# METABOLIC CONTROL OF PATIENTS WITH PKU WHEN FRUIT AND VEGETABLE CONSUMPTION IS UNCOUNTED

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## ABSRACT

**Introduction**

Dietary management for PKU is complex and many barriers arise for the caregiver, patient, and the clinician in achieving optimal blood phe levels and clinical outcomes for this patient population. Compliance can decline over time and the quality of foods and nutrients also suffer. Noncompliance to diet increases blood phe levels and executive functioning skills worsen, such as the ability to plan, reason, and problem solving. With worsened executive functioning skills, compliance becomes more difficult and motivation to return to diet declines further.

**Methods**

A systematic review of available evidence was conducted based the Academy of Nutrition and Dietetics' Evidence Analysis Process to answer the following question: Will allowing the consumption of unlimited fruits and vegetables worsen metabolic control among PKU patients when compared to counting the phe consumed from fruits and vegetables in the daily phe allowance? Evidence Worksheets and Quality Criteria Checklists from the Academy of Nutrition and Dietetics' Evidence Analysis Process were used to critically analyze relevant research articles. A conclusion statement was written and graded based on the findings and quality of available research.

**Results**

Five studies were systematically reviewed using the criteria established by AND's Evidence Analysis Library. These five studies sought to identify the potential metabolic effects of allowing uncounted fruit and vegetable consumption has for people with PKU. Although the study designs and interventions differed between each study, they all came to the same general conclusion: allowing consumption of uncounted fruits and vegetables does not have a negative impact on the metabolic control of those with PKU.

**Conclusion**

All of the studies concluded that consumption of uncounted fruits and vegetables at varying amounts did not compromise metabolic control and even improved control in some groups of people with PKU. These studies motivate future research to find opportunities to improve the quality of life of those with PKU by simplifying diet management and encouraging variety of foods consumed. These studies also encourage practitioners to develop creative solutions when providing the most effective dietary treatment for their patients with PKU. Grade: I (good/strong evidence)

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# CHAPTER 1: INTRODUCTION

Phenylketonuria (PKU) is a genetic disorder caused by a mutation in the gene for the enzyme phenylalanine hydroxylase (PAH), which is responsible for converting phenylalanine (phe) to tyrosine in the liver (Walter, 2002). PKU presents as a spectrum disorder, with varying degrees of symptom severity depending on residual amounts of PAH remaining (Vockley, 2014). The American College of Medical Genetics and Genomics (ACMG) Practice Guidelines recommend maintaining blood phe levels within the goal range of 120-360 umol/L through all age ranges, as well as pregnancy, to achieve optimal treatment outcomes (Vockley, 2014). Manipulation of the diet is needed to maintain optimal blood phe levels and to achieve normal growth and development.

Traditional diet management for people with PKU consists of lifelong natural protein restriction coupled with a synthetic protein formula that excludes phe, has additional tyrosine, calories, fat, vitamins and minerals for all age ranges to achieve blood phe levels within the optimal treatment range, adequate growth, development and nutrient intake. When untreated in infancy, PKU can cause severe and irreversible mental retardation (Vockley, 2014). With the development and implementation of newborn screening, infants with PKU are detected within their first week of life and treatment is started immediately to prevent cognitive deficits (Vockley, 2014). Intellectual and neuropsychiatric problems can still occur among patients who have optimal blood phe levels during infancy and childhood, but have elevated blood phe levels and worsened metabolic control later in life (Vockley, 2014).

Dietary management for PKU is complex and many barriers arise for the caregiver, patient, and the clinician in achieving optimal blood phe levels and clinical outcomes for this

patient population. Compliance can decline over time and the quality of foods and nutrients also suffer. Even with adequate synthetic protein formula consumption, avoiding natural protein increases the risk of consuming a diet deprived in essential nutrients. The independence that comes with adolescence and adult years bring new challenges for diet compliance for many patients. Noncompliance to diet increases blood phe levels and executive functioning skills worsen, such as the ability to plan, reason, and problem solving. With worsened executive functioning skills, compliance becomes more difficult and motivation to return to diet declines further.

In the United States, treatment of PKU has typically included an individualized prescription for phe intake in milligrams per day from naturally lower protein foods. Phe content of fruits and vegetables are counted in milligrams and included in the total daily dietary phe prescription. Specialty produced low protein food products and foods containing only carbohydrate and fat are consumed without restriction. All other foods are weighed on a gram scale, or measured using household measurements in an effort to quantify phe intake. These foods include low protein grains, fruits and vegetables. High protein foods must be avoided due to their phe content, and includes all meats, dairy products, eggs, nuts, seeds and many grains. Food labels can help assist some patients in determining how much protein or phe is in a product and can ease the burden of measuring those foods. Most fruits and vegetables, however, do not have food labels and, therefore, need to be measured by gram scale or household measurements when consumed to account for the amount of phe. The traditional diet approach for PKU management is difficult for caregivers and patients, resulting in poor compliance especially among adolescents (Zimmermann, 2012). A less complicated approach to managing PKU diet

may improve compliance in those that do not follow the traditional restricted diet recommendations precisely enough to reduce their blood phe levels to the treatment range. The purpose of this Evidence Analysis Library project is to explore alternative PKU dietary practices and the impact of simplified dietary approach on metabolic control. Specifically, this project will collect evidence of how the liberalization of the standard PKU diet, by allowing a degree of free consumption of fruits and vegetables, affects metabolic control.

*Research Question*

Will allowing the consumption of unlimited fruits and vegetables worsen metabolic control among PKU patients when compared to counting the phe consumed from fruits and vegetables in the daily phe allowance?

*Subproblems*

A subproblem relating to the research question is to define the alternative PKU diet interventions in question. The simplified dietary approaches for this analysis include the consumption of uncounted fruits and vegetables and counting grams of protein instead of milligrams of phe as a means of tracking daily phe intake and meal planning to achieve phe prescription goals.

*Limitations*

The limitations of this project include the small number of primary research articles available in this area. The number of subjects participating in these studies is small, which can limit the ability to generalize the results towards the greater PKU population. The diversity of the subjects may also be limited due to small sample sizes, further decreasing the ability to generalize the found results. Potential bias because of inability to fully blind the researchers and

the participating subjects could also be a limitation for this project. Long term effects of these simplified diets are unknown, due to the short term study design of the research conducted in this area.

*Delimitations*

Delimitations have been set to find articles relevant to the purpose of this project. Inclusionary delimitations include: subjects who have any degree of phenylalanine hydroxylase deficiency, subjects who are over one year of age, metabolic control measured through blood phe levels, and studies published within the last ten years. Exclusionary delimitations include: studies that do not use the free consumption of fruits and vegetables as a variable, animal studies, or studies that include female subjects who are pregnant. This project will also not take into account alternative dietary management for other rare metabolic disorders as PKU is the main population of focus for this project.

*Assumptions*

It is assumed that the articles under investigation provide relevant and honest information as they will all be found in peer reviewed journals. It is also assumed that patients and caregivers find the traditional PKU diet to be difficult and time consuming and would prefer an easier method of managing this diet while keeping adequate metabolic control. Current intake for fruits and vegetables are assumed to be inadequate due to the burden of having to weigh and measure these foods before consumption.

*Definitions*

**Hyperphenylalaninemia (HPA):** patients with PAH deficiency whose maximum blood phe concentration is between 120 and 600 umol/L

**Phenylalanine (phe):** an essential amino acid

**Phenylalanine Hydroxylase (PAH):** an enzyme that is responsible for converting phenylalanine to tyrosine

**Phenylketonuria (PKU):** a genetic disorder caused by a mutation in the gene for the enzyme PAH

**Tetrahydrobiopterin (BH4):** an essential cofactor for PAH whose deficiency can result in secondary PAH deficiency and elevated blood phe levels

# CHAPTER 2: REVIEW OF LITERATURE

Traditional diet management for people with Phenylketonuria (PKU), a genetic metabolic disorder, includes lifelong severe natural protein restriction to decrease intake of phenylalanine (phe), an essential amino acid, for maintenance of blood phe levels within optimal ranges. Many challenges arise for the caregiver, patient and the clinician in achieving optimal blood levels and outcomes for this patient population. To improve growth, development and optimal blood phe levels, dietary management for PKU has become stricter to meet optimal blood phe levels (Feillet, 2010). Strict diet control attempts to improve outcomes for this patient population. However, compliance under these conditions often declines and the quality of foods and nutrients consumed can also decline. Especially as patients gain independence in teenage and adult years, compliance to diet often falters resulting in increased blood phe levels and negatively impacting cognition. As blood phe levels increase, executive functioning skills worsen in this population, making compliance to a strict, complicated diet even more difficult and further decreasing motivation to return to this diet.

PKU became the first inborn error of metabolism to be screened in newborns (Vockley, 2014). It is most common in Caucasians with an overall incidence of 1 in 10,000 live births (Vockley, 2014). When left untreated in infancy, PKU can cause severe and irreversible mental retardation. Newborn Screening allows for detecting positive screens for PKU shortly after birth and initiating treatment using natural protein restriction in combination with a synthetic protein formula has been effective in preventing devastating cognitive deficits (Vockley, 2014). However, even with treatment, intellectual and neuropsychiatric issues can still occur overtime (Vockley, 2014). Those with good metabolic control early in life who later decline control can

have irreversible and reversible neuropsychiatric issues (Vockley, 2014). Impaired intellectual and neuropsychiatric abilities make following a complex restrictive diet difficult for the PKU patient population, resulting in poor compliance and even poorer quality of life.

Traditionally, phe content of fruits and vegetables are counted in milligrams and included as part of the prescribed dietary phe goal. Foods that contain phe are weighed on a gram scale or measured using household measurements. Food labels can help assist some patients in determining how much protein or phe is in a product and can ease the burden of measuring those foods. Most fruits and vegetables, however, do not have food labels and therefore need to be measured by gram scale or household measurements when consumed. By liberalizing fruits and vegetables from total phe count and eliminating the need to weigh and measure them on a gram scale or household measurements, diet quality could potentially improve as patients may be more willing to consume fruits and vegetables if it would take less time and work when planning and tracking phe intake. The purpose of this literature review is to explore liberalization of the standard PKU diet by allowing unlimited consumption of fruits and vegetables and the effects this approach has on metabolic control.

## Background

PKU is a genetic disorder caused by a mutation in the gene for the enzyme phenylalanine hydroxylase (PAH) which is responsible for converting phe to tyrosine in the liver (Walter, 2002). It is an autosomal recessive disorder that results from more than 500 known gene mutations (Vockley, 2014). PKU presents as a spectrum disorder, with varying degrees of severity depending on residual amounts of PAH remaining (Vockley, 2014). Classical PKU describes patients with the most severe form of PKU who possess no enzyme activity, resulting

in blood phe levels greater than 1,200 umol/L when the condition is left untreated (Vockley, 2014). A maximum blood phe level between 800-1200 umol/L represents moderate PKU, 600-800 umol/L represents mild PKU, and 120-600 umol/L represents the least severe form of the condition and is called hyperphenylalaninemia (HPA) (Zimmermann, 2012).

