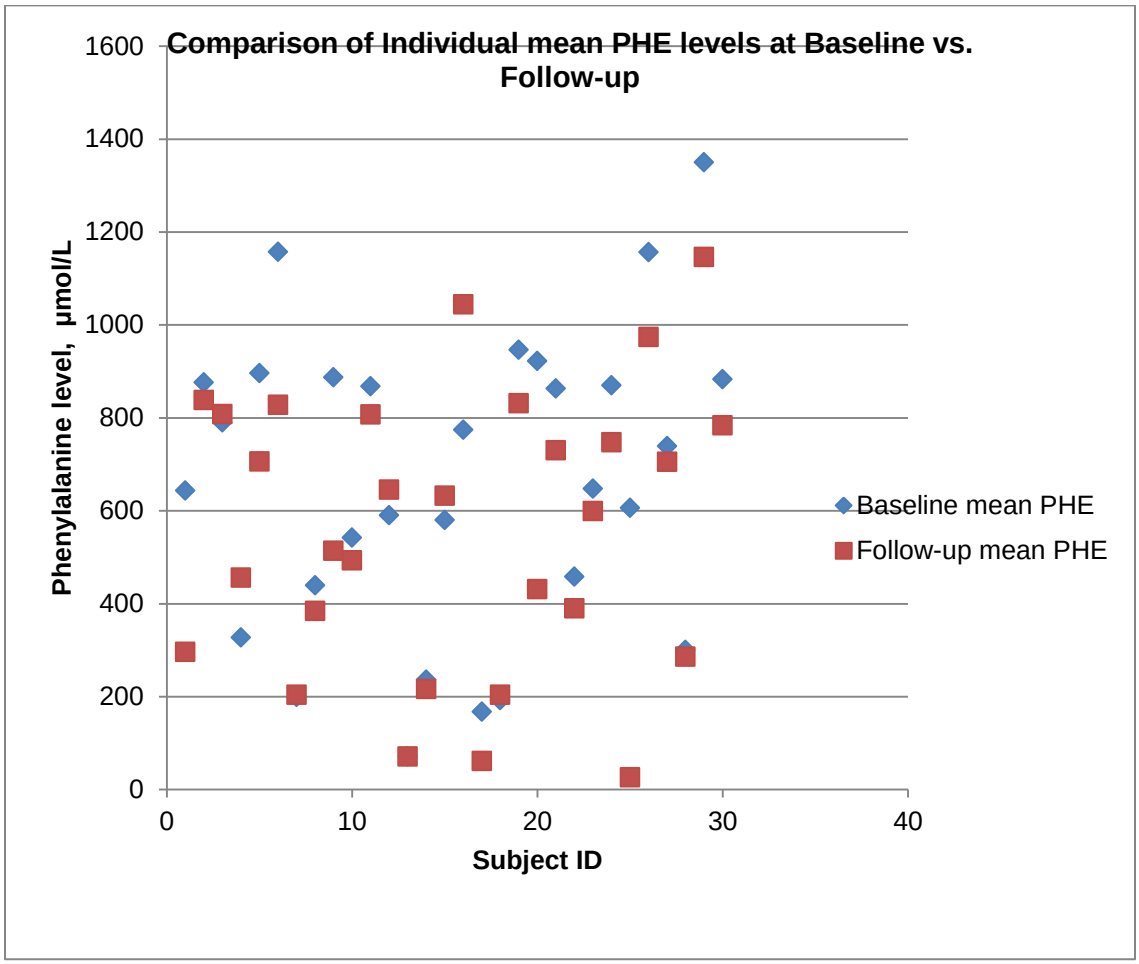
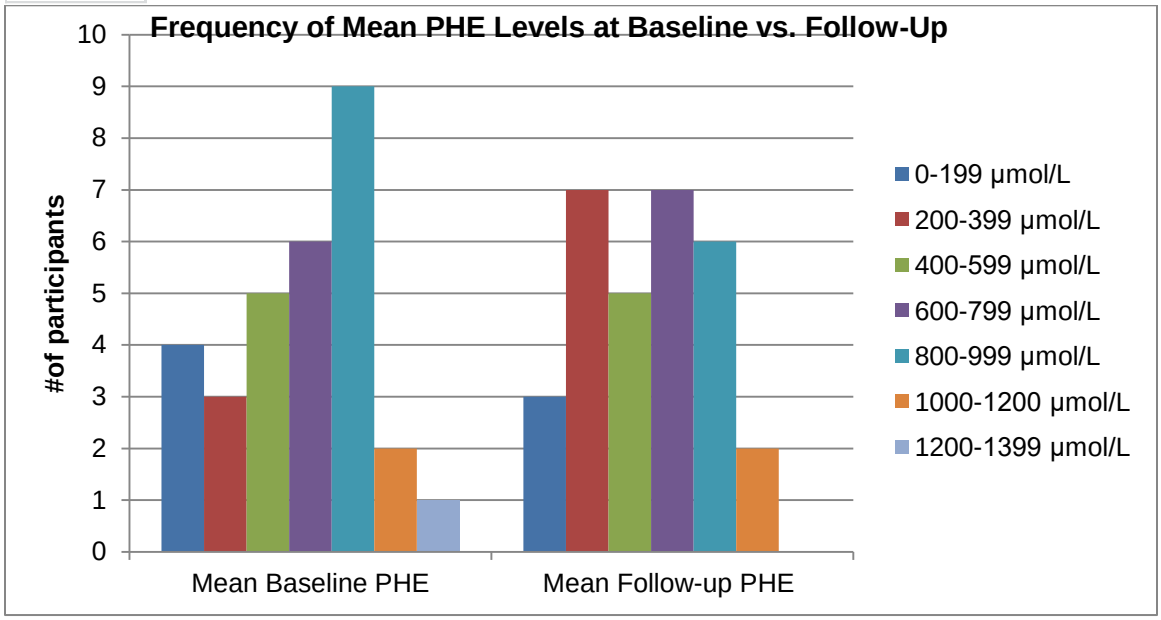


Figure 5 demonstrates the frequency as well as the distribution of PHE levels at baseline compared with follow-up data. At baseline, the highest frequency of PHE levels (30%) were in the 800-999 µmol/L range, while at follow-up, a greater frequency of PHE levels were in the 200-399 µmol/L and 600-799 µmol/L ranges (23%). Figure 5 also

illustrates the decrease in PHE levels and the shift towards lower PHE levels among the group and it shows the more evenly distributed PHE levels as compared to Figure 4.

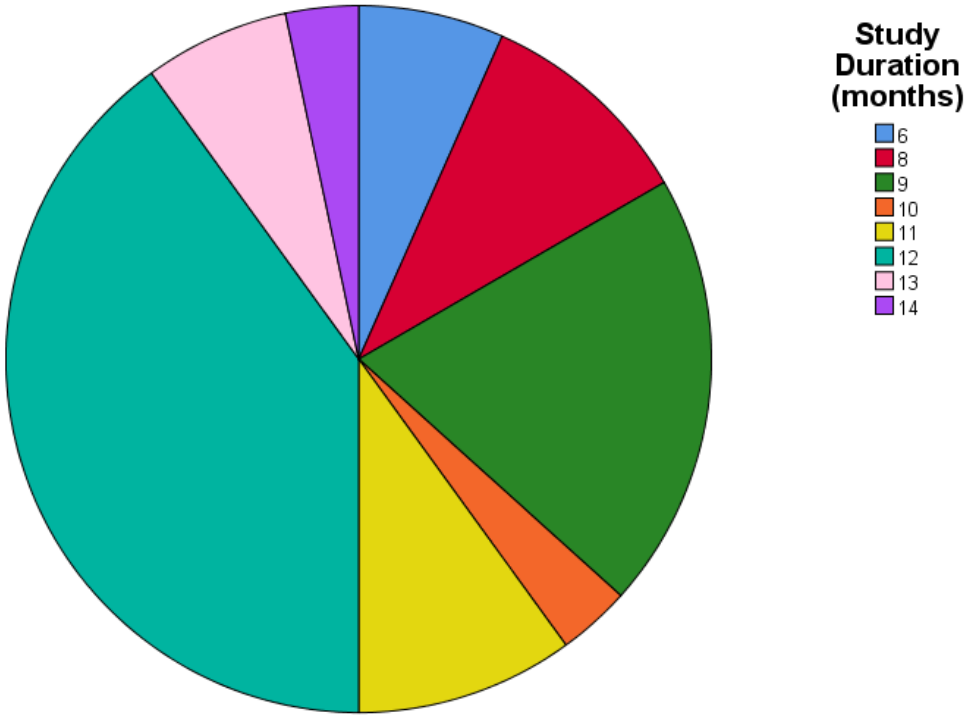
Figure 5: Distribution of Mean PHE levels at Baseline vs. Follow-up



Study Duration

Figure 6 shows the duration of time spent using the Simplified Diet method, as well as the distribution of study duration among the group. The median length of time patients followed the intervention was 10.5 months (range: 6-14 months). Two participants (6.1%) followed the intervention for 6 months, three (9.1%) followed for 8 months, six (18.2%) followed for 9 months, one (3%) followed for 10 months, three (9.1%) followed for 11 months, twelve participants (36.4%) followed for 12 months, two (6.1%) followed for 13 months, and 1 participant (3%) followed the Simplified Diet method for 14 months.