Tetrahydrobiopterin (BH4) is an essential cofactor for PAH whose deficiency can result in secondary PAH deficiency and elevated blood phe levels (Vockley, 2014). Sapropterin (Kuvan) is a synthetic form of BH4 used as a pharmacological chaperone to allow improved function of any remaining PAH enzyme activity in people with PKU (Vockley, 2014). An estimated 25-50% of patients with PKU respond to Kuvan, resulting in an improvement in neuropsychiatric symptoms and/or increased phe tolerance without increased blood phe levels (Vockley, 2014). The American College of Medical Genetics and Genomics (ACMG) Practice Guidelines recommend all patients with PKU undergo a trial on Kuvan to determine if they are responders to BH4 (Vockley, 2014).

ACMG Practice Guidelines recommend maintaining blood phe levels within a goal range of 120-360 umol/L regardless of age including during pregnancy to achieve optimal treatment outcomes (Vockley, 2014). Manipulation of the diet is needed to maintain optimal phe levels and to achieve normal growth and development. A synthetic protein formula fortified in phe-free protein, calories, fat, vitamins and minerals is needed for individuals of all ages to achieve adequate growth, development and nutrient intake. Blood phe levels are monitored weekly to monthly, depending on age and pregnancy status (Vockley, 2014). Even with consumption of synthetic protein formula, avoiding natural protein increases the risk of consuming a diet deficient in essential nutrients. Additional laboratory tests to determine nutrient status should be

done routinely, including a full panel of plasma amino acids, complete blood count, ferritin, vitamin D, trace elements, a comprehensive metabolic panel, essential fatty acids as well as routine DEXA scans to determine bone density (Vockley, 2014).

*Establishing the PKU diet*

Nutritional management goals for people with PKU aim to provide a nutritionally adequate diet while maintaining blood phe levels within the optimal range of 120-360 umol/L and supporting adequate growth and mental function (MacLeod, 2010). The PKU diet is started as soon as possible after an infant is found to test positive for PKU from the Newborn Screening blood test. A phe prescription is developed by adjusting the amount of phe provided through standard infant formula or breast milk to achieve optimal blood phe levels. A synthetic protein formula that is phe-free is added to a standard infant formula or breast milk to provide adequate calories, synthetic protein, fat, vitamins and minerals. The use of this synthetic protein formula is essential for the infant to grow and develop. Natural protein is limited, but not totally eliminated, to provide a prescribed amount of phe while still providing an adequate amount of this essential amino acid to avoid phe deficiency (MacLeod, 2010).

The PKU diet becomes more complicated with the addition of solid foods. The limitation of food is extreme and typically eliminates all meat, dairy, egg, nut, and seed products as well as most bread, pasta, rice and higher protein vegetable items (MacLeod, 2010). Thus, the diet is limited to foods that are higher in sugar and fat, fruits, cereals, crackers, specially made low protein bread and pasta products, limited vegetables and the synthetic protein formula. When starting solid foods, the dietary source of phe begins to transition from infant formula or breast milk to phe from fruits, vegetables, small amounts of rice cereal and low protein pasta, porridge

and bread products (MacLeod, 2010). Parents are taught to weigh and measure foods and to calculate phe content of foods in order to provide the child's exact phe prescription daily. Keeping daily diet records is often essential in maintaining the diet accurately and to keep track of phe intake throughout the day. Once established, a person's phe prescription remains relatively unchanged except during times of extreme growth and rapid cell turnover (infancy, early childhood, puberty, and pregnancy).

*Nutritional Issues with the PKU Diet*

To optimize the growth and development of patients with PKU, diet therapy has become more complex and restrictive (Feillet, 2010). Once solid foods are introduced, the foods allowed in the traditional PKU diet resemble a strict vegan diet (Feillet, 2010). The PKU and vegan diets share similar nutritional benefits, including lower intake of saturated fat and cholesterol and higher intake of fiber, magnesium, potassium, folate, vitamins C and E, and phytochemicals (Feillet, 2010). The PKU diet, however, is more restrictive than the vegan diet as high protein grains must also be restricted; this further limits essential micronutrients (Feillet, 2010). Lower intake of Vitamins A, C and E, selenium, coenzyme Q, Vitamins B2, B6 and B12, folate, iron, zinc and carnitine have been observed in patients following the PKU diet (Feillet, 2010). Genetic polymorphism concerning cholesterol metabolism has been reported in PKU patients associated with low cholesterol levels and micronutrient status (Verduci, 2004).

Because the PKU diet does not allow animal fats, it tends to be low in long chain polyunsaturated fatty acids (LCPUFA), including arachidonic acid and docosahexaenoic acid (DHA) which have essential roles in neurological development and protection of neural damage from peaks in blood phe levels (Feillet, 2010). Both children and adults who follow the PKU diet

have been found to be deficient in LCPUFA, requiring supplementation of DHA and fish oils (Feillet, 2010). Another complication of PKU is the progressive reduction in bone mineral density which is thought to potentially be caused by poor dietary compliance and the restrictive diet, though the exact mechanism is unknown (Feillet, 2010).

Oxidative stress has been observed in some inborn errors of metabolism, including PKU (Feillet, 2010). Antioxidant status may be altered in those following the PKU diet due to deficiencies in selenium or coenzyme Q10 (Feillet 2010). Because of the severe restriction of the PKU diet, reduced synthesis of endogenous antioxidants, ubiquinone-10 and glutathione, may also occur (Rocha and Martins, 2012). Poor metabolic control, as evidenced by elevated blood phe levels, may also enhance the endogenous synthesis of free radicals, which in turn can increase oxidative stress (Rocha and Martins, 2012). Dietary guidance and evaluation of nutritional status should occur regularly throughout the life of people with PKU to avoid devastating consequences of the nutrient deficiencies described.

*Challenges of PKU Diet through the Life Cycle*

People with PKU are recommended to stay complaint to the PKU diet for their whole life to achieve the best outcomes. It is especially important to continue this diet because of the extreme difficulty of returning to the strict diet after periods of noncompliance (MacLeod, 2010). Compliance is measured in a variety of ways including blood phe concentration, frequency of monitoring blood phe levels, attendance at outpatient PKU clinic visits, diet records, and patient and caregiver self- report of level of compliance to diet and treatment (MacDonald, 2010). Blood phe concentration is thought to be the best measurement of compliance to diet, but it is not without its shortcomings. Blood phe levels must be repeated at regular intervals to show the

trend of blood phe levels (MacDonald, 2010). But patients often avoid drawing blood samples if they suspect their blood phe levels will be elevated or will intentionally decrease their phe intake prior to a blood draw (MacDonald, 2010). Infections, hourly changes in blood phe levels, energy intake and timing of synthetic protein formula intake can also impact the results of blood phe concentration, further challenging the practitioners' ability to assess level of dietary compliance (MacDonald, 2010).

Many factors can contribute to patients' and their caregivers' noncompliance to PKU diet recommendations. Limited access to synthetic protein formula, illiteracy of patient or caregiver, language barriers, family dysfunction, poor social and family support, inability to cook or prepare recommended foods and general disinterest in following the recommendations can all lead to worsened compliance (MacDonald, 2010). Noncompliance can be intentional as when the patient consciously does not take the amount of synthetic protein formula as prescribed, as well as unintentional as when a patient forgets to take the recommended amount of synthetic protein formula (MacDonald, 2010). It is essential for healthcare professionals working with patients with PKU to find collaborative solutions to compliance barriers and to improve patient-provider communications so that patients adhere to recommendations (MacDonald, 2010).

In early childhood, compliance to PKU dietary treatment is generally considered to be good (MacLeod, 2010). Parental control is at its highest during this life stage and the child is confronted with minimal peer pressure related to the rarity of the prescribed diet (MacLeod, 2010). As children approach adolescence, they begin to develop taste preferences similar to other children their age and start to make their own decisions regarding food choices (MacLeod, 2010). Timing and frequency of the synthetic protein formula intake now must coordinate with

school and extracurricular activities. The synthetic protein formula looks and smells unusual compared to other beverages, increasing peer pressure from other children and leading to refusal of the child with PKU to drink their synthetic protein formula at school (MacLeod, 2010). Without a sufficient protein source for over eight hours, catabolism occurs, releasing phe into the bloodstream and increasing blood phe concentrations (MacLeod, 2010). Calorie intake also declines without the calorie dense synthetic protein formula, potentially causing the child to consume more natural foods and exceeding their daily phe prescription, also increasing their blood phe concentrations (MacLeod, 2010). With increased blood phe levels, the child can experience academic challenges in school that continue into adulthood.

Diet monitoring and management starts to transition from the parent to the patient as the patient grows into teenage years and adulthood. Continued diet education and slow transfer of roles and responsibilities is essential for continuing adherence of diet in the teenager and young adult. If the PKU diet is no longer followed, the patient will experience great difficulty when attempting to return to diet. A survey showed that 31% of patients who attempted to return to diet again discontinued the diet 10 months later due to a lack of motivation (MacLeod, 2010). The consequences of dietary nonadherence are not immediate or easily recognized by patients, which can decrease motivation to stay on diet (MacLeod, 2010). Symptoms of elevated blood phe levels often include increased irritability, difficulty concentrating, and headaches (MacLeod, 2010).

When considering blood phe levels as an indicator of compliance, Gokmen-Ozel et al (2009) found these concentrations to be well controlled but to worsen with age. The average blood phe level over the course of a year was calculated for 1,921 participants from ten centers in

ten countries (Gokmen-Ozel, 2009). These researchers found that 88% of children <1 years old met their center's target blood phe range, 74% of those 1-10 years old, 89% of those 11-16 years old and only 65% for adults (Gokmen-Ozel, 2009). In another study conducted at four PKU clinics, researchers Walter, et al (2002) found that mean blood phe concentrations increased with age. Seventy percent of subjects <10 years of age on average met their center's target phe range, while only 20% of subjects >15 years old had average blood phe levels within the target range (Walter 2002). Walter et al (2002) suggested the increase in mean blood phe concentration in those >15 years of age was due to noncompliance to diet.

A critical stage for PKU patients to maintain goal blood phe concentrations is during pregnancy. When a woman with PKU becomes pregnant, dietary compliance is essential to avoid the devastating effects of high blood phe levels on the fetus (MacLeod, 2010). The possible symptoms in the offspring include microcephaly, congenital heart defects, intrauterine growth retardation and learning disabilities (MacLeod, 2010). Achieving optimal blood phe levels before conception is essential and can be very challenging for the patient who has not consistently followed the diet optimally. Consumption of synthetic protein formula is needed to provide adequate calories, phe free protein, vitamins and minerals for the pregnant patient and the amount increases to meet the increased requirements during pregnancy (MacLeod, 2010). Temporary insertion of a gastrostomy tube is recommended for patients who are unable or unwilling to consume the prescribed amount of synthetic protein formula by mouth during the length of their pregnancy.

The traditional diet approach for PKU management is difficult for caregivers and patients, resulting in poor compliance especially in adolescents (Zimmermann, 2012). A less

complicated approach in managing PKU diet is needed to improve compliance in those that fail to follow the traditional restricted diet recommendations. A simpler method of managing the PKU diet will likely help improve compliance when compared to a more complex method (MacDonald, 2010). Fruits and vegetables are rich in essential vitamins and minerals but are often very low in protein. Fruits and vegetables are also good sources of dietary fiber; however, fiber could impede the level of protein absorption (Gilani et al., 2005). All of these factors support the clinician in encouraging people with PKU to consume more fruits and vegetables and for those with PKU to rely on fruits and vegetables for a large portion of their daily intake. The need to measure all fruits and vegetables can be a large burden on those with PKU or the caregivers who provided and track their intake, and can even deter the consumption of foods from these food groups. If a less complicated approach can be used while keeping blood phe levels within goal ranges, compliance and thus quality of life could potentially improve (Rohde, 2012). Although evidence is limited, five studies specifically looked at unrestricted consumption of fruits and vegetables among PKU patients.

## Unrestricted Consumption of Fruits and Vegetables

Two clinics set out to study and record the effects of consumption of unlimited fruits and vegetables on metabolic control of PKU. MacDonald et al. (2003) from The Children's Hospital in Birmingham, United Kingdom conducted a three part, open, prospective crossover study in the outpatient setting to evaluate unrestricted fruit and vegetable consumption among PKU Patients. Inclusion criteria included patients with 70% of blood phe levels within the recommended range six months prior to start of the study, the ability to take own blood phe levels or have caregiver who could, over 1 year of age, and the ability to consume fruits and vegetables within amounts

of 50 to 100 milligrams phe per 100 grams (MacDonald et al., 2003). Fifteen patients with moderate to severe PKU, 13 girls and 2 boys aged 1 to 24 years, were recruited into the study (MacDonald et al., 2003). Synthetic protein formula was kept consistent and within original diet prescription and consumed at the same times on blood sampling days (MacDonald et al., 2003).

The first phase was conducted for weeks 1 through 3 in which subjects were allowed to eat unlimited amounts of fruits and vegetables containing less than 50 milligrams phe per 100 grams (MacDonald et al., 2003). Blood phe levels were collected twice daily on the last three days of weeks one and week three before breakfast and before dinner (MacDonald et al., 2003). Subjects received a standardized meal on blood sampling days (MacDonald et al., 2003). Phase two was conducted over weeks four through eight, in which subjects ate one or more portions of fruits and vegetables containing 50 to 75 milligrams phe per 100 grams per day (MacDonald et al., 2003). Blood samples were taken during weeks six and eight in the same fashion as phase one, including the same standardized meal but with one additional portion of fruit or vegetable containing 50 to 75 milligrams phe per 100 grams (MacDonald et al., 2003).

Phase three participation was optional for the subjects and consisted of seven weeks of consuming at least three portions per week of vegetables containing 75 to 100 milligrams phe per 100 grams (MacDonald et al., 2003). Blood phe levels were drawn at weeks 11, 13, and 15 in the same way as phase one and phase two with a standardized meal which included a serving of vegetables with 75-100 milligrams phe per 100 grams (MacDonald et al., 2003). Few fruits contain greater than 75 milligrams phe per 100 grams food, which is why only vegetables were added to the final phase of this study. Through all three phases, subjects kept food records of consumed fruits and vegetables with 50 to 100 milligrams phe per 100 grams (MacDonald et al.,

2003). On blood sampling days, all foods and drinks were weighed on gram scales to 5 gram accuracy with only foods and drinks consumed included in the diet record (MacDonald et al., 2003).

Unlimited consumption of fruits and vegetables with 51-100 milligrams phe per 100 grams did not compromise control in plasma phenylalanine levels in this study and significant changes were not found between the different phases of this study (MacDonald et al., 2003). Average phe intake increased above baseline by 54 mg per day in the second phase and 39 mg per day in phase three with a significant increase in natural protein (MacDonald et al., 2003). The authors concluded that unlimited fruit and vegetable intake of items with 51-100 milligrams phe per 100 grams does not compromise plasma phenylalanine levels (MacDonald et al., 2003).

The use of standardized meals is a strength of this study as it leaves less room for error in recording diet and analyzing nutrient intake. The amount of synthetic protein formula and timing of intake were consistent and in accordance to recommended intakes. Laboratory staff was blinded to blood results and families were blinded to phe results during the study to help decrease bias. The study was not randomized as the control period was always before the test period, weakening the results. The sample size was small and diminished further in the third study phase with only 12 participants, further decreasing the strength of the found results due to small sample size (MacDonald et al., 2003). The study was short term which can be a weakness, as long term effects are unknown, but provides evidence for the need for further long term studies.

In another study, Rohde et al. (2012) investigated whether the PKU diet can be liberalized to include unlimited consumption of fruits and vegetables containing less than 75

milligrams of phe per 100 grams of food in the short term without negatively impacting metabolic control. They conducted an open clinical trial in the outpatient setting (Rohde et al., 2012). Inclusion criteria were restricted to PKU patients between 2-10 years of age with average blood phe levels less than 360 umol/L over at least 6 months (Rohde et al., 2012). Exclusion criteria included patients with any diseases or abnormalities in general or found on neurological examination (Rhode et al., 2012). Twenty-eight patients were screened for the study with only 16 meeting inclusion criteria (Rohde et al., 2012). Two patients were excluded during the study leaving 14 total participants (8 females and 6 males) with a mean age of 5.7 ± 2.4 years (Rohde et al., 2012).

For Days 1-3 of the study, patients followed their current dietary phe and synthetic protein formula prescription, kept daily diet records of all foods consumed, and took daily dried blood samples to determine blood phe concentration (Rohde et al., 2012). On Day 4, patients were randomized to either continue their current diet plan of restriction of all foods including fruits and vegetables or were instructed that they could consume unlimited fruits and vegetables (Rohde et al., 2012). The groups switched diets on the eighteenth day and continued this dietary treatment for another 2 weeks (Rohde et al., 2012). Individual synthetic protein intake from synthetic protein formula prescription was unchanged throughout the entire study (Rohde et al., 2012).

During the fruit and vegetable restriction phase, all foods were weighed to determine phe content (Rohde et al., 2012). Unlimited consumption of fruits and vegetables with less than 75 milligrams of phe per 100 grams of food was allowed while staying within phe prescription and weighing and measuring all other foods during the unlimited fruits and vegetables phase (Rohde

et al., 2012). No set amount of additional intake of fruits and vegetables was recommended, but participants were encouraged to consume more if they desired (Rohde et al., 2012). Dried blood phe content was measured daily between 0700 and 0900 after an overnight fast and diet records were recorded on days 5, 6, 15, 16, 17, 19, 20, 29, 30, and 31 (Rohde et al., 2012). Foods and beverages were measured with a gram scale to the nearest 1.0 gram on diet record days (Rohde et al., 2012).

The data from each phase was averaged and the changes between the study phases were analyzed using Wilk's multivariate analysis of variance (MANOVA) with a level of $P<0.05$ considered significant (Rohde et al., 2012). The total amount of fruits and vegetables consumed and types consumed did not change significantly through the phases and intake only reached 76-83% of recommended intake of fruits and vegetables for German children (Rohde et al., 2012). When unrestricted, amounts of fruit and vegetable intake did not increase and the phe usually allotted for fruits and vegetables was used for consumption of other foods (Rohde et al., 2012). Phe intake was significantly increased by an average of 58 milligrams per day during the free fruits and vegetables study phase, but did not negatively impact metabolic control as average blood phe concentrations remained within the recommended range (Rohde et al., 2012). The authors, therefore, concluded that free consumption of fruits and vegetables does not negatively impact short term metabolic control in children with PKU (Rohde et al., 2012).

A strength of the study is the frequency of blood phe levels, which were optimal as they were taken daily and coincided with diet records to accurately assess significant changes. The researchers showed their hypotheses to be accurate and furthered the discussion on improving the quality of life for patients with PKU through simplifying the diet. This was an open study

without blinding, which could have increased the risk of bias from all parties involved. Parents knew their child's intake was being analyzed during the study, which could have resulted in following a more restrictive diet than their baseline. The researchers were also not blinded to any phase or part of the study, which could cause bias in interpreting results. Standardized meals were not provided, and diet records were self recorded, which could have produced inaccurate accounts of actual intake.

The sample size and subjects were also a weakness for this study. There were only 14 patients who were 2-10 years old with caregivers picking out most meals and providing all foods. Participants' blood phe levels already were within the normal range of <360 umol/L. These patients are already receiving the best care to manage PKU and therefore already have great control of PKU to provide optimal clinical outcomes. It would be adventitious to try this study in a group of teenage and adult patients who do not have optimal levels, have difficulty managing their own diet, and whose quality of life has declined because of poor clinical outcomes. A longer study would be beneficial to show long term effects of following this unlimited fruit and vegetable diet.

Rhode et al (2014) continued to gather data on these patients and published a one year follow up of their original study. Phase 3 of this study lasted for months 1-6 post the original study and phase 4 lasted for months 7-12 (Rohde et al., 2014). Only nineteen subjects from the original study were included in the data analysis and three subjects did not participate in phase 4 (Rohde et al., 2014). The mean age of the subjects was 4.7 years, plus or minus 2.1 years, with a mean phe tolerance of 357 mg phe per day (Rohde et al., 2014).

Patients in both phase 3 and 4 continued to follow free consumption of fruits and vegetables with less than 75 milligrams of phe per 100 grams of food (Rohde et al., 2014). Blood phe levels were collected every two to four weeks for the 12 month follow up (Rohde et al., 2014). Three day diet records and dried blood phe concentrations were collected at the end of phase three, 6 months after the starting date and at the end of phase 4, twelve months after the starting date (Rohde et al., 2014).

The data from each study phase was averaged with longitudinal changes analyzed by the Friedman test (Rohde et al., 2014). The Wilcoxon test was conducted to compare individual study phases if there were significant differences with a level of $P < 0.05$ showing significance (Rohde et al., 2014). Mean dried blood phe concentrations remained within the recommended range of 40-240 umol/L throughout the study (Rohde et al., 2014). The frequency of elevated levels decreased but not significantly (Rohde et al., 2014). Intake of fruits and vegetables met 91% of the recommended amount for German children following the restricted phase of the study and remained consistent throughout all 4 study phases (Rohde et al., 2014). Phe intake increased significantly by an average of 68 mg per day, a 28% increase, while maintaining blood phe levels within the recommended range (Rohde et al., 2014). Total protein intake increased significantly in phases 3 and 4, but this was due to an increase in synthetic protein formula which is increased as the child grows to meet total protein requirements for growth and development (Rohde et al., 2014).

This one year follow up showed no worsening of metabolic control with liberalization of fruit and vegetable intake (Rohde et al., 2014). The authors conclude that the phe utilization from fruits and vegetables was poor due to the decreased ability for the body to break down and digest

the protein from these foods (Rohde et al., 2014). Therefore, patients with PKU who consume more fruits and vegetables may have a phe prescription that is higher than their actual tolerance due to the poor utilization of phe from fruits and vegetables (Rohde et al., 2014). The source of additional phe was not from increased fruit and vegetable intake, but rather from consumption of higher phe containing foods that required weighing, measuring and recording (Rohde et al., 2014). This increase in phe increases the choices and variety in diet for a patient with PKU by allowing children with PKU to gain more freedom with managing their diet and being able to choose foods without limitations (Rohde et al., 2014).