Figure 6: Proportion and Duration of Time PKU Patients Followed the Simplified Diet Method



Correlation Between Duration of Study and Change in PHE levels

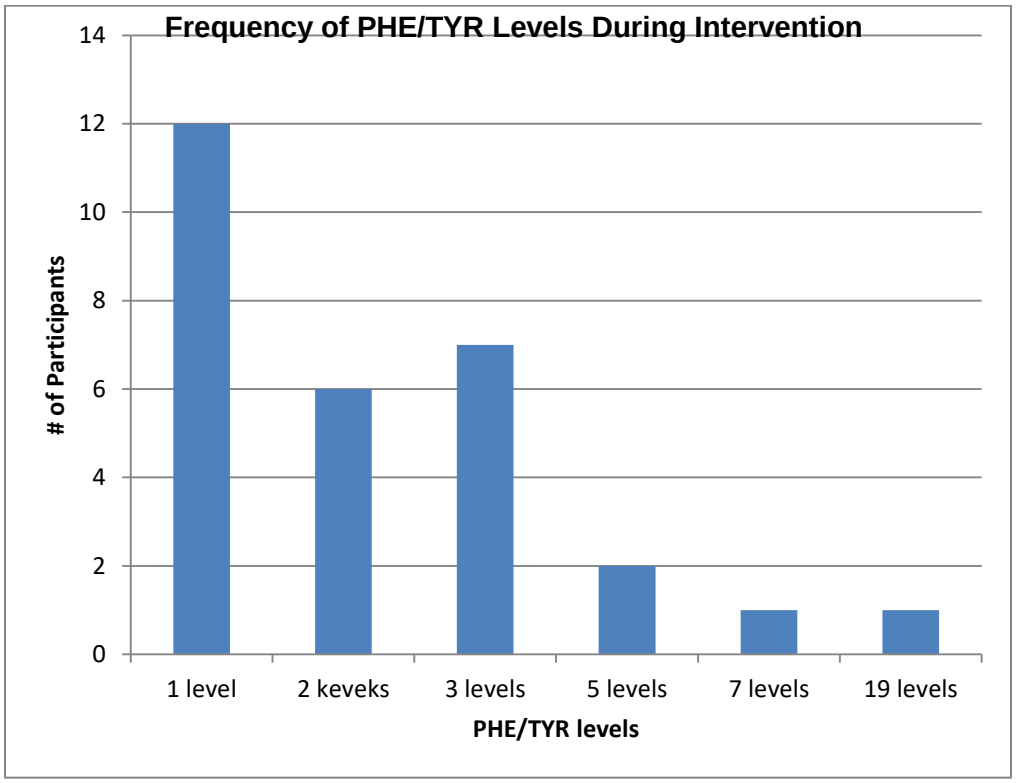
Results of the Pearson correlation test indicated that there was no correlation between duration of time on the study and change in PHE level ($r = -0.031$, $p=0.873$).

Frequency of PHE/TYR Testing

Figure 7 illustrate the distribution and frequency of testing. At baseline, it was determined from the historical data from the previous year that the mean number of blood PHE/TYR measurements (levels) was 3.5/year, with a range of 1 to 40 tests. PHE/TYR measurement frequency during the time the Simplified Diet method was used

was 2.9 levels measured per participant, with a range of 1 to 19 tests. Twelve (40%) only collected 1 PHE measurement during the intervention period; 6 participants (20%) had 2 measurements; 7 (23%) had 3 measurements; and 1 participant (3%) had 6 and 19 levels each. The participant who had 40 PHE/TYR levels the year before was only in the study for 8 months, reflecting the decrease to 19 during the intervention period. Eleven of 12 participants with only 1 PHE/TYR level from the previous year had one level during the study. Four of 30 participants (13%) increased frequency of testing during the intervention period. Five participants (17%) showed a reduction in PHE/TYR testing during the intervention period; however, only 1 remained in the study for less than 12 months.

Figure 7: Frequency of PHE/TYR level Testing During Intervention



Associations in PHE Reduction Among Treatment Groups

To determine if demographic groups influenced response to the intervention, a Mann-Whitney U Test and Kruskal-Wallis Test U Test were used to determine if the designated groups had the same distribution of scores, which was a change in PHE level. Table 3 shows the designated groups and type of statistical test used, along with the statistical significance. Among gender, marital status, education level, Kuvan usage, current protein tracking system, age, BMI category and current protein prescription group, none of these groups showed a greater difference in PHE levels.

Table 3: Patient Characteristics Compared with Change in PHE level

Test: Mann-Whitney U Test		Test: Kruskal-Wallis U Test	
Group	**p value**	**Group**	**p value**
Gender	0.580	Age (years)	0.473
Male		13-18	
Female		18-34	
Marital Status	0.067	35-50	
Married		BMI (category)	0.702
Unmarried		Underweight	
Education level	0.120	Normal weight	
High School diploma or less		Overweight	
College degree or higher		Obese	
Kuvan usage	0.250	Current Protein Prescription	0.543
Currently taking Kuvan		10 grams or less	
Not on Kuvan		11-24 grams	
Current tracking method	0.497	24-40	
Counting mg/g protein			
Avoidance of high protein foods			

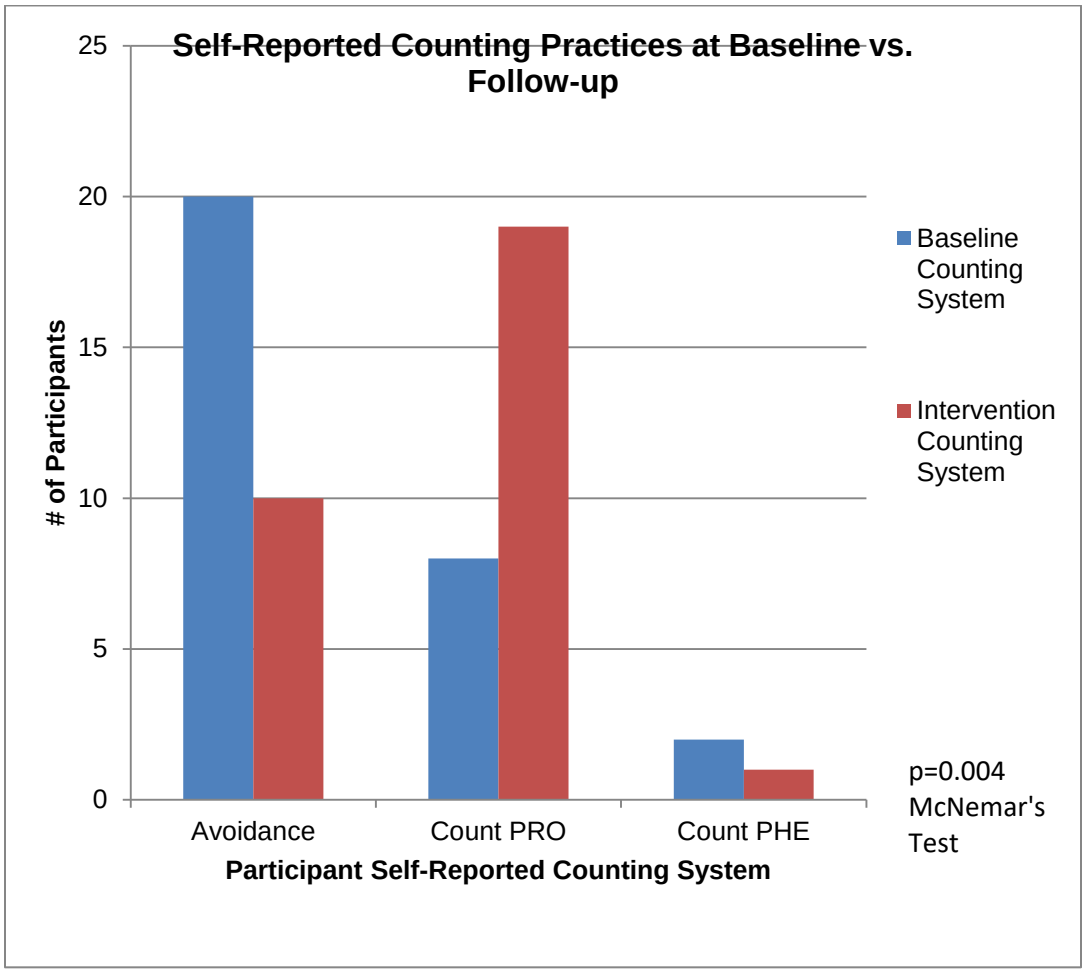
Self-Reported Counting Method Analysis

Participants were asked to answer the following question at enrollment and after using the Simplified Diet as part of the Demographic/Nutrition Practices Questionnaire. (Appendix E). Participants were asked "What is your current method of keeping track of your PHE or protein? (circle 1) a. I count mg of PHE, b. I count grams of protein or c. I don't count, I just avoid high protein foods." Answers were divided into participants who count, combining those who count milligrams of PHE with those who count grams of protein, and those who don't count, and just avoid high protein foods. Figure 8 summarizes the enrollment and Simplified Diet responses. At enrollment, 19 participants (63%) reported they didn't count and 11 (37%) reported counting their protein intake. After using the Simplified Diet, these numbers changed and only 10 participants (30%) were still not counting with an increase to 20 participants (70%) who were counting their protein intake. Using McNemar's Test, it showed that the Simplified Diet had a significant difference ($p=0.004$) on counting practices, which resulted in more participants keeping track of their protein intake.