## Simplified PKU Diet

The potential benefits of a simplified diet for PKU management were also noted in a study conducted by Zimmermann et al. (2012) at the University Children's Hospital in Zurich. Through a detailed PKU cohort followed over three years, they sought to further investigate control of PKU when fruits and vegetables with less than 100 milligrams phe per 100 grams are eaten in amounts according to the World Health Organization (WHO) (Zimmermann et al., 2012). According to WHO (2004), daily consumption of 400 grams of fruits and vegetables is recommended. Age of diagnosis, phenylalanine hydroxylase mutations, BH4 responsiveness and diet plans of 80 patients with PKU at the University Children's Hospital were collected retrospectively (Zimmermann et al., 2012).

Up until six months of life, breastfeeding in combination with a synthetic protein formula is encouraged for all patients (Zimmermann et al., 2012). This is followed by the introduction of potatoes, vegetables and fruit with increased monitoring of blood phe levels (Zimmermann et al., 2012). Patients are traditionally then transitioned to a strict, traditional PKU diet. Only fruits,

vegetables, limited grains, and low protein food products are allowed and all foods must be weighed and measured to meet a predetermined phe prescription (Zimmermann et al., 2012). Zimmermann's simplified diet allows an unrestricted intake of fruits, vegetables and low protein food products (Zimmermann et al., 2012). Following WHO recommendations to consume 400 grams of fruits and vegetables daily, the simplified diet encouraged consumption of five fist-sized servings of fruits and vegetables that contain up to 100 milligrams phe per 100 grams daily (Zimmermann et al., 2012). This approach eliminated measuring many foods and the need for counting each milligram of phe from fruits and vegetables consumed. Avocado, broccoli, brussels sprout, passion fruit, kale, peas, sprouts, potatoes and corn are measured and were not eaten freely as they contain greater than 100 milligrams phe per 100 grams (Zimmermann et al., 2012).

A total of 80 subjects were included in this study with half being male, 41 with classical PKU, 16 with moderate PKU, 10 with mild PKU and 13 with mild hyperphenylalaninemia (HPA) (Zimmermann et al., 2012). At birth, seventy-three patients were on the traditional diet restriction, while seven were started on the simplified diet (Zimmermann et al., 2012). Fifty patients on the traditional diet agreed to switch to the simplified diet, while 23 decided to remain on the traditional diet restriction (Zimmermann et al., 2012).

The amount of potatoes, rice, and maize consumed are set at a specific amount depending on the patient's phe tolerance (Zimmermann et al., 2012). Intake of these fixed amounts are estimated daily by caregivers and/or patients but are occasionally checked using stricter weighing techniques as needed (Zimmermann et al., 2012). Blood levels are taken using Guthrie cards with dried blood spots one hour after breakfast or dinner and are monitored every 1-2

weeks for patients 0-2 years old, 1-4 weeks for patients 2-10 years old and at least monthly for those over 10 years old (Zimmermann et al., 2012).

Median blood phe levels for patients on the simplified diet did not differ significantly from those on the traditional diet for those with classical, moderate, and mild PKU (Zimmermann et al., 2012). Four patients with HPA switched to the simplified diet and were not included in the statistical analysis (Zimmermann et al., 2012). Blood phe levels compared between age groups showed a significant difference in blood phe levels in those over 16 years old with those on the simplified diet having more blood phe levels in the recommend ranges than those who were on the traditional diet (Zimmermann et al., 2012). The researchers concluded that consuming of fruits and vegetables with less than 100 milligrams phe per 100 grams according to WHO recommendations did not worsen blood phe levels independent of classification of PKU and age of when the diet was changed from traditional to simplified (Zimmermann et al., 2012).

Although this study had a large sample size in comparison to other studies in this area, it is still relatively small with only 50 participants willing to trial a simplified diet. The goal of this simplified approach is to maintain blood phe levels in goal range without having to measure certain foods, but by omitting this information this research is not able to quantify the amount of fruits and vegetables consumed. Calculation of phe intake therefore becomes an estimate and decreases the validity of the findings. The study did not include blinding in any part of the study, which could increase risk of bias in the study.

These researchers took the most relevant studies already conducted in this area to form their purpose and methodology. They added classification of severity of PKU, described as

classical, moderate, mild PKU and HPA, to determine if this technique is suitable for people with varying PAH enzyme activity. Findings were also evaluated by age group of patients which offers confidence in future research and recommendations for this technique in all age ranges.

These researchers stressed that their technique of a simplified PKU diet should not be confused with a liberalized PKU diet. Patients are still expected to take regular blood phe levels and maintain blood phe level within treatment range. Occasionally, all foods are weighed and measured as a way to check current intake. This study prompts the need for future larger scale, longer term studies to further discover the efficacy of a simplified approach in PKU diet management that could potentially improve the quality of life for this population.

### Protein counting with use of free fruits and vegetables

The hypothesis that dietary treatment of PKU should be simplified to improve dietary compliance was investigated by Sweeney et al. (2011) at the Women's and Children's Hospital in Adelaide, Australia. In Australia, the traditional PKU diet also consists of a synthetic protein formula, vitamins, minerals and trace elements as well as specially made low protein foods (Sweeney et al., 2011). A limited amount of phe is provided from naturally low in protein foods (fruits, vegetables and limited grains) and phe intake is counted using an exchange system in which one exchange provides 15 mg phe (Sweeney et al., 2011). For example, if a child is determined by blood phe levels to tolerate 360 mg phe, then the number of 15 mg phe exchanges provided would equal 24. The researchers also noted that a 50 mg phe exchanges system is used in the UK where one gram of protein equals 50 mg phe (Sweeney et al., 2011).

Current research comparing the strengths and weaknesses of these dietary methods for PKU management are lacking; therefore, the purpose of this study was to measure the impact on

metabolic control each of these methods have on patients with PKU (Sweeney et al., 2011). They also sought to increase variety in the PKU diet by offering varying amounts of unlimited fruits and vegetables (Sweeney et al., 2011). Their prediction was that the 50 mg phe exchange system would not adversely affect metabolic control when compared to the 15 mg phe exchange system and that it would be easier to follow per parent and patient report (Sweeney et al., 2011).

In Phase One, all subjects completed a baseline 3 day diet diary and PKU Diet Attitudes Questionnaire (Sweeney et al., 2011). Subjects were then randomized into two groups: continue current phe exchange system (control group) or change to counting grams of protein (study group) (Sweeney et al., 2011). Foods with <20 mg phe per serving were free and a diet chart of free foods was provided (Sweeney et al., 2011). Six months after the initial appointment, questionnaires and diet diaries were completed for both groups. The control group was then educated on counting grams of protein. Six months later, diet diaries and questionnaires were again collected. Phase Two assessed the impact on blood phe levels of a further liberalization of uncounted foods. Foods with 40-50 mg phe/serving were uncounted but given a serving limit and foods with >50 mg phe/ serving were counted at 0.5 g protein increments (Sweeney et al., 2011). An extensive diet chart of free foods was provided. All subjects completed a baseline 3 day diet diary and PKU Diet Attitudes Questionnaire. Six months later, 3 day diet diaries and questionnaires were again completed (Sweeney et al., 2011).

Blood spot filter paper cards were collected weekly and were analyzed for blood phe levels using tandem mass spectrometry (Sweeney et al., 2011). A PKU attitudes questionnaire, consisting of 18 questions based on a five-point Likert scale and three free answer questions, was completed by the main caregiver and participants with PKU school aged or older (Sweeney et

al., 2011). The questionnaire was designed to determine their attitudes on food preparation, diet monitoring, collecting blood tests, dietary variety, metabolic control, stressfulness of diet and quality of life (Sweeney et al., 2011). A three day diet diary was also collected in both phases, but data were not reported due to lack of dietary detail (Sweeney et al., 2011).

Eighteen patients with PKU with a median age of 10 years 1 month from the Women's and Children's Hospital Metabolic Unit where consented into the study (Sweeney et al., 2011). The participants consisted of 5 males and 13 females with 16 having the classic PKU phenotype and two having the moderate PKU phenotype (Sweeney et al., 2011). All 18 participants had initially been taught the 15 mg phe exchange method and were on a synthetic protein formula (Sweeney et al., 2011). Participants were randomly assigned to either the control group where they continued their current 15 mg phe exchange counting or the study group where they were educated to follow the 50 mg phe exchange counting method (Sweeney et al., 2011). Both groups met with the same dietitian to be educated on their assigned diet and synthetic protein formula prescriptions were unchanged (Sweeney et al., 2011). After 6 months of the phase one study, the control group was educated on the 50 mg phe exchange method and both groups completed three day diet diaries and the study questionnaire (Sweeney et al., 2011). Seventeen participants advanced to phase two where all participants followed the 50 mg phe exchange counting method and foods with <50 mg phe per serving were considered free (Sweeney et al., 2011). After six months, three day diet diaries and questionnaires were completed by the participants and their caregivers (Sweeney et al., 2011).

The results for phase one of this study showed no significant differences in the participants' blood phe levels from the six months before and six months after using the new

exchange method (Sweeney et al., 2011). The control participants' blood phe levels also did not differ significantly before or during phase one of the study (Sweeney et al., 2011). The participants and their parents indicated a preference for the 50 mg phe exchange method as it made tracking phe intake easier and increased the ability to consume commercial products as food labels could be used to determine phe intake (Sweeney et al., 2011). They also noted that their children became more interested in their diet as they now understood how to manage the diet with this easier method (Sweeney et al., 2011). The children felt there was a significant increase in variety of their diet while caregivers reported only a slight increase (Sweeney et al., 2011).

The results for phase two showed a significant improvement in blood phe levels for four of the participants, no change for nine of the participants and significantly worse blood phe levels for one patient (Sweeney et al., 2011). All participants and caregivers preferred the phase two diet chart which allowed unlimited consumption of fruits and vegetables with <50 mg phe per serving (Sweeney et al., 2011). Parents reported a decrease in time to prepare foods as less foods needed to be weighed and measured, lessening the burden of mealtime preparation (Sweeney et al., 2011). Children reported being more accurate with tracking the non-free foods in their diet with this simpler method and being more involved in their diet management (Sweeney et al., 2011).

The positive results from this study led some Australian PKU centers to adopt the United Kingdom's established practice of using the 50 mg phe exchange system (Sweeney et al., 2011). This study adds important insight into quality of life issues with its use of the PKU Diet Attitudes Questionnaire. Although the questionnaire only found a slight significant increase in

attitudes towards perceived variety in the diet, it shows patient and caregiver opinions and feelings towards the PKU diet and diet changes. Participants were randomized in phase one of this study, decreasing potential bias of the allocation of patients between the study and control groups. The format of the diet charts were kept consistent between the two study phases making the transition from phase one free foods to phase two free foods easier for patients and caregivers.

Blinding was not used in this study, leading to potential bias. For example, the researchers provided background information on the patient in phase two who had worsening phe levels, reporting he was sick during this phase and this is why his levels increased (Sweeney et al., 2011). However, there is no way for the researchers to differentiate whether it was the illness or the change in phe exchange counting method that caused the increase in levels. The inability to have proper blinding may be an inherent limitation for these types of studies. This study also had a very small number of participants due to being conducted at only one metabolic center, potentially decreasing the validity of its findings.

Three day diet records were recorded and collected but results were not published or analyzed due to poor quality (Sweeney et al., 2011). Without accurate diet records, actual phe intake during the study phases is unknown and no correlation between phe intake and blood phe levels could be made. The researchers noted that multicenter collaboration and longer term studies are needed to evaluate changes in metabolic control with allowance of increased free consumption of fruits and vegetables (Sweeney et al., 2011).