Self-Reported Counting System Compared with Mean PHE Levels

At baseline, 2 participants were counting PHE and 8 were counting grams of protein. At the follow-up appointment, only one participant counted PHE, with 19 counting protein. No participants switched from counting PHE/protein to estimation/avoidance of high protein foods. At enrollment, mean PHE level for those counting PHE was 402 µmol/L, 770 µmol/L for participants using protein counting, and 604 µmol/L for participants' who estimate/avoid high protein foods. After the intervention, the mean PHE level utilizing PHE counting was 26 µmol/L, 490 µmol/L for participants utilizing protein counting, and 615 µmol/L for the estimate/avoid high protein food group.

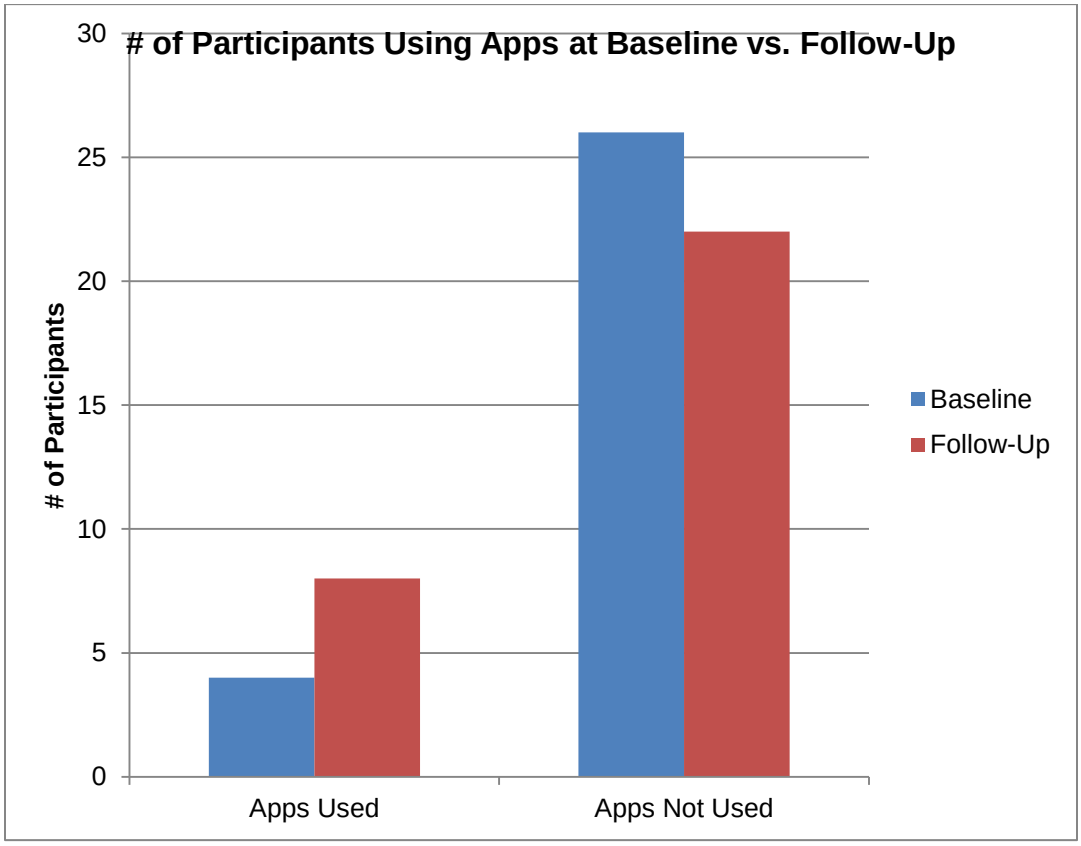
Figure 8: Self-reported Counting Practices at Baseline vs Follow-up



Use of Apps to Track Intake

Participants were also asked to report current use of apps at both enrollment and after use of the Simplified Diet. This question was listed on the Demographic/Nutrition Practices Questionnaire (Appendix E). Figure 9 displays the change in app usage between Enrollment and after use of the Simplified Diet. At enrollment, only four (13%) participants were using apps, whereas after using the Simplified Diet, this usage increased to eight (27%) participants. This increase was not significant (p=0.125) based on McNemar's Test. Apps reported being used were My Fitness Pal, How Much Phe, Weight Watcher's app, and PKUGo.

Figure 9: Use of Apps at Baseline vs. Follow-Up



Nutrient Intakes

All participants were asked to provide a 3-day food record prior to baseline and at follow-up appointment (Appendix D). Twelve participants (40%) provided food records at enrollment and 5 (17%) provided food records after using the Simplified Diet method. Only 4 participants provided food records at both time points. Using the Wilcoxen Signed-Ranks Test to determine if differences existed in related samples, there were no significant differences in any of the nutrients analyzed. Nutrients analyzed included calories, protein, PHE, TYR, carbohydrate, total fat, Vitamin C, calcium, fiber, and zinc.

At baseline, the mean daily caloric intake was 1713 kcals; IQR 1252-1916, and this met 86% DRI for calories. At follow-up, mean daily calories was 1801; IQR 1597-

2097, meeting 90% DRI. Protein intake included protein from metabolic formula as well as PHE from foods eaten. Mean baseline protein intake was 71.4 grams; IQR 47-58, 140% DRI. At follow-up, mean protein intake increased to 76.2 grams; with an IQR of 40-108, 140% DRI. Mean PHE intake at baseline was 835 mg; IQR 402-1498, and at follow-up, mean PHE intake increased to 1160 mg with an IQR of 413-1601. Mean TYR intake at baseline was 2032 mg and at follow-up, TYR mean increased to 2949 mg, IQR 1714-5251. Mean fiber intake at baseline was 8.44 g; (IQR 3-14), and at follow-up, mean fiber intake increased to 16 g; IQR 11-23 g. Calcium intake also increased with use of Simplified Diet method, with mean baseline intake at 955 mg; IQR 354-1175, and at follow-up, mean intake was at 1202 mg; IQR 420-2274. Mean Vitamin C intake was 58 mg at baseline; IQR 20-81, and at follow-up, the mean intake was 60 mg; IQR 26-84. Mean Vitamin B12 intake at enrollment was 2.29 mcg; IQR 0.7-3.7, and this intake dropped to a mean of 1.66 mcg; IQR 0.5-3, 69% DRI at follow-up.

Qualitative Results

Verification Procedures

Several verification procedures were used in this study. These procedures included triangulation, member checks, researcher's position or reflexivity, and rich, thick descriptions.[73]

Triangulation was used by asking all 30 participants the same three questions twice, once at baseline, and again after using the Simplified Diet method. This technique was used to confirm emerging findings. The principal investigator was the only researcher involved in the data collection.

Researcher's position or reflexivity was used as I have very close communication with all of the participants and they are all comfortable with sharing all facets of their PKU management with me.

Rich, thick descriptions were utilized in providing enough descriptions to contextualize the study so that readers will be able to determine the extent to which their situations match the research context, and whether the study findings can be transferred.[73]

The lens used for this study included utilizing the constructivist or interpretive position. Constructivists believe in pluralistic, interpretive, open-ended, and contextualized perspectives towards reality.[64] The lens of the researcher included disconfirming evidence. The lens of the study participants' included prolonged engagements in the field. The lens of the people external to the study included thick, rich description.

Alyson Hanish, PhD MSN, RN, who is a Nurse Specialist/Instructor at the University of Nebraska Medical Center with qualitative research experience provided the member-check for this study and reviewed all transcription, as well as provided validation for codes and themes.

Summary of Themes

Following the completion of the all interviews, the data was compiled and coded. The codes developed into five themes- two themes from baseline interviews, and three from participants' experience with the Simplified Diet method. See Table 4 for a list of themes as well as frequency among participants. All names have been changed for the purposes of describing patients' experiences while protecting their identity (Appendix H).

Themes at Enrollment Visit

Theme 1: Challenges

One of the main elements taken from the baseline interview was that following any consistent counting system was a challenge, and resulted in a lot of inconsistency. This feeling occurred at all ages, and highlighted the struggles of many patients trying to follow the PKU diet.

Table 4: List of Themes and Theme Frequency

THEME	EXAMPLE	n
Baseline Theme 1: Challenges	"Keeping track of my own diet is challenging, and I haven't made the effort to figure out a counting system that works well for me now."	15
Baseline Theme 2: Counting System: Responsibility and Transition	"My mom used to keep track of everything I ate and drank but now I don't keep track of anything	11
Post-Simplified Diet Theme 1: Awareness	"The Simplified Diet has allowed me to know more about the foods I eat, and keep better control of my PKU"	14
Post-Simplified Diet Theme 2: Easier	"The Simplified Diet method has helped with less stress, and more creativity in my diet with more fruits and vegetables"	12
Post-Simplified Diet Theme 3: Realistic	It's easier to keep track of protein instead of tracking everything I put in my mouth and I'm actually using this system daily now"	9

Matt stated,

> “My mom used to keep track of everything. Once I went to college, I didn’t count anything. I still ate a low-protein diet, but I just avoided high-protein foods, and didn’t make any other attempts to know exactly what I was eating”

Sarah shared similar feelings

> “My parents kept track of all of my food, but now that I’m on my own, I struggle to keep track of my own diet. I don’t eat anything high in protein, but I don’t look up anything like my parents used to, and if I feel like my level is high, I usually cut back on a few things and I feel better in a couple of days”

Paul described his experience with his struggles with transitioning responsibility:

> “My parents always managed my PKU until I graduated high school and moved away to college. I still ate a low-protein diet once I was on my own; but I didn’t count anything, I just avoided high-protein foods and didn’t make any attempts to figure out exactly how much protein I was eating. If my level didn’t come back high, I didn’t really worry about it.”