## Summary and Conclusion

The studies reviewed sought to find ways to improve the quality of life for PKU patients by increasing dietary choices and making the PKU diet easier to follow. Their aim was to simplify the traditional PKU diet, while keeping metabolic control within recommended ranges. All five studies noted the challenge in maintaining the traditionally strict PKU diet. They also discussed the risk of poor compliance and poor outcomes for this population, especially for those in adolescence and adulthood. Study sample sizes were small, which is not surprising as PKU is a rare genetic disorder and the number of patients seen by each clinic is likely limited.

These studies were not blinded, which can lead to bias in interpreting results. MacDonald et al. (2003) used standardized meals for a portion of its study, while the other four studies did not. Diet records were kept in the MacDonald et al. (2003), the Rohde et al. (2012) and the Rohde et al. (2014) studies and blood phe levels were monitored more frequently. Zimmermann et al. (2012) did not have diet records, per design, and continued to monitor blood phe levels at usually recommended frequencies based on age. Sweeney et al. (2011) collected diet records but did not publish the findings from them due to inaccuracy of these records. Synthetic protein formula prescriptions were kept consistent throughout all five studies in an attempt to avoid changes in blood phe levels due to changes in synthetic protein formula intake.

Rhode et al. (2012), Rhode et al. (2014), Sweeney et al. (2011) and MacDonald et al. (2003) used open, cross over studies with multiple phases while Zimmermann et al. (2012) did not. MacDonald et al. (2003), Sweeney et al. (2011) and Zimmermann et al. (2012) divided patients by severity of PAH deficiency to determine the effects of changes in diet management techniques. Rhode et al. (2012) and Rhode et al. (2014) only included participants with average blood phe levels <360 umol/L and did not differentiate between degree of PAH deficiency. All

of the studies concluded that consumption of uncounted fruits and vegetables at varying amounts did not compromise metabolic control and even improved control in some groups of people with PKU. These studies motivate future studies to find opportunities to improve the quality of life of those with PKU by simplifying diet and encouraging variety of foods consumed. The findings of these studies will be further analyzed as described in the Methods section.

# CHAPTER 3: METHODS

Registered Dietitians play an important role in managing the health and well being of the patients they serve. It is essential that recommendations from Registered Dietitians not only come from expert clinical judgment, but also to be derived from the best known evidenced based practices. The Academy of Nutrition and Dietetics (AND) has developed the Evidence Analysis Library (EAL) as a resource and tool to provide guidelines for dietetic evidence based practice. Evidence based practice in dietetics involves a systematic review and analysis of scientific evidence to form nutrition recommendations (AND, 2015). This process is used to ensure that the best and most up to date evidence is used in combination with professional expertise to improve patient outcomes (AND, 2015). The methodology of this EAL project will follow the five complex steps outlined by AND to provide a methodical review of relevant research regarding the effect of unlimited consumption of fruits and vegetables on metabolic control for patients with PKU.

**Step One: Formulate Evidence Analysis Question**

A good research question is focused on finding evidence that will impact the nutrition interventions provided to patients (AND, 2012). The steps within the nutrition care process have been considered in formulating the question and strengthen the relevance of the question for its impact on patient care. The PICO Format (population, intervention, comparison intervention, and outcome) is also a useful tool that ensures the question is focused and relevant.

Maintaining goal blood phe levels through diet is essential to achieve the best physical, mental and emotional outcomes for patients with PKU. One method of teaching this severely restricted diet does not necessarily provide the best outcomes for all patients and their families.

Diet goals and education must be catered towards the abilities and needs of the patients and caregivers. The use of unlimited fruit and vegetable consumption is being used as a way to increase variety of foods consumed is described. Unrestricted fruit and vegetable intake may have advantages of lessening the burden of following the diet, and improving compliance and tighten metabolic control.

The following research question was developed: Will allowing the consumption of unlimited fruits and vegetables worsen metabolic control in patients with PKU when compared to counting the phe consumed from fruits and vegetables in the daily phe allowance?

PICO Format:

Population: Patients with PKU

Intervention: Unlimited consumption of fruits and vegetables

Comparison Intervention: quantified consumption of fruits and vegetables

Outcomes: Metabolic control as defined by blood phe concentrations

**Step Two: Gather and Classify Evidence**

The purpose of Step Two is to find the most relevant and appropriate research to analyze. The databases used for the search included PubMed and Ovid MEDLINE with the general search terms “PKU” and “Fruits and Vegetables”. The sources found in the search have been documented and titles and abstracts have been reviewed to determine if the article meets inclusion criteria. Inclusion criteria includes subjects over 12 months of age, studies in the ambulatory setting, subjects with any degree of PAH deficiency, outcomes measured by blood phe level, studies published in the years 2000 through 2015 and those published in a peer reviewed journal. Only primary articles were considered as the research question is intervention

based. Exclusion criteria included subjects less than 12 months old, pregnant women, studies not conducted in the outpatient setting, and studies published before the year 2000. The time was purposefully kept broad in an attempt to capture as many studies as possible as the number of studies in this population is often limited. All of the steps used to gather the evidence are documented in the Search Plan and Results (Figure 1), including a list of excluded articles and reasons for exclusion.

In total, eleven articles were found using the described search databases and terms. An additional article was found by reaching out to the Metabolic Dietitian community, for a total of twelve studies in total to be considered. Seven of these studies were excluded, mainly due to the fact that they did not incorporate an intervention using the uncounted consumption of fruits and vegetables in the PKU population. Five articles were found to meet the inclusion criteria and were included in this EAL project. Each accepted article has been classified by type of research design using the EAL Research Design Algorithm (Appendix A). The articles are graded based on a hierarchy of the study's strength in design. Randomized controlled trials, cluster randomized trials, and randomized crossover trials receive an 'A' rating, prospective and retrospective cohort studies receive a 'B' rating, non-randomized controlled trials, non-randomized crossover trials, case control studies, time series studies, diagnostic, validity or reliability studies receive a 'C', and non controlled trial, case study or case series, other descriptive studies, cross sectional studies, trend studies, and before-after studies receive a 'D' (AND, 2012).

**Figure 1.** Search and Plan Results

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|---|---|
| *Question:* | Will allowing the consumption of unlimited fruits and vegetables worsen metabolic control in patients with PKU when compared to counting the |

| | |
|---|---|
| | phe consumed from fruits and vegetables in the daily phe allowance? |
| *Date of Literature Review for the Evidence Analysis:* | April 2015 |
| *Inclusion Criteria:* | ● Age: over 12 months of age<br>● Setting: ambulatory<br>● Nutrition Related Problem: Patients with any degree of phenylalanine hydroxylase deficiency<br>● Metabolic control measured through blood phe concentrations<br>● Study Design: Primary reports<br>● Size of study groups: all sizes<br>● Year range: 2000-2015<br>● Language: English<br>● Published in a peer reviewed journal |
| *Exclusion Criteria:* | ● Participant Age: less than 12 months of age<br>● Setting: inpatient<br>● Health Status: pregnant females<br>● Year range: prior to 2000<br>● Language: articles not published in English |
| *Search Terms: Search Vocabulary* | Health Condition: PKU<br>Intervention Terms: Fruits and Vegetables |
| *Electronic Databases:* | Database: PubMed<br>Search Terms: (PKU) and (fruits) and (vegetables)<br>Hits: 11<br>Database: Ovid MEDLINE<br>Search Terms: (PKU) and (fruits) and (vegetables)<br>Hits: 7 |
| *List of Articles Included from Electronic Database*: | MacDonald, A., Rylance, G., Davies, P., Asplin, D., Hall, S.K., Booth, I.W. (2003). Free use of fruits and vegetables in phenylketonuria. *Journal of Inherited Metabolic Disorders*, *26*, 327-338.<br><br>Rohde, C., Mutze, U., Weigel, J.F.W., Ceglarek, U., Thiery, J., Kiess, W., Beblo, S. (2012). Unrestricted consumption of fruits and vegetables in phenylketonuria: no major impact on metabolic control. *European Journal of Clinical Nutrition, 66*, 633-638.<br><br>Rohde, C., Mutze, U., Schulz, S., Thiele, A.G., Ceglarek, U., Thiery, J., |

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|---|---|
| | Mueller, A.S., Kiess, W., Beblo, S. (2014). Unrestricted fruits and vegetables in the PKU diet: a 1-year follow-up. *European Journal of Clinical Nutrition, 68,* 401-403.<br><br>Zimmermann, M., Jacobs, P., Fingerhut, R., Torresani, T., Thöny, B., Blau, N., Baumgartner, M.R., Rohrbach, M. (2012). Positive effect of a simplified diet on blood phenylalanine control in different phenylketonuria variants, characterized by newborn BH4 loading test and PAH analysis. *Molecular Genetics Metabolism, 106,* 264-268. |
| *List of Articles Included from Citation Search or other Means:* | Sweeney, A.L., Roberts, R.M., Fletcher, J.M. (2011). Dietary Protein Counting as an Alternative Way of Maintaining Metabolic Control in Phenylketonuria. *Journal of Inherited Metabolic Disorders,* DOI 10.1007/8904. |
| *List of Articles Excluded from Electronic Database and Reason:* | Rocha, J.C., Martins, M.J. (2012). Oxidative stress in phenylketonuria: future directions. *Journal of Inherited Metabolic Disorders, 35,* 381-98.<br>● Review article, did not assess blood phe concentration as an outcome in allowing liberalized fruits and vegetables<br>Shi, L., Mao, Y. (2010). Excessive recreational computer use and food consumption behavior among adolescents. *Italian Journal of Pediatrics, 5,* 36-52.<br>● Did not assess patients with PKU, did not assess blood phe concentration as an outcome in allowing liberalized fruits and vegetables<br>Weetch, E., Macdonald, A. (2006). The determination of phenylalanine content of foods suitable for phenylketonuria. *Hum Nutr Diet, 3,* 229-36.<br>● Did not assess blood phe concentration as an outcome in allowing liberalized fruits and vegetables<br>Tao, S., Liu, W.X., Chen, Y.J., Xu, F.L., Dawson, R.W., Li, B.G., Cao, J., Wang, X.J., Hu, J.Y., Fang, J.Y. (2004). Evaluation of factors influencing root-induced changes of copper fractionation in rhizosphere of a calcareous soil. *Environ Pollut, 129,* 5-12.<br>● Did not assess patients with PKU, did not assess blood phe concentration as an outcome in allowing liberalized fruits and vegetables<br>Rao, G.Y., Andersson, S., Widén, B. (2002). Flower and cotyledon asymmetry in Brassica cretica: genetic variation and relationships with fitness. *Evolution, 56,* 690-698.<br>● Did not assess patients with PKU, did not assess blood phe |

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| | concentration as an outcome in allowing liberalized fruits and vegetables<br>Garriga, M.M., Metcalfe, D.D. (1988). Aspartame intolerance. *Ann Allergy, 61,* 63-9<br>● Did not assess blood phe concentration as an outcome in allowing liberalized fruits and vegetables, published in 1988<br>Kindt, E., Motzfeldt, K., Halvorsen, S., Lie, S.O. (1984). Is phenylalanine requirement in infants and children related to protein intake? *Br J Nutr, 51,* 435-442.<br>● Did not assess blood phe concentration as an outcome in allowing liberalized fruits and vegetables, published in 1984 |
| *Summary of Articles Identified to Review* | ● Included Primary Research Articles Identified: 5<br>● Included Review Articles Identified: 0<br>● Total Number of Included Articles: 5<br>● Number of Articles Considered but Excluded: 7<br>● Total Number of Articles Considered: 12 |

**Step Three: Critically Appraise Each Article**

The five articles that meet inclusion and exclusion criteria have been read and critically appraised using the EAL Evidence Worksheet and Quality Criteria Checklist. The purpose of the EAL Evidence Worksheet is to abstract key information for future reference, identify study details to determine the study's quality, summarize major findings, record the author's conclusions, note study limitations and applicability, and to note the funding source (AND, 2012). The EAL Worksheets for all five articles can be found in Appendix A.