Beth described her experience:

> “Pregnancy was the only reason I got back on the diet. My husband helped, but I felt the diet was my sole responsibility, and it was completely overwhelming and challenging to figure out how to do everything since I had never managed anything as a kid. I knew I had to figure it out for the health of my baby.”

Phrases like “My mom used to make me write everything down at the end of the day, and “I never keep track of my diet” were commonly heard.

Theme 2: Counting system: Responsibility & Transition:

This theme became very apparent after the patient interviews. Most patients related the lack of any counting system, and just "avoided high protein foods." Many described their inefficiency as a product of their transition to self-care, and described their feelings of inefficiency as compared to their parent or caretaker's efforts when they were younger.

Sam described his experience:

> When I was younger, my parents counted every milligram of phenylalanine, and they weighed everything out. When I started counting myself, I couldn't keep it up like they had, and I don't think my current counting system is very effective because I just avoid high protein foods, which makes it really hard to make changes, since I don't really keep track of what I'm eating from day to day."

Deb states,

> "I remember my mom weighing out my food and formula. I am so busy with my job, that I just can't maintain that same system she used to have. I know my current system is very ineffective, and pretty much nonexistent."

Richard describes his experience since taking over his PKU diet:

> "I don't think my current counting system is very effective because I just avoid high-protein foods right now. I don't count, and my level is always higher than it should be even though I feel fine."

Themes After Using the Simplified Diet

Theme 1: "Awareness"

This theme became apparent after patients used the simplified diet and appeared in most frequently in patient responses. Many described the idea that they were actually aware of how much protein they were eating on a daily basis through the use of label reading.

Isabel described her experience:

> "The simplified diet is a much better system than what I was previously using and has made me more aware of what I'm eating." I can now find the protein content on almost anything I eat and should be counting, and have become more consistent with my protein intake."

Ben also described his new-found awareness:

> "I used to just think my old system was sufficient. I avoided high protein foods and my level was ok. Now, I can look at food labels and am more aware of what I'm putting in my mouth as well as how much protein I'm eating. It's been interesting to really see how much protein I am actually eating. Even potatoes and grains have put my protein intake over the limit. I'm now using more low-protein foods, and my level has decreased."

Julie shared this experience:

> "When my parents used to count milligrams of phenylalanine, it just seemed so foreign to me, and I knew I could never maintain that system. Now that I can look at labels and know what my protein goal is, I am so much more aware of what I'm eating, and why my phenylalanine levels have been higher than goal. It's not easy trying to stay under that goal, but I have the tools to do it now."

Theme 2: "Easier"

Many of those interviewed discussed their feelings towards this theme after using the simplified diet.

Joe explains,

> "The simplified diet is much easier and encourages me to eat more fruits and vegetables. I can focus my attention on counting the protein that needs to be counted from reading labels, and just eat foods naturally low in protein."

Several felt similarly about the theme "Easier"

> "The simplified diet is so much easier since I don't have to count the small stuff."

> "Things have been easier since starting Kuvan, but now the Simplified Diet has made PKU management easier too."

Theme 3: "Realistic"

This theme was articulated by many as they had previously struggled with older counting systems they felt were unrealistic to maintain as they have gotten older and have assumed responsibility over their PKU diet management.

Scott discussed his feelings about this:

> "The simplified diet makes more sense to use, especially in social situations" It makes so much more sense to count grams of protein, and much more realistic to add everything up at the end of the day, without having to count every milligram of food I've eaten."

Mary related similar feelings:

> "I don't worry about counting all of the fruits and vegetables anymore, and can focus on foods that need to be counted."

Natalie described her feelings about using the Simplified Diet

> "The Simplified Diet is easy to use and has become a part of my routine now. I find it easy to track my protein intake using my app, and if I don't see if in the database, I can use the label on the food. It's so much more practical, especially since I'm busy with work."

Table 5: Summary of Converged Data

Time of Intervention	**Qualitative Theme**	**Quantitative Finding**
Baseline	Challenges	Mean PHE = 666 µmol/L
Baseline	Counting System: Responsibility and Transition	Only 10 of 30 (33%) counting protein/PHE, 67% estimating/avoiding high-protein foods
Follow-up	Awareness	20 of 30 (67%) now counting PHE/protein
Follow-up	Easier	Statistically significant reduction in PHE level
Follow-up	Realistic	Change in PHE occurred across all demographic groups, 22 of 30 (73%)

CHAPTER 4

DISCUSSION

The results of this study have important implications for clinical practice. This study demonstrated that the Simplified Diet method, including the use of protein counting as well as free use of fruits and vegetables containing phenylalanine of 50 mg PHE/100 g food or less, improved metabolic control in teenagers and adults with PKU. This study also described several positive attitudes and behaviors towards using the PKU Diet while using the Simplified Diet method.

There are numerous results which converge and connect the qualitative and quantitative data from this study. First, the themes of “Challenges” at baseline, describing the challenges and inconsistency of the PKU diet were apparent in the suboptimal PHE levels, as well as the lack of tracking protein intake by most individuals. Mean PHE levels were almost two times above the therapeutic goal of 120-360 umol/L, and only one-third of the participants were tracking their protein intake.

Based on the Nebraska Newborn Screening outcomes data, there isn’t sufficient evidence to use the Simplified Diet method in the young group of birth to 12 years of age. PHE control in this age group is readily achieved by parents or caregivers who are concerned about brain development and consequences as a result of suboptimal PHE control. This was apparent in the study’s qualitative theme of “Counting System: Responsibility and Transition”. Many described at both enrollment and after use of the Simplified Diet that their parents had weighed and measured everything they had consumed as a child, and they weren’t willing to continue doing this as they got older. Even though there was a significant improvement in PHE levels after using the Simplified Diet method, metabolic control was still not in the optimal 120-360 µmol/L

range. Positive attitudes towards managing their diet were observed, with positive themes of the Simplified Diet method being realistic, easier, and awareness of their diet being felt by many. As children start school, and become more independent from their parent's PKU diet diligence, perhaps the Simplified Diet method needs to be initiated at a much earlier age to both the parents/caregivers and children, so that diet management can be easier as the children get older and eventually transition to self-care.

The decrease in PHE levels was seen in all demographic groups. This is encouraging from a patient education standpoint, in that it showed that the educational materials developed to teach the Simplified Diet method were appropriate and effective for all patient groups. There was no significant difference in TYR levels and there was an increase in BMI after use of the Simplified Diet method, although not significant. This increase in BMI was corroborated with an increase in caloric intake after use of the Simplified Diet method. Even though PHE levels decreased among the group, participants were able to eat foods without counting them, including fruits and vegetables, and this caloric increase could have accounted for the increase in BMI.

The length of time participating in the study varied among participants. When the study was initially designed, the intent was to follow each participant as part of their routine medical care, assuming most were seen on a yearly basis. It was soon realized, that this standard has more variability in it, and individualizing each patient's plan of care was the first priority. Even with this variability in length of participation, there was no correlation between length of study participation and decrease in PHE level.

There was little variability in the frequency of blood PHE levels during the study period. Even with a small $5 gift card incentive to get blood levels while using the Simplified Diet method, there wasn't a significant increase in blood level testing. One

could make the argument that diet had no effect on the frequency of blood PHE measurements with a majority of participants testing only once per year; however, not only did the mean PHE decrease among study participants compared with the previous year, the average PHE level using the Simplified Diet method was lower than the previous 3 years among those included in the Nebraska Newborn Screen Program Annual Report from 2014-2016. Figure 1 provided us with the data from the Nebraska Newborn Screen Program. Optimal PHE range of 2-6 mg/dl equates to 120-360 μmol/L. The percentage of adults who were able to achieve an optimal level in the 120-360 μmol/L increased from 9% at enrollment to 32% after using the Simplified Diet method, and was reported as 25-39% by the Nebraska Newborn Screening Program.

Figure 1: Percent of Nebraskans with PKU whose average PHE levels were in the optimal 2-6 mg/dL range for their age group.[52-54]

This study population included patients from different states, where metabolic food and formula programs do not offer the same level of support compared to Nebraska, which may further affect adherence to the PKU diet. This was highlighted in the theme of “Easier” after use of the Simplified Diet method, when a study participant from a surrounding state who does not receive any funding for low-protein foods, suggested that counting protein offered a much easier system to track his protein intake, since he was couldn’t afford special low-protein foods on his own, and purchased “regular foods” at the grocery store and could still easily track his protein intake.

Stratifying the data into various patient categories to determine if the change in PHE was more significant did not indicate any particular group that had a greater decrease in PHE. The PHE decrease in the marital status ($p=0.067$) and education level ($p=0.120$) groups did not reach statistical significance. Among the marital status group,

those who were married tended to show a larger difference in PHE levels compared to the unmarried counterparts. This could be due to an increased support system, especially with reading labels and food selection. Among the education group, those with the least amount of education tended to show a larger but not statistically significant difference in PHE. This finding is optimistic, as I feel the Simplified Diet method has given patients a useful tool to use in their everyday lives, instead of assuming they were using the traditional system of counting milligrams of phenylalanine, even though they have probably never used this system as they have gotten older, but parents followed consistently when they were younger. By teaching label reading and protein counting, the group with the least amount of education may have proven they had more to learn and apply to their daily lives. The theme of "realistic" was discussed by several of the participants as it related to finding effective tools to manage their PKU, and the Simplified Diet method helped them to keep track of a diet they may have never been given the tools to use in the past.