The Quality Criteria Checklist designed by the EAL has also been used to rate the overall quality of the article in a systematic manner (AND, 2012). The questions are written in 'yes/no' form and work to examine the study design and its execution in an objective manner (AND, 2012). The questions in the Quality Criteria Checklist have been developed specifically to identify observations that are generally accepted as fundamentals of sound scientific

investigation (AND, 2012). Once completed, the checklist is used to give each article an overall rating of positive, neutral, or negative (AND, 2012). The Quality Criteria Checklists from these five articles can also be found in Appendix A. The results have been summarized in a single table to allow for a side by side comparison of each article (Table 2).

**Step Four: Summarize Evidence**

Step Four further summarizes the evidence found by creating an overview table and narrative synthesis (AND, 2012). The purpose of the overview table and narrative synthesis is to combine the relevant information into a brief, articulate, easy- to- read summary (AND, 2012). The overview table allows comparison of the five studies at a glance and helps determine which articles best answer the research question (AND, 2012). The information gathered on each article in the EAL Evidence Worksheets is transferred into the overview table and includes the author, year, study design, class rating, study type, purpose, populations, intervention, outcomes and limitations (AND, 2012).

Brief summaries of each study have also been included to provide a narrative of the relevant findings from each article in an attempt to provide an answer to the research question. After summarizing each article, comparison and patterns between articles and themes are described in the Evidence Summary (AND, 2012). The overview table has been used to help find similarities and differences between the articles. The Evidence Summary includes an overall summary statement, comparison factors statement, methodology statement, and outcome impact statement (AND, 2012). The overall summary statement is brief and focuses on any general agreement between the studies (AND, 2012). The comparison factors statement is a more detailed presentation of the associations between the outcomes of the studies and goes into detail

on the agreements and disagreements between the articles (AND, 2012). The methodology statement describes the types of research designs used and further discusses the strengths or weaknesses of each article based on study design (AND, 2012). Lastly, the outcome impact statements describe any interventions, research procedures, or intervening factors that have affected the outcomes of each study (AND, 2012).

**Step Five: Write and Grade the Conclusion Statement**

In Step Five, a conclusion statement using all of the gathered information is stated. Whether or not the study question was answered and what this evidence states is defined in the conclusion statement (AND, 2012). The overall strength of the evidence supporting the conclusion statement is graded on a scale of I to V where I is good, II is fair, III is limited, IV is expert opinion only, and V is not assignable (AND, 2012). The elements being graded include quality, consistency, quantity, clinical impact and how well the conclusions can be generalized to the broader population (AND, 2012).

# CHAPTER 4: RESULTS

Five studies fit the outlined restrictions of the research question as outlined in the Search and Plan Results figure. Evidence Worksheets and Quality Criteria Checklists have been completed for these five studies and are located in Appendix A. A brief description of the relative findings from each study is provided below in the Narrative of Relevant Findings. A summary of the results from the Quality Criteria Checklists have been summarized in Table 1. The Overview Table, Table 2, provides an outline of the five studies for the purpose of comparing them at a glance.

## Narrative of Relevant Findings

### Rohde et al., (2012)

Rohde et al. (2012) investigated whether the PKU diet can be liberalized to include unlimited consumption of fruits and vegetables containing less than 75 milligrams of phe per 100 grams of food in the short term without negatively impacting metabolic control. They conducted a randomized crossover trial in the outpatient setting (Rohde et al., 2012). Twenty-eight patients were screened for the study with only 16 meeting inclusion criteria (Rohde et al., 2012). Two patients were excluded during the study leaving 14 to complete it; the mean age of patients was 5.7 ± 2.4 years, 8 females and 6 males (Rohde et al., 2012).

For the first three days of the study, all patients followed their current dietary phe and synthetic protein formula prescription (Rohde et al., 2012). On the fourth day, patients were randomized to either continue their classic PKU diet plan or were instructed to consume unlimited fruits and vegetables with <75 milligrams phe per 100 grams (Rohde et al., 2012). The

two groups switched diets on the eighteenth day and continued this dietary treatment for another 2 weeks (Rohde et al., 2012).

The data from each phase was averaged and the changes between the study phases were analyzed using Wilk's multivariate analysis of variance (MANOVA) with a level of $p<0.05$ considered significant (Rohde et al., 2012). The total amount of fruits and vegetables consumed and types consumed did not change significantly through the phases and intake only reached 76-83% of recommended intake of fruits and vegetables for German children, as this study was conducted in Germany (Rohde et al., 2012).

When unrestricted, amounts of fruit and vegetable intake did not increase and the phe usually allotted for fruits and vegetables was used for consumption of other foods (Rohde et al., 2012). As a result, phe intake significantly increased (P=0.037) by an average of 58 milligrams per day (18% increase) during the free fruits and vegetables study phase, but did not negatively impact metabolic control as average blood phe concentrations remained within the recommended range (Rohde et al., 2012). Average blood phe concentrations remained stable (P= 0.76): restricted group phe 246 umol/l +/- 140 and unrestricted group phe 243 umol/L, +/- 137 (Rohde et al., 2012). The frequency of blood levels above the ideal range increased slightly, but not significantly (P=0.123): restricted group 30% of levels above ideal range, unrestricted group 37% of levels above ideal range (Rohde et al., 2012). The study sequence had no effect on dietary or laboratory variables and there was no influence on average dried blood tyrosine levels throughout all study phases (Rohde et al., 2012). The authors, therefore, concluded that free consumption of fruits and vegetables does not negatively impact short term metabolic control in people with PKU (Rohde et al., 2012).

The frequency of blood phe levels was optimal as they were taken daily and coincided with diet records to accurately assess significant changes. The study groups were comparable and randomization was used in determining study groups and order of study phases. This is an open study without blinding. Standardized meals were not provided, and diet records were self recorded which could produce inaccurate accounts of actual intake. The sample size was also a limiting factor for this study as there were only 14 patients participating. Participants' blood phe levels already were within the normal range of <360 umol/L. The study was only 2 weeks long and it is not clear if that is sufficient time to change dietary habits or determine impact on long term metabolic control.

**Rhode et al., (2014)**

Rhode et al (2014) continued to gather data on these patients and published a one year follow up of their original study. Phase 3 of this study lasted for 1-6 months past the original study and phase 4 lasted for months 7-12 (Rohde et al., 2014). Only nineteen subjects from the original study were included in data analysis and three subjects did not participate in phase 4 (Rohde et al., 2014). The mean age of the subjects was 4.7 years, +/- 2.1 years, with a mean phe tolerance of 357 mg phe per day (Rohde et al., 2014).

Patients in both phase 3 and 4 continued to follow unlimited consumption of fruits and vegetables with less than 75 milligrams of phe per 100 grams of food (Rohde et al., 2014). Blood phe concentrations were collected every two to four weeks for the 12 month follow up (Rohde et al., 2014). Three day diet records and blood phe concentrations were collected at the end of phase three, 6 months after the starting date, and at the end of phase 4, twelve months after the starting date (Rohde et al., 2014).

The data from each study phase was averaged with longitudinal changes analyzed by the Friedman test (Rohde et al., 2014). The Wilcoxon test was conducted to compare individual study phases if there were significant differences with a level of $P < 0.05$ showing significance (Rohde et al., 2014). Mean dried blood phe concentrations remained within the recommended range of 40-240 umol/L throughout the study (Rohde et al., 2014). The frequency of elevated levels decreased but not significantly (Rohde et al., 2014). Intake of fruits and vegetables met 91% of the recommended amount for German children following the restricted phase of the study and remained consistent throughout all 4 study phases (Rohde et al., 2014). Phe tolerance increased significantly by an average of 68 mg per day, a 28% increase, while maintaining blood phe levels within the recommended range (Rohde et al., 2014). This one year follow up showed no worsening of metabolic control with liberalization of fruit and vegetable intake (Rohde et al., 2014). The authors conclude that the phe utilization from fruits and vegetables was poor due to the decreased ability for the body to digest the protein from these foods (Rohde et al., 2014).

**MacDonald et al., (2003)**

Researchers MacDonald et al. (2003) from The Children's Hospital in Birmingham, United Kingdom conducted a three part, open, prospective non- randomized crossover study in the outpatient setting to evaluate unrestricted fruit and vegetable consumption among PKU patients. Fifteen patients with moderate to severe PKU, 13 girls and 2 boys, with an age range of 1 to 24 years were recruited into the study (MacDonald et al., 2003). Synthetic protein formula was kept consistent and within original diet prescription from before entering the study and consumed at the same times on blood sampling days (MacDonald et al., 2003).

The first phase was conducted over weeks 1 through 3 in which subjects were allowed to eat unlimited amounts of fruits and vegetables containing less than 50 milligrams phe per 100 grams (MacDonald et al., 2003). Subjects received standardized meals on blood sampling days (MacDonald et al., 2003). Phase two was conducted over weeks 4 through 8, where subjects ate one or more portions of fruits and vegetables containing 50 to 75 milligrams phe per 100 grams daily (MacDonald et al., 2003). Blood samples were taken at weeks six and eight in the same fashion as phase one, including the same standardized meal but with one additional portion of fruit or vegetable containing 50 to 75 milligrams phe per 100 grams (MacDonald et al., 2003). Twelve subjects participated in the optional Phase 3 of the study, which consisted of seven weeks of consuming at least three portions per week of vegetables containing 75 to 100 milligra ms phe per 100 grams (MacDonald et al., 2003). Blood phe levels were drawn at weeks 11, 13, and 15 in the same way as phase one and phase two with standardized meals which included one serving of vegetables with 75-100 milligrams phe per 100 grams (MacDonald et al., 2003).

Paired t-tests were used to compare differences in nutritional intake between weeks 1-3 and 4-8 as well as between weeks 1-3 and 9-15 (MacDonald et al., 2003). Unlimited consumption of fruits and vegetables with 51-100 milligrams phe per 100 grams did not compromise control in plasma phenylalanine levels in this study and significant changes were not found between the different phases of this study (MacDonald et al., 2003). Average phe intake increased above baseline by 54 mg in the second phase and 39 mg in phase three, a significant increase in natural protein (MacDonald et al., 2003).