Self-reported counting methods provided incidental, insightful results. Asking each patient to answer this simple question was initially intended to match the amount stated as part of each participant's routine clinic visit, but transformed into a much different story about how patients were actually managing their diet. Observances had been made in years past, when patients would try and quantify their protein intake in clinic. A protein amount was "estimated" by the nutrition therapist instead of patients actually knowing exactly how much protein they were consuming. This curiosity in knowing current practice provided much-needed evidence that patients needed to be re-educated with a system actually applicable to their current lifestyles. The fact that there was a statistically significant increase in the number of participants who actually counted their diet, as compared to those who "just avoided high protein foods" showed the

success of the Simplified Diet method in terms of its practical application to patients' lives. The theme of "awareness" eluded to the results of the self-reported counting methods. Several participants felt that the Simplified Diet method actually made them look at what they were eating, whereas before, they may have only relied on old lists of "good" and "bad" foods. I feel the Simplified Diet method made them aware of the reality of what they were actually eating, and not by assumptions of what they thought they were supposed to be eating. This improvement did not mean all participants reported counting their intake after using the Simplified Diet method, which means additional efforts are needed to empower our patients to educate, and re-educate, depending on the circumstances of each patient.

It is important to look at all three counting systems (counting PHE, protein counting, and estimation) compared to mean PHE levels at both baseline and follow-up time periods. At baseline, all three systems had a mean PHE level above 120-360 µmol/L range. PHE counting had a mean PHE level of 402 µmol/L; protein counting had a mean PHE level of 770 µmol/L, and mean PHE was 604 µmol/L for the estimate/avoidance of high protein foods. After use of the Simplified Diet method intervention, the PHE counting group had a mean PHE of 26 µmol/L, while the protein counting group was greatly reduced to 490 µmol/L, and mean PHE for the group who estimated was similar to baseline at 615 µmol/L. This is encouraging data, suggesting effective educational efforts.

Use of apps to track protein intake was limited, even after the use of the Simplified Diet method. There has been no research published on the use of apps to manage PKU, but there is evolving evidence on the use of apps to manage chronic health conditions. This technology could become a very useful management tool, and will need to be studied for its place and effectiveness with the PKU diet.

As there is no “gold standard” method of dietary intake assessment, the method chosen largely depends on the research question, population, group, available resources, and the foods and nutrient of interest.[74,76] In the area of metabolic nutrition and PKU, the three-day food record has long been recognized as the “gold standard”, which was the reason it was used for this study. Unfortunately, the small numbers of food records collected at both enrollment and at the follow-up visit made it very difficult to make many clinical and statistical decisions about patients’ intake and the effect of the Simplified Diet method on patients’ nutritional status. This data was first analyzed based on only 4 paired food records to determine if there were any differences among the participants. With no significant differences detected, the nutrient intakes were analyzed using all food records received in order to gather a better snap-shot of current intakes compared with the DRI. The DRI values were based on the average age of the study, and midpoints were used to capture any differences between men and women. There were little differences in calorie, protein, carbohydrate, or Vitamin C intakes. There was only an average increase of 84 calories/day using the Simplified Diet method and both calorie intakes at enrollment and after Simplified Diet were less than 100% DRI. Despite the small caloric increase, BMI increased, and the average BMI for the group is considered overweight. Mean PHE intakes increased 325 mg per day, despite the significant decrease in phenylalanine levels. Owing to the small number of food records turned in after following the Simplified Diet method, it is difficult to understand the large increase in PHE intake. Tyrosine intake also significantly increased after use of the Simplified Diet method, and likely coincided with reported increase in PHE. Total fat, fiber, and calcium intakes all increased after use of the Simplified Diet method. It is likely that fiber intake increased due to the increased of fruits and vegetables, since patients could use many fruits and vegetables freely. Several participants reinforced the

theme “easier” by stating they ate more fruits and vegetables using the Simplified Diet method.

It is possible that PHE from fruits and vegetables may not be absorbed efficiently. It is known that vegetable protein is the least digestible of all proteins and a correction factor of 85% is given for digestibility for diets based mainly on vegetables and unrefined carbohydrates.[74] The nonstarch polysaccharide content of some of the fruits and vegetables may also reduce apparent digestibility by up to 10% and increase nitrogen excretion in the feces.[76]

Based on the fact that many patients self-reported their estimated intakes and avoidance of high protein foods at enrollment and due to the lack of food records analyzed in this study, it is hard to know if allowing free use of fruits and vegetables with ≤50 mg PHE/100 g food actually increased their PHE consumption, although the blood PHE levels would strongly argue otherwise. It is unlikely that any change in energy intake explained the lack of effect of extra dietary phenylalanine on plasma phenylalanine, as the caloric intake and mean BMI did increase with use of the Simplified Diet method.

There were significant changes in the attitudes in all age groups using the Simplified Diet. In the younger participants, the positivity was felt in their realization they had the proper tools to manage their own PKU diet, and served as a powerful transition tool for parents who had managed everything regarding their PKU management prior to starting this study. In the adults, it was realizing they could effectively manage their diet, without expecting them to count everything they put in their mouths, and understanding that a simpler system was essentially more effective since many weren’t using any system at all prior to the start of this study.

In summary, this study demonstrated that the Simplified Diet diet utilized in this study was an effective approach to manage the PKU diet that allowed teens and adults with PKU to consume foods that contained lower amounts of PHE without measuring or counting them. It provided increased flexibility, promoted healthy food choices and was an easier PKU diet management tool than the traditional method of counting all PHE consumed.

Challenges

Challenges with teaching and implementing the Simplified Diet method were minimal. Changing to the new system was well-accepted by the study participants, but seemed to pose a greater challenge with parents who had followed the diet meticulously and carefully for many years. The parents' attitudes were not collected or analyzed, but their initial hesitancy to what they thought was "giving up control" was noted. After seeing the success their teenage children had with improved PHE levels, along with new-found independence, parents provided another source of support for continued use of the Simplified Diet method.

Another potential challenge is using the Simplified Diet method with individuals who have a low tolerance to phenylalanine, less than 250-300 mg/day. For these individuals, the Simplified Diet method becomes more limited in the remaining amount of protein patients are allowed to use labels for, since a majority of their protein could come from the "free" use of fruits and vegetables. Additional education may be necessary to find the right balance of the number of grams allowed per day. Using the Simplified Diet method will still provide a more effective and consistent way to achieve PHE level control, as many of these patients may have just estimated their intakes in the past and

not actually counted their intake, making it very difficult to titrate protein intake to optimize metabolic control.

Limitations

This study had several limitations. First, it was an open study, and no one was blinded from the results. There was not a separate control group. Each patient served as their own control, utilizing their historical PHE control from the year prior to enrolling in the study. During food record analysis and data analyses, participants were given a study number, so identities were not readily known during the study. The study involved motivated PKU patients, which may have introduced ascertainment bias favoring success. There will still be PKU patients who are non-compliant with even the Simplified Diet method.

The limited number of food records obtained from participants at both time points limited the ability to quantify any significant differences the Simplified Diet method had on participants' nutrient intakes. The limited number of individuals completing food records highlighted the need for an accurate 24-hour recall tool that can be used in the PKU population for easier and a more accurate way to predict the effect of diet on PKU control.

Future Directions

PKU is the "poster child" for much of our understanding of inborn errors of metabolism (IEM), and exemplifies successful disease management by dietary manipulation and its impact on the patient, family, and society.[79] The use of the Simplified Diet only adds to this understanding of PKU and provides abundant new opportunities to be utilized and studied in the future as shown in Figure 10.

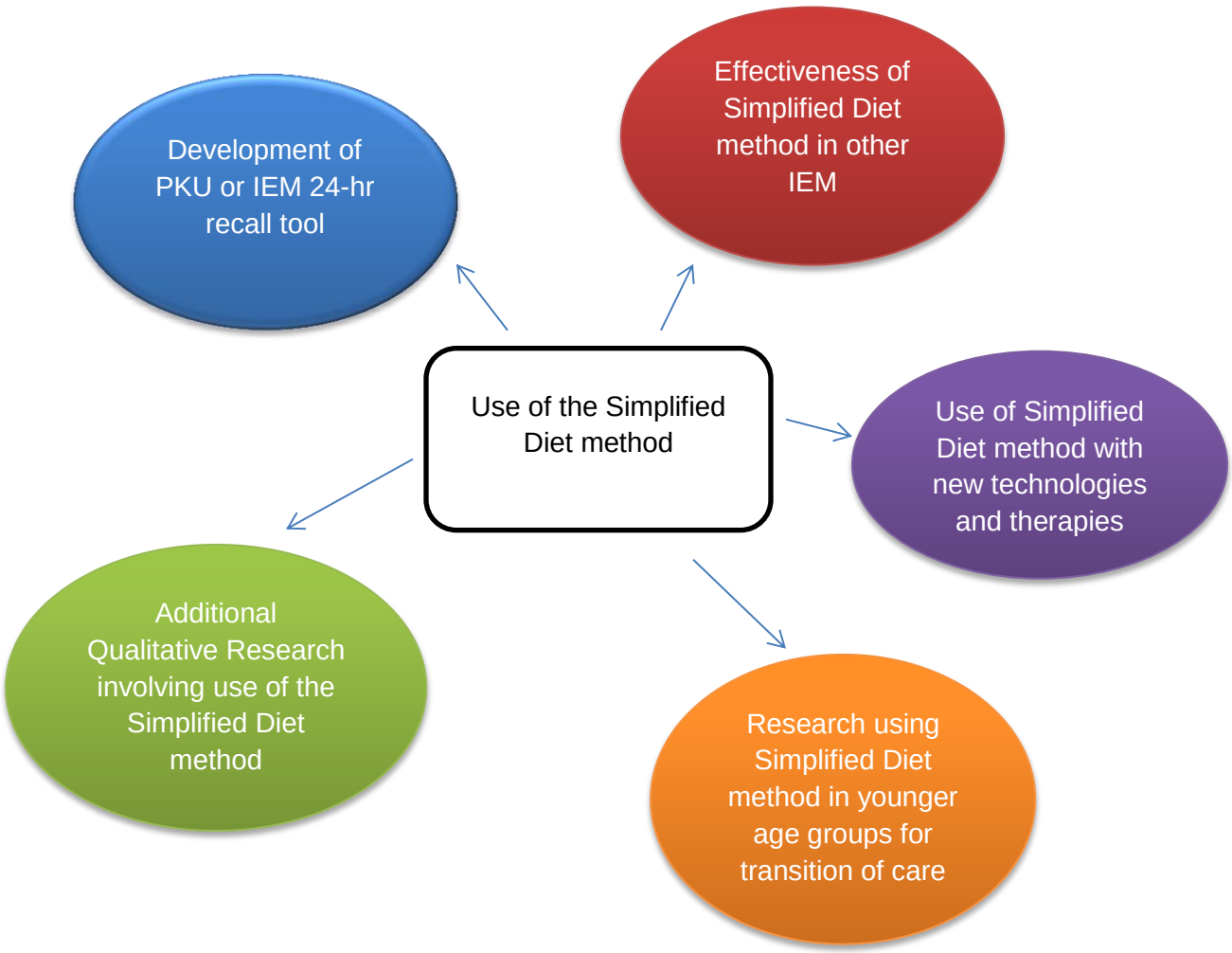
Future research utilizing the Simplified Diet method is multi-faceted. The Simplified Diet should be studied in other IEMs including Maple Syrup Urine Disease (MSUD), Glutaric Acidemia Type 1 (GA-1), Propionic Acidemia (PA) and Methylmalonic Acidemia (MMA) where traditional diet approaches with counting specific amounts of offending amino acids may not be a realistic or effective approach to maintaining metabolic control for all patients. Research using younger age groups is also needed, not only to improve metabolic control, but also to develop effective approaches to transition of care from parents or caregiver to child, and determining most appropriate age to start this transition in order to maintain optimal metabolic control throughout their lifetime.

Using the Simplified Diet method to help develop a a concise food list for use in a web-based 24-hour dietary recall tool could be very beneficial for PKU patients and others with an IEM. This new dietary tool could provide a much more accurate picture of current nutrient intakes as compared to three-day food records and further enhance opportunities for effective medical nutrition therapy for all IEM's.

Additional qualitative research is needed to help describe the attitudes of those using the Simplified Diet method. Whether qualitative alone, or mixed, this much-needed research can help shape future techniques and recommendations based on patients' experiences and feedback.

Using the Simplified Diet method with research involving new technologies and therapies could provide critical evidence needed for future development and treatment for PKU. The Simplified Diet method could be used as new apps are developed to make tracking the diet easier on smartphones or any other portable devices. The Simplified

Figure 10: The Future of Research Involving the Use of the Simplified Diet Method



Diet method could also be utilized with use of a home PHE monitor, currently under development. The Simplified Diet method could also be used with future new treatments for PKU, such as enzyme therapy, that have the potential to significantly increase the PHE intakes of patients with PKU. With new PKU treatments on the horizon, dietary treatment will need to evolve and keep up with the changing needs of our patients. In this respect, the Simplified Diet method will be utilized to optimize patients' outcomes.

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APPENDIX A: ADULT CONSENT FORM

ADULT CONSENT - CLINICAL BIOMEDICAL

Title of this Research Study

Use of Dietary Protein Counting and Free Fruits and Vegetables to Improve Metabolic Control Among Teens and Adults With Phenylketonuria: a Mixed Methods Approach

Invitation

You are invited to take part in this research study. You have a copy of the following, which is meant to help you decide whether or not to take part:

- Informed consent form
- "What Do I need to Know Before Being in a Research Study?"
- The Rights of Research Subjects

Why are you being asked to be in this research study?

You are being asked to be in this study because you have phenylketonuria (PKU) and you are an adult patient 19-65 years of age and are seen at the Metabolic Clinic at the University of Nebraska Medical Center (UNMC).

If you are pregnant, nursing an infant, or plan to become pregnant during this study, you may not be in this study.

What is the reason for doing this research study?

The purpose of this study is to determine if phenylalanine levels can be improved using a simplified protein counting system, including unlimited use of some fruits and vegetables naturally low in protein. This study will also look at attitudes and feelings towards current and new protein counting systems.

What will be done during this research study?

In this study, you will be educated on using a new protein counting system, which includes counting grams of protein, as well as unlimited use of several fruits and vegetables, which are very low in protein. You will be given a handout, called "Diet for Life--the PKU Way" and a compact food list you can keep with you to help track your protein intake. In addition to the 3-day food record you bring to clinic, you will also be

asked to answer a few questions about yourself and your current nutrition practices. You will also be asked several questions about your attitudes and feelings towards your current counting system, and after following this new system for 1 year, you will be asked the same questions again.

You should follow the recommendations the clinic gives you for getting your phenylalanine levels drawn over the course of a year, and we will be following these levels closely. We will use your phenylalanine levels from the previous year and while you are in the study to see if the new system of counting has any impact on your Phe levels.

What are the possible risks of being in this research study?

The risks associated with this study include the possibility of your phenylalanine level becoming elevated, which is also possible with standard of care diet treatment. This elevation could cause you to have issues with concentration, as well as mood and memory changes. If you feel these symptoms, please call us.

There is also a risk for the the loss of confidentiality.

What are the possible benefits to you?

Your Phe levels may decrease, improving metabolic control of your PKU.
You may not get any benefit from being in this research study.

What are the possible benefits to other people?

The results of this study may help scientists learn more about PKU and how to manage and treat it.

What are the alternatives to being in this research study?

If you choose not to participate in this study, you can ~~will~~ continue with your current way of tracking your phenylalanine or protein intake.

What will being in this research study cost you?

There is no cost for you to participate in this research study. All of your regular medical care you are currently receiving for your PKU will be billed to insurance.

Will you be paid for being in this research study?

You will receive a $5 gift card for each phenylalanine level collected while you are in this study.

Who is paying for this research?

This research is being paid for by the Principal Investigator.

What should you do if you are injured or have a medical problem during this research study?

Your welfare is the main concern of every member of the research team. If you are injured or have a medical problem or some other kind of problem as a direct result of being in this study, you should immediately contact one of the people listed at the end of this consent form.

How will information about you be protected?

You have rights regarding the protection and privacy of your medical information collected before and during this research. This medical information is called "protected health information" (PHI). PHI used in this study may include your medical record number, address, birth date, medical history, the results of physical exams, blood tests, x-rays as well as the results of other diagnostic medical or research procedures. Only the minimum amount of PHI will be collected for this research. Your research and medical records will be maintained in a secure manner.

Who will have access to information about you?

By signing this consent form, you are allowing the research team to have access to your PHI. The research team includes the investigators listed on this consent form and other personnel involved in this specific study at the Institution.

Your PHI will be used only for the purpose(s) described in the section What is the reason

for doing this research study?

You are also allowing the research team to share your PHI, as necessary, with other people or groups listed below:

- The UNMC Institutional Review Board (IRB)
- Institutional officials designated by the UNMC IRB
- Federal law requires that your information may be shared with these groups:
    - The HHS Office of Human Research Protections (OHRP)

You are authorizing us to use and disclose your PHI for as long as the research study is being conducted.

You may cancel your authorization for further collection of PHI for use in this research at any time by contacting the principal investigator in writing. However, the PHI which is included in the research data obtained to date may still be used. If you cancel this authorization, you will no longer be able to participate in this research.

How will results of the research be made available to you during and after the study is finished?

In most cases, the results of the research can be made available to you when the study is completed, and all the results are analyzed by the investigator or the sponsor of the research. The information from this study may be published in scientific journals or presented at scientific meetings, but your identity will be kept strictly confidential.

If you want the results of the study, contact the Principal Investigator at the phone number given at the end of this form or by writing to the Principal Investigator at the following address: Jill Skrabal, MS, RD, LMNT, CDE, 981200 Nebraska Medical Center, Omaha, NE 68198-1200.

What will happen if you decide not to be in this research study?

You can decide not to be in this research study. Deciding not to be in this research will not affect your medical care or your relationship with the investigator or the Institution. Your doctor will still take care of you and you will not lose any benefits to which you are entitled.

What will happen if you decide to stop participating once you start?

You can stop participating in this research ("withdraw") at any time by contacting the Principal Investigator.

Deciding to withdraw will otherwise not affect your care or your relationship with the investigator or this institution. You will not lose any benefits to which you are entitled.

Any research data obtained to date may still be used in the research.

Will you be given any important information during the study?

You will be informed promptly if the research team gets any new information during this research study that may affect your treatment plan.

What should you do if you have any questions about the study?

You have been given a copy of "*What Do I Need to Know Before Being in a Research Study?*" If you have any questions at any time about this study, you should contact the Principal Investigator or any of the study personnel listed on this consent form or any other documents that you have been given.