The use of standardized meals is a strength as it leaves less room for error in recording diet and analyzing nutrient intake. Amount of synthetic protein intake from synthetic protein formula and timing of intake were consistent and in accordance to recommended intakes. The few blinded parts of this study included laboratory staff to blood results and families to phe results during the study to help decrease bias. The study was not randomized as the control period was always before the test period, weakening the results. The sample size was small and diminished further in the third study phase with only 12 participants, further decreasing the strength of the results due to small sample size (MacDonald et al., 2003). This study was short term in design which can be a weakness, as long term effects are unknown.

**Sweeney et al., (2011)**

The purpose of this randomized control trial was to measure the impact on metabolic control from using the following phe counting methods: counting grams of protein (1 gram= 50 mg phe) and counting mg phe exchanges (1 unit = 15 mg phe) (Sweeney et al., 2011). They also sought to increase variety and test metabolic impact by offering varying amounts of free fruits and vegetables (Sweeney et al., 2011).

In Phase One, all subjects completed a baseline 3 day diet diary and PKU Diet Attitudes Questionnaire (Sweeney et al., 2011). Subjects were then randomized into two groups: continue current phe exchange system (control group) or change to counting grams of protein (study group) (Sweeney et al., 2011). Foods with <20 mg phe per serving were free and a diet chart of free foods was provided (Sweeney et al., 2011). Six months after the initial appointment, questionnaires and diet diaries were completed for both groups. The control group was then educated on counting grams of protein. Six months later, diet diaries and questionnaires were

again collected. Phase Two assessed the impact on blood phe levels of a further liberalization of uncounted foods. Foods with 40-50 mg phe/serving were free but given a serving limit and foods with >50 mg phe/ serving were counted at 0.5 g protein increments (Sweeney et al., 2011). An extensive diet chart of free foods was provided. All subjects completed a baseline 3 day diet diary and PKU Diet Attitudes Questionnaire. Six months later, 3 day diet diaries and questionnaires were again completed (Sweeney et al., 2011).

Eighteen patients with PKU and a median age of 10 years 1 month from the Women's and Children's Hospital Metabolic Unit where consented into the study (Sweeney et al., 2011). The participants consisted of 5 males and 13 females with 16 having the classic PKU phenotype and two having the moderate PKU phenotype (Sweeney et al., 2011). All 18 participants had initially been taught the 15 mg phe exchange method and were taking a synthetic protein formula (Sweeney et al., 2011). Seventeen participants advanced to phase two where all participants followed the 50 mg phe exchange counting method and foods with <50 mg phe per serving were considered free (Sweeney et al., 2011).

Analysis for both phase 1 and phase 2 blood tests and questionnaires was conducted using a non-parametric statistic, the Wilcoxon Signed Rank Test with a statistical significance of $p<0.05$ (Sweeney et al., 2011). The results for phase one of this study showed no significant differences between the study participants' blood phe levels from the six months before and six months after using the new exchange method (Sweeney et al., 2011). The control participants' blood phe levels also did not differ significantly before or during phase one of the study (Sweeney et al., 2011). The participants and their parents indicated a preference for the new exchange method as it made tracking phe intake easier and increased the ability to consume

commercial products (Sweeney et al., 2011). They also noted that their children became more interested in their diet as they now understood how to manage the diet with this easier method (Sweeney et al., 2011). The children felt there was a significant increase in variety of their diet while caregivers reported only a slight increase (Sweeney et al., 2011).

The results for phase two showed a significant improvement in blood phe levels for four of the participants, no change for nine of the participants and significantly elevated blood phe levels for one patient (Sweeney et al., 2011). All participants and caregivers preferred the phase two diet chart which allowed unlimited consumption of fruits and vegetables with <50 mg phe per serving (Sweeney et al., 2011). Parents reported a decrease in time to prepare foods as less foods needed to be weighed and measured, lessening the burden of mealtime preparation (Sweeney et al., 2011). Children reported being more accurate with tracking the non-free foods in their diet with this simpler method and being more involved in their diet management (Sweeney et al., 2011).

This study adds important insight with its use of the PKU Diet Attitudes Questionnaire. Participants were randomized in phase one of this study, decreasing potential bias of the allocation of patients between the study and control groups. The format of the diet charts were kept consistent between the two study phases making the transition from phase one free foods to phase two free foods easier for patients and caregivers. Blinding was not used in this study, leading to potential bias. This study also had a very small number of participants due to being conducted at only one metabolic center, potentially decreasing the validity of its findings. Three day diet records were recorded and collected but results were not published or analyzed due to poor quality (Sweeney et al., 2011).

**Zimmermann et al., (2012)**

Potential benefits of a simplified diet in PKU management was also noted in a study conducted by Zimmermann et al. (2012) at the University Children's Hospital in Zurich. Through a detailed PKU cohort followed over three years, they sought to further investigate control of PKU when intake of fruits and vegetables with less than 100 milligrams phe per 100 grams are eaten in amounts recommended by the World Health Organization (WHO) (Zimmermann et al., 2012). According to WHO (2004), daily consumption of 400 grams of fruits and vegetables is recommended. Age of diagnosis, phenylalanine hydroxylase mutations, BH4 responsiveness and diet plans of 80 patients with PKU at the University Children's Hospital were collected retrospectively (Zimmermann et al., 2012).

Zimmermann's proposed simplified diet allows unrestricted intake of fruits, vegetables and low protein food products (Zimmermann et al., 2012). Following WHO recommendations to consume 400 grams of fruits and vegetables daily, the simplified diet encouraged consumption of five fist-sized servings of fruits and vegetables that contain up to 100 milligrams phe per 100 grams daily (Zimmermann et al., 2012). This approach simplified measuring techniques and eliminated the need for counting each milligram of phe from fruits and vegetables consumed.

A total of 80 subjects were included in this study with half being male, 41 with classical PKU, 16 with moderate PKU, 10 with mild PKU and 13 with mild hyperphenylalaninemia (HPA) (Zimmermann et al., 2012). At birth, seventy-three patients were on the traditional diet restriction, while seven were started on the simplified diet (Zimmermann et al., 2012). Fifty patients on the traditional diet agreed to switch to the simplified diet, while 23 decided to remain on the traditional diet restriction (Zimmermann et al., 2012). Blood phe levels are taken using

Guthrie cards with dried blood spots one hour after breakfast or dinner and are monitored every 1-2 weeks for patients 0-2 years old, 1-4 weeks for patients 2-10 years old and at least monthly for those over 10 years old (Zimmermann et al., 2012).

Median blood phe levels for patients on the simplified diet did not differ significantly from those on the traditional diet for those with classical, moderate, and mild PKU (Zimmermann et al., 2012). Four patients with HPA switched to the simplified diet and were not included in the statistical analysis (Zimmermann et al., 2012). Blood phe levels compared between age groups showed a significant difference in those over 16 years old with those on the simplified diet having more blood phe levels in the recommend than those who were on the traditional diet (Zimmermann et al., 2012). The researchers concluded that consumption of fruits and vegetables with less than 100 milligrams phe per 100 grams within WHO recommendations did not worsen blood phe levels independent of classification of PKU and age at which the diet was changed from traditional to simplified (Zimmermann et al., 2012).

Although this study had a large sample size in comparison to other studies in this population, it is still relatively small with only 50 participants willing to trial the simplified diet. The goal of this simplified approach is to maintain blood phe levels in goal range without having to measure certain foods, but by omitting this information this research is not able to quantify the amount of fruits and vegetables consumed. Calculation of phe intake therefore becomes an estimate and decreases the validity of the findings. The study did not include blinding in any part of the study, which could increase risk of bias in the study.

Classification of severity of PKU, described as classical, moderate, mild and HPA, was taken into account to discover if this technique is suitable for people with varying PAH enzyme

activity. Findings were also divided into age group of patients which offers additional confidence in future research for this technique in all age ranges. These researchers emphasized that their technique of a simplified PKU diet should not be confused with a liberalized PKU diet. Patients are still expected to take regular blood phe levels and stay within target range. This study prompts the need for future larger scale, longer term studies to further study the efficacy of a simplified approach to PKU diet management that could potentially improve the quality of life for this population.

**Table 1.** Quality Criteria Summary

| | **MacDonald et al., (2003)** | **Rhode et al., (2014)** | **Rhode et al., (2012)** | **Sweeney et al., (2011)** | **Zimmerman n et al., (2013)** |
|---|---|---|---|---|---|
| Overall Quality Rating | + | + | + | + | + |
| **Relevance Questions** | | | | | |
| 1. Would implementing the studied intervention or procedure result in improved outcomes for the patients/ clients/ population group? | Yes | Yes | Yes | Yes | Yes |
| 2. Did the authors study an outcome or topic that the patients / clients / population group would care about? | Yes | Yes | Yes | Yes | Yes |
| 3. Is the focus of the intervention or procedure or topic of study a common issue of concern to dietetics practice? | Yes | Yes | Yes | Yes | Yes |
| 4. Is the intervention or procedure feasible? | Yes | Yes | Yes | Yes | Yes |
| **Validity Outcomes** | | | | | |
| 1. Was the research question | Yes | Yes | Yes | Yes | Yes |

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| clearly stated? | | | | | |
| 2. Was the selection of study subjects / patients free from bias? | Yes | Yes | Yes | Yes | Yes |
| 3. Were study groups comparable? | Yes | Yes | Yes | Yes | Yes |
| 4. Was method of handling withdrawals described? | Yes | Yes | Yes | Yes | Yes |
| 5. Was blinding used to prevent introduction of bias? | Yes | No | No | No | No |
| 6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described? | Yes | Yes | Yes | Yes | Yes |
| 7. Were outcomes clearly defined and the measurements valid and reliable? | Yes | Yes | Yes | Yes | Yes |
| 8. Was the statistical analysis appropriate for the study design and type of outcome indicators? | Yes | Yes | Yes | Yes | Yes |
| 9. Are conclusions supported by results with biases and limitations taken into consideration? | Yes | Yes | Yes | Yes | Yes |
| 10. Is bias due to study's funding or sponsorship unlikely? | Yes | Yes | Yes | Yes | Yes |

**Table 2.** Overview Table

| Author, Year, Study Design, Class Rating | Study Type/ Purpose | Study Populations | Intervention | Outcomes | Limitations |
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| **Author:** MacDonald, | This study aimed to | **Initial n:** 15 (13 girls, 2 | Three part 15 week study, part three | Free use of fruits and vegetables | *This was an open study, not blinded* |

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| A., Rylance, G., Davies, P., Asplin, D., Hall, S.K., Booth, I. W.<br><br>**Year:** 2003<br><br>**Study Design:** Non Randomized Crossover Trial<br><br>**Class:** C<br><br>**Rating:** + | evaluate the effect of the free use of fruits and vegetables containing 51-100 mg phe per 100g on biochemical control of children with PKU. | boys)<br><br>**Final n:** 15 (13 girls, 2 boys) for phase 1 and phase 2<br>12 (10 girls, 2 boys) for phase 3<br><br>**Age:** Mean 6 years old, range 1-24 years<br><br>-All had moderate to severe PKU<br>-Median phe intake of 50 mg phe exchanges was 6 (300 mg phe), range 5 (250 mg phe) to 16 (800 mg phe)<br>- synthetic protein formula was consumed by all subjects | optional<br>Part 1: weeks 1-3 patients ate freely fruits and vegetables only containing 0-50mg/100g. Twice daily blood phe concentrations were collected the last three days of weeks 1 and 3. Standardized meals were given on blood sampling days.<br>Part 2: weeks 4-8 patients ate at least one daily portion of fruits and vegetables containing 51-75 mg/100g. Twice daily blood phe concentrations were collected the last three days of weeks 6 and 8. The same standardized meals from Part 1 were given on blood sampling days, but with at least one portion of fruits and vegetables containing phe 51-75 mg/100 g added to each meal.<br>Part 3: Optional. Weeks 9-15 patients ate at least three portions per week of vegetables containing 76-100 mg phe per | containing 51-75 mg/100 g and 76-100 mg/100g did not adversely affect blood phe control. Repeated measures analysis of variance did not show any significant changes in plasma phe within or between the three parts of the study<br>Part 2: extra median intake of 54 mg (range 30-138 mg) phe on days of dietary assessment. Significant increase in natural protein ($p<0.005$), energy ($p<0.001$), and carbohydrate ($P<0.005$) intake between weeks 1-3 and 4-8.<br>Part 3: extra median intake of 39 mg (range 13-143 mg) per day.<br>Overall, natural protein intake provided by all free foods in excess of prescribed phe increased from a median of 36% between weeks 1 and 3 to 63% | *except for analyzing blood phe levels and reporting levels to patients and families.*<br><br>*Non-randomized trial, test period always followed the control period.*<br><br>*Small sample size*<br><br>*Short term* |

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| | | | 100g. Twice daily blood phe concentrations were collected the last three days of weeks 11, 13, and 15. The same standardized meals from Part 1 were given on blood sampling days, but with at least one portion of vegetables containing phe 76-100 mg/100 g added to each meal. When possible they also continued to eat fruits and vegetables with 51-75 mg phe per 100 g. | between weeks 4 and 8 and weeks 9 and 15. Between weeks 1-15, natural protein intake increased significantly ($p<0.005$), carbohydrate intake only increase slightly and fat intake remained unchanged. Patients did not decrease intake of other foods to compensate for the extra volume from fruits and vegetables. | |
| **Author:** Rohde, C., Mutze, U., Weigel, J.F.W., Ceglarek, U., Thiery, J., Kiess, W., Beblo, S.<br><br>**Year:** 2012<br><br>**Study Design:** Randomized Cross over study<br><br>**Class:** A<br><br>**Rating:** + | To asses if free consumption of fruits and vegetables containing less than 75 mg phe per 100 g affects metabolic control in children with PKU. | **Initial n:** 16<br><br>**Final n:**14 (8 female, 6 male)<br><br>**Age:** Range 2-10, mean 5.7 years<br><br>Patients were regularly followed in clinic<br><br>Phe tolerance 335 mg plus or minus 48 mg<br><br>Dried blood phe | Day 1-3: all patients followed classic PKU treatment, recorded daily diet records and obtained daily dried blood phe concentrations.<br>Day 4-18: Patents are randomized into one of two groups, free fruit and vegetable consumption (those with less than 75 mg phe per 100 g) or restricted fruit and vegetable consumption. Daily dried blood he levels | Total phe intake increased significantly: P= 0.037, average increase of 58 mg per day, 18% increase<br>Average blood phe levels remained stable: P= 0.76, restricted group 246 umol/l +/- 140, unrestricted: 243 umol/L, +/- 137<br>Frequency of blood levels above ideal range increased slightly but not | *Small sample size*<br>*No blinding took place*<br>*The study was only 2 weeks long, question if sufficient time to change dietary habits or determine impact on metabolic control*<br>*Parents might have been keeping their children on a more strict diet during the trial, blood phe levels may have been lower than usual because of this*<br>*No standardized* |

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| | | concentrations 230 umol/l, plus or minus 63 | obtained, diet records recorded on days 5, 6, 15, 16, and 17.<br>Day 19-32: Patients switched to opposite group. Daily dried blood he levels obtained, diet records recorded on days 19, 20, 29, 30, 31. | significantly: P=0.123, restricted 30%, unrestricted 37%<br>Study sequence had no effect on dietary or laboratory variables<br>No influence on average dried blood tyrosine levels | *meals* |
| **Author:** Rohde, C., Mutze, U., Schulz, S., Thiele, A.G., Ceglarek, U., Thiery, J., Mueller, A.S., Kiess, W., Beblo, S.<br><br>**Year:** 2014<br><br>**Study Design:** Randomized Cross over study<br><br>**Class:** A<br><br>**Rating:** + | To asses if free consumption of fruits and vegetables containing less than 75 mg phe per 100 g affects long term metabolic control in children with PKU. | **Initial n:** 25<br><br>**Final n:** 19, but 3 did not complete the final Phase 4<br><br>**Age:** Range 2.6-6.8 years, mean 4.7years<br><br>Patients were regularly followed in clinic<br><br>Mean phe tolerance 357 mg/day, ranging from 215-660 mg/day<br><br>Dried blood phe concentrations 230 umol/l, plus or minus 63 | After the core trial, remaining subjects continued the free fruit and vegetable diet for another 12 months.<br>Blood phe concentrations were collected every 2 to 4 weeks.<br>Daily diet records and daily blood phe concentrations were collected over a 3 day period at the end of 6 months (Phase 3) and at the end of 12 months (Phase 4). | Mean blood phe concentrations remained within the recommended range (40-240 umol/L)<br>Frequency of blood phe concentrations decreased slightly but not significantly<br>The weight of fruits and vegetables consumed as well as the mg phe consumed from them remained stable, despite liberalization of fruits and vegetables<br>Phe tolerance increased significantly, $p<0.001$, by an average of 68 mg/day<br>Total protein intake increased in study phases 3 and 4 due | *Small sample size*<br>*No blinding took place*<br>*Parents might have been keeping their children on a more strict diet during the diet record and blood phe monitoring days, blood phe levels may have been lower than usual because of this*<br>*No standardized meals* |

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| | | | | to increase in synthetic protein formula to maintain total protein requirements | |
| **Author:** Sweeney , A.L., Roberts, R.M., Fletcher, J.M.<br><br>**Year:** 2011<br><br>**Study Design:** Randomized Crossover Trial<br><br>**Class:** A<br><br>**Rating:** + | To compare a gram protein exchange system (1 gram protein = 50 mg phe) with a unit exchange system (1 unit = 15 mg phe) and its effect on metabolic control measured by blood phe levels as well as acceptance from children and adolescents with PKU. | **Initial n:** Phase 1: 18 (13 female, 5 male) Phase 2: 18 (13 female, 5 male)<br><br>**Final n:** Phase 1: 17 Phase 2: 18, data available for only 14 due to irregular blood phe levels and incomplete questionnaires.<br><br>**Age:** Phase 1: median age of 10 years 1 month, range 2 years 5 months to 17 years 6 months Phase 2: median age of 11 years 6 month, range 1 years 7 months to 20 years 3 months<br><br>Phase 1: 16 had classic PKU phenotype, 2 | Phase One: All subjects completed a baseline 3 day diet diary and PKU Diet Attitudes Questionnaire. Subjects were then randomized into two groups: continue current phe exchange system (control group) or change to counting grams of protein (study group). Foods with <20 mg phe/ serving were free, diet chart of free foods was provided. Six months after initial appointment, questionnaires and diet diaries were completed for both groups. The control group was then educated on counting grams of protein. Six months later, diet diaries and questionnaires were again collected. Phase Two: Assessed the impact on blood | Phase 1: Phe levels over 6 months were comparable to pre-study levels (mean phe pre 366 umol/L +/- 169, mean phe post change 388 umol/L +/- 160). Families and patients preferred the protein counting method per questionnaire. Patients reported significant increase in variety in diet using protein counting method. Phase 2: four participants had a significant improvement on blood phe levels, nine showed no significant change and one participant's levels were significantly higher. All participants preferred the freer diet chart. | *Small sample size* *No blinding took place* *No standardized meals* *No standardized timing for blood draws* |

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| | | had moderate PKU phenotype<br>Phase 2: 16 had classic PKU phenotype, 2 had moderate PKU phenotype | phe levels of a further liberalization of uncounted foods. Foods with 40-50 mg phe/serving were free but given a serving limit. Foods with >50 mg phe/ serving were counted at 0.5 g increments. Extensive diet chart of free foods was provided. All subjects completed a baseline 3 day diet diary and PKU Diet Attitudes Questionnaire.<br>Six months later, 3 day diet diaries and questionnaires were again completed. | | |
| **Author:** Zimmermann, M., Jacobs, P., Fingerhut, R., Torresani, T., Thöny, B., Blau, N. Baumgartner, M.R., Rohrbach, M.<br><br>**Year:** 2012<br><br>**Study Design:** Retrospective Cohort Study<br><br>**Class:** B | The aim of this study was to investigate the effect of the intake of fruits and vegetables containing less than 100 mg phe per 100 g weight, in quantities recommended by WHO, on the course and treatment | **Initial n:** 80 (40 female, 40 male)<br><br>**Final n:** 50<br><br>**Age:** Age 2-9 years (12 patients), Age 10-16 years (13 patients), Age >16 years (25 patients)<br><br>Classical PKU: 41 subjects<br>Moderate PKU: 16 subjects | First months of life: advocate breastfeeding in combination with synthetic protein formula for first 6 months, followed by potatoes mixed with vegetables and fruits with monitoring blood phe levels every 3-7 days.<br>After weaning period: Encouraged 5 servings of fruits and vegetables with <100 mg phe per 100 g | 73 out of 80 patients were on traditional diet upon starting treatment at diagnosis<br>7 out of 80 were on the simplified diet since birth<br>50 patients chose to switch to the simplified diet, 23 refused and remained on the classical diet (used to traditional diet, lack of motivation to change) | *Small sample size*<br>*Possible misclassification of phenotypes due to classification of highest blood phe level before treatment*<br>*Possible bias from small sample size after switch to liberalized diet*<br>*Lack of diet records, inability to quantify phe consumed after diet switch*<br>*No blinding was used* |

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| **Rating:** + | control of the disease. | Mild PKU: 10 subjects<br>Mild HPA: 13 subjects<br>Consanguinity was confirmed in 8 families<br>51 subjects had a 48 hour BH4 loading tests<br>32 subjects had molecular analysis of the PAH gene to identify disease causing mutation | weight. Generally, all fruits and vegetables were recommended if consumed with variation. Special low protein foods could are eaten ad libitum. The quantities of potatoes, rice and maize are fixed for a period of time; parents/patients estimate the fixed quantities and check these quantities with a scale periodically. Synthetic protein formula is the only component that needs to be weighed. Quantity of synthetic protein formula, potatoes, rice and maize are adjusted based on blood phe concentrations. A lump sum of 25 mg phe for fruits, vegetables, and protein free foods was counted for toddlers, 50 mg for older children and adults. | **Blood phe Levels by Severity of PKU:**<br>Classical PKU (26 patients): Median age 19 years, median blood phe level on traditional diet 275 umol/L, median phe on simplified diet 355 umol/L, p=0.96<br>Moderate PKU (13 patients): Median age 10 years, median blood phe level on traditional diet 200 umol/L, median phe on simplified diet 313 umol/L, p=0.06<br>Mild PKU (7 patients): Median age 16.5 years, median blood phe level on traditional diet 300 umol/L, median phe