What are your rights as a research participant?

You have rights as a research subject. These rights have been explained in this consent form and in The Rights of Research Subjects that you have been given. If you have any questions concerning your rights or complaints about the research, you can contact any of the following:

- The investigator or other study personnel
- Institutional Review Board (IRB)
    - Telephone: (402) 559-6463
    - Email: IRBORA@unmc.edu
    - Mail: UNMC Institutional Review Board, 987830 Nebraska Medical Center, Omaha, NE 68198-7830
- Research Subject Advocate
    - Telephone: (402) 559-6941
    - Email: unmcrsa@unmc.edu

Documentation of informed consent

You are freely making a decision whether to be in this research study. Signing this form means that:

- You have read and understood this consent form.
- You have had the consent form explained to you.
- You have been given a copy of The Rights of Research Subjects
- You have had your questions answered.
- You have decided to be in the research study.
- If you have any questions during the study, you have been directed to talk to one of the investigators listed below on this consent form.
- You will be given a signed and dated copy of this consent form to keep.

Signature of Subject _____
Date _____

My signature certifies that all the elements of informed consent described on this consent form have been explained fully to the subject. In my judgment, the subject possesses the legal capacity to give informed consent to participate in this research and is voluntarily and knowingly giving informed consent to participate.

Signature of Person obtaining consent _____
Date _____

Authorized Study Personnel

Principal

* Skrabal, Jill
phone: 402-559-3660
alt #: 402-559-2550
degree: RD

Faculty Advisor

Rizzo, William
phone: 402-559-2560
alt #: 402-559-2560
degree: MD

University of Nebraska Medical Center

IRB PROTOCOL #451-15-EP

IRBVersion: 2

APPENDIX B: PARENTAL CONSENT FORM

PARENTAL CONSENT - CLINICAL BIOMEDICAL

Title of this Research Study

Use of Dietary Protein Counting and Free Fruits and Vegetables to Improve Metabolic Control Among Teens and Adults With Phenylketonuria: a Mixed Methods Approach

Invitation

You are invited to allow your child to take part in this research study. You have a copy of the following, which is meant to help you decide whether or not to allow your child to take part:

- Informed consent form
- "What Do I need to Know Before Being in a Research Study?"
- The Rights of Research Subjects

Why is your child being asked to be in this research study?

Your child is being asked to be in this study because he/she has phenylketonruia (PKU)~~, and they are~~ is between the ages of 13-18 years of age and is seen at the Metabolic Management Clinic at Children's Hospital and Medical Center.

If your child is pregnant, nursing an infant, or plans to become pregnant during this study, she may not be in this study.

What is the reason for doing this research study?

The purpose of this study is to determine if your child's phenylalanine levels can be improved using a simplified protein counting system, including unlimited use of some fruits and vegetables naturally low in protein. This study will also look at your child's attitudes and feelings towards his/her current and new protein counting system.

What will be done during this research study?

In this study, your child will be taught how to use a new protein counting system, which includes counting grams of protein, as well as unlimited use of several fruits and vegetables, which are very low in protein. Your child will be given a handout, called "Diet for Life-the PKU Way" and a compact food list he/she can keep to help track protein intake. In addition to the 3-day food record your child brings to clinic, he/she will also be asked to answer a few questions about him/herself and his/her current nutrition practices. Your child will also be asked several questions about his/her attitudes and feelings towards their current counting system, and after following this new system for 1 year, your child will be asked the same questions again.

Your child should follow the recommendations the clinic gives for getting their phenylalanine levels drawn over the course of a year, and we will be following these levels closely. We will use your child's phenylalanine levels from the previous year and while your child is in the study to see if the new system of counting has any impact on your child's Phe levels.

What are the possible risks of being in this research study?

The risks associated with this study include the possibility of your child's phenylalanine level becoming elevated, which is also possible with standard of care diet treatment. This elevation could cause your child to have issues with concentration, as well as mood and memory changes. If you feel like your child is having these symptoms, please call us.

There is also a risk for the loss of confidentiality. .

What are the possible benefits to your child?

Your child's Phe levels may decrease, improving metabolic control of his/her PKU.

Your child may not get any benefit from being in this research study.

What are the possible benefits to other people?

The results of this study may help scientists learn more about PKU and how to manage and treat it.

What are the alternatives to being in this research study?

If you choose for your child to not participate in this study, your child cancontinue with his/her current way of tracking ~~their~~ phenylalanine or protein intake.

What will allowing your child to be in this research study cost you?

There is no cost for your child to participate in this research study. All of your child's regular medical care he/she is currently receiving for their PKU will be billed to insurance.

Will you or your child be paid for being in this research study?

You will receive a $5 gift card for each phenylalanine level collected while your child is in this study.

Who is paying for this research?

This research is being paid for by the Principal Investigator.

What should you do if your child is injured or has a medical problem during this research study?

Your child's welfare is the main concern of every member of the research team. If he/she is injured or has a medical problem or some other kind of problem as a direct result of being in this study, you should immediately contact one of the people listed at the end of this consent form.

How will information about your child be protected?

Your child has rights regarding the protection and privacy of his/her medical information collected before and during this research. This medical information is called "protected health information" (PHI). PHI used in this study may include his/her medical record number, address, birth date, medical history, the results of physical exams, blood tests, x-rays as well as the results of other diagnostic medical or research procedures. Only the minimum amount of PHI will be collected for this research. Your child's research and medical records will be maintained in a secure manner.

Who will have access to information about your child?

By signing this consent form, you are allowing the research team to have access to your child's PHI. The research team includes the investigators listed on this consent form and other personnel involved in this specific study at the Institution.

Your child's PHI will be used only for the purpose(s) described in the section "What is the reason for doing this research study?"

You are also allowing the research team to share his/her PHI, as necessary, with other people or groups listed below:

- The UNMC Institutional Review Board (IRB)
- Institutional officials designated by the UNMC IRB
- Federal law requires that the subject's information may be shared with these groups:
    - The HHS Office of Human Research Protections (OHRP)

You are authorizing us to use and disclose your child's PHI for as long as the research study is being conducted.

You may cancel your authorization for further collection of your child's PHI for use in this research at any time by contacting the principal investigator in writing. However, the PHI which is included in the research data obtained to date may still be used. If you cancel this authorization, your child will no longer be able to participate in this research.

How will results of the research be made available to you during and after the study is finished?

In most cases, the results of the research can be made available to you when the study is completed, and all the results are analyzed by the investigator or the sponsor of the research. The information from this study may be published in scientific journals or presented at scientific meetings, but your child's identity will be kept strictly confidential.

If you want the results of the study, contact the Principal Investigator at the phone number given at the end of this form or by writing to the Principal Investigator at the following address: Jill Skrabal, MS, RD, LMNT, CDE, 981200 Nebraska Medical Center, Omaha, NE 68198-1200.

What will happen if you decide not to give permission for your child to be in this

research study?

You can decide not to give permission for your child to be in this research study. Deciding not to be in this research will not affect your child's medical care or his/her relationship with the investigator or the Institution. Your child's doctor will still take care of him/her. Your child will not lose any benefits to which he/she is entitled.

What will happen if you decide to stop your child's participation once it starts?

You can stop your child's participation in this research ("withdraw") at any time by contacting the Principal Investigator.

Deciding to withdraw will otherwise not affect your child's care or relationship with the investigator or this institution. Your child will not lose any benefits to which he/she is entitled.

Any research data obtained to date may still be used in the research.

Will you be given any important information during the study?

You and your child will be informed promptly if the research team gets any new information during this research study that may affect your child's treatment plan..

What should you do if you have any questions about the study?

You have been given a copy of *"What Do I Need to Know Before Being in a Research Study?"* If you have any questions at any time about this study, you should contact the Principal Investigator or any of the study personnel listed on this consent form or any other documents that you have been given.

What are your child's rights as a research subject?

Your child has rights as a research subject. These rights have been explained in this consent form and in The Rights of Research Subjects that you have been given. If you have any questions concerning his/her rights or complaints about the research, you can contact any of the following:

- The investigator or other study personnel
- Institutional Review Board (IRB)

    - Telephone: (402) 559-6463.
    - Email: IRBORA@unmc.edu
    - Mail: UNMC Institutional Review Board, 987830 Nebraska Medical Center, Omaha, NE 68198-7830
- Research Subject Advocate
    - Telephone: (402) 559-6941
    - Email: unmcrsa@unmc.edu

Documentation of informed consent

You are freely making a decision whether to give permission for your child to be in this research study. Signing this form means that:

- You have read and understood this consent form.
- You have had the consent form explained to you.
- You have been given a copy of The Rights of Research Subjects
- You have had your questions answered.
- You have decided to permit your child to be in the research study.
- If you have any questions during the study, you have been directed to talk to one of the investigators listed below on this consent form.
- You will be given a signed and dated copy of this consent form to keep.

Signature of Parent/Guardian _____
Date _____

You are agreeing to be in this research study. You have had someone explain the study to you, and answer your questions.

Signature of Subject _____
Date _____

My signature certifies that all the elements of informed consent described on this consent form have been explained fully to the parent(s)/guardian(s) of the subject. In my judgment, the parent(s)/guardian(s) possesses the legal capacity to give informed consent for the subject to participate in this research and is voluntarily and knowingly giving informed consent.

Signature of Person obtaining consent _____
Date _____

Authorized Study Personnel

Principal

* Skrabal, Jill
phone: 402-559-3660
alt #: 402-559-2550
degree: RD

Faculty Advisor

Rizzo, William
phone: 402-559-2560
alt #: 402-559-2560
degree: MD

PT NAME

MR #

IRB PROTOCOL #451-15-EP

IRBVersion: 2

APPENDIX C: YOUTH INFORMATON SHEET

YOUTH INFORMATION SHEET

Title

Use of Dietary Protein Counting and Free Fruits and Vegetables to Improve Metabolic Control Among Teens and Adults With Phenylketonuria: a Mixed Methods Approach

Description

You are invited to be in this research study. Being in this research study is voluntary you don't have to be in this research study to get treated. If you decide not to be in the study your doctor will still take care of you.

The goal of this study will be to see if your phenylalanine levels improve by using a simplified protein counting system with free fruits and vegetables.

You will be taught how to use a new counting system to help manage your PKU. You will be given a handout and a food list you can use to help track your protein intake. In addition to the 3-day food record you bring to clinic, you will be asked asked several questions about your attitudes and feelings towards your current counting system,. You will also be asked questions about your attitudes and feelings towards your current system, and after following this new system for 1 year, you will be asked the same questions again. We will look in your medical records at your phenylalanine levels one year before you started being in this study and then for the whole time you are in the study. We will compare these to see if there is any effect on your levels when you follow the simplified counting system.

The main risk of being in this research is that others may find out you are in this study. This could occur because this research will occur with standard of care treatment, including at least monthly phenylalanine levels. .

You can stop being in this study at any time.

PT NAME

MR #

IRB PROTOCOL #451-15-EP

APPENDIX D: 3-DAY FOOD RECORD

CHILDREN'S HOSPITAL AND MEDICAL CENTER
UNIVERISITY OF NEBRASKA MEDICAL CENTER
PEDIATRIC AND ADULT METABOLIC MANAGEMENT CLINICS
3-day food record**

Please record food and drink consumed for **three (3) consecutive days before a phenylalanine level or before a clinic visit. Bring this form with you or email to **JiSkrabal@nebraskamed.com**

****

Name:______________________________

How is formula mixed? (make sure to include formula intake on food record)

____

______oz water to make ______oz formula

DATE /TIME	FOOD/DRINK (please include brand names if available)	AMOUNT	PHE(mg) or PRO (g)

APPENDIX E: QUALITATIVE RESEARCH QUESTIONS

Qualitative Study Questions:

Central question:

How do you feel about your current phe/protein counting system?

Sub-questions:

How has your role in managing your PKU changed as you have gotten older?

If you could design the “perfect” phe/protein counting system you could teach to other patients, describe to me what that would be.

APPENDIX F: PATIENT EDUCATION HANDOUT

"DIET FOR LIFE—THE PKU WAY"

3 simple steps to managing your PKU

P- Plenty of "free" fruits and vegetables

K- Keep track of your protein intake

U- Use of PKU formula

This handout is designed to give you additional tools to help manage your PKU. You may be managing your diet differently now, but please follow these steps and direction from your dietitian to help manage your PKU in a different way…

Turn the page to get started…….

Step 1:

P- Plenty of "free" fruits and vegetables

The fruits and vegetables listed below contain small amounts of protein, whether they are fresh, frozen, canned, or in juice form. You can eat these fruits and vegetables without worrying about weighing measuring or counting.

FRUITS

Apples
Apple chips
Applesauce
Apricots
Bananas
Blackberries
Blueberries
PearBoysenberries
Breadfruit
Cantaloupe
Casaba Melon
Cherimoya
Cherries
Crabapples
Cranberries
Elderberries
Crenshaw Melon
Figs
Fruit Cocktail
Gooseberries
Grapefruit
Grapes
Guava
Honeydew Melon
Kiwi
Kumquats
Lemons
Limes
Loganberries
Mandarin oranges
Mangos
Mulberries
Nectarines
Oranges
Papaya
Passion-fruit
Peaches
Persimmons
Pineapple
Plaintains
Plums
Pomegranate
Prickly
Prunes
Pummelo
Quince
Raisins
Raspberries
Rhubarb
Star Fruit
Strawberries
Tangelo
Tangerines
Watermelon

VEGETABLES

Beets
Bok Choy
Cabbage, Chinese
Cabbage, green
Cabbage, red
Carrots
Cassava root
Celery
Chard Swiss
Chayote
Chicory greens
Cucumbers
Eggplant
Grape leaves
Jicama
Kohlrabi
Leeks
Lemon Grass
Lettuce
Lotus Root
Mushrooms (Crimini)
Onions
Parsley
Parsnips
Peppers (Bell)
Pimentos
Pumpkin
Radicchio
Radishes
Rutabaga
Sauerkraut
Squash, Acorn
Squash, Butternut
Squash, Spaghetti
Taro
Tomatillo
Tomato Sauce
Tomatoes
Turnips
Waterchestnuts
Yuca (Cassava)

Step 2:
K- Keep track of your protein intake

Keeping track of your protein intake is essential to keeping your phenylalanine level in the goal range.

Tips:

- **You do not need to include the free fruits and vegetables.**
- **You do need to count fruits and vegetables higher in protein, This includes corn, potatoes, mushrooms, and other fruits and vegetables not listed on the "free" list**
- **You also need to count any other foods you eat. Use the food label to help you determine how much protein you are eating or drinking.**
- **In addition to the food label, use "PKU Food List" or other PKU resource to help you look up protein content of foods, and track your daily intake**

Using a food label to track protein intake:

Nutrition Facts

Serving Size 1 ounce Servings in bag 4

Amount Per Serving	
Calories 155	Calories from Fat 93
	% Daily Value*
Total Fat 11g	16%
Saturated Fat 3g	15%
Trans Fat	
Cholesterol 0mg	0%
Sodium 148mg	6%
Total Carbohydrate 14g	5%
Dietary Fiber 1g	5%
Sugars 1g	
Protein 2g	

Vitamin A	0%	• Vitamin C	9%
Calcium	1%	• Iron	3%

* Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

1. **Look at serving size**
2. **Look at protein grams**
3. **Multiply number of grams as needed based on number of servings consumed**

Your daily protein limit is _____grams.

Step 3:
U: Use of PKU formula

Use of PKU formula is critical for you to get enough protein your body can use. Without your PKU formula, you will not be able to achieve target phenylalanine levels, build or maintain your strength, and in many cases, consume adequate vitamins and minerals.

Below is a list of all available PKU formulas.

Your PKU Formula Prescription is:

_____

_____

Mixed in _____ oz water (if applicable)

Formula Options:

Abbott
Phenex-2 unflavored
Phenex-2 vanilla

Cambrooke
Camino Pro- pina colada, fruit
Glytactin BetterMilk
Glytactin Complete 10/15
Glytactin Restore
Glytactin Restorelite
Glytactin Swirl

Mead Johnson
Phenyl-Free 2
Phenyl-Free 2 HP

Nutricia
PhenylAde Amino Acid Blend
PKU Lophlex LQ- juicy orange, mixed berry blast, tropical
Periflex Junior- unflavored, orange
Periflex Advance-unflavored, punch, o
Periflex LQ-berry cream, orange cream
PhenylAde Essential- unflavored, chocolate, vanilla, strawberry, orange cream
PhenylAde GMP
PhenylAde 40-unflavored, citrus
PhenylAde 60-citrus, unflav
PhenylAde RTD
Phlexy-10 blackcurrant/apple, tropical surprise
Phlexy-10 tablets
PhenylAde PheBLOC- tablets, powder

Vitaflo
PKU Express 15
PKU Gel
PKU Cooler, orange, purple, white, red

The best formula to use is the one you will use daily! You can use more than 1 product if it helps you reach your prescription goal!!

APPENDIX G: DEMOGRAPHIC/NUTRITION PRACTICES

Demographic/Nutrition Practices Information

Pt ID Number:____ Date:_____
++
Ht:_____ Weight:_____

Current age:____
Highest education level:

a. Current grade level:_____ Current GPA:____
b. High school diploma or GED
c. Some college
d. Bachelor's Degree
e. Master's Degree
f. Doctoral Degree (PhD, MD, or other professional doctorate)

Current relationship status:

a. Never married
b. Never married but in a relationship
c. Married __ years
d. Divorced

What is your current method of keeping track of your phenylalanine (phe) or protein? (circle one)

a. I count mg of phenylalanine (example: no more than 300 mg/day)
b. I count grams of protein (example: no more than 5 grams protein/day)
c. I don't count—I just avoid high protein foods
d. Other: (please explain)_____
_____

Do you use any apps to keep track of your phenylalanine (phe) or protein intake?

a. No

Yes: List app names:_____

APPENDIX H: SUMMARY OF CODES/THEMES

Codes/Themes

At Enrollment

1. Challenges - “My mom used to keep track of everything, but now I don’t count anything”

   “My parents kept track of all of my food, but now that I’m on my own, I struggle to keep track of my own diet”

2. Counting System: Responsibility and Transition: “My current counting system is not accurate and I don’t take the time to look up the phe content”

After Use of Simplified Diet

1. Awareness: The simplified diet is a much better system than what I was previously using and has made me more aware of what I’m eating”
   “I am aware of the protein content of foods and I look at labels more”

2. Easier- The simplified diet is much easier and encourages me to eat more fruits and vegetables

   “The simplified diet is so much easier since I don’t have to count the small stuff”

3. Realistic- The simplified diet makes more sense to use, especially in social situations”

   It is much more realistic to add everything up from food labels, and I’m able to know how much I’m eating daily, instead of right before a phe level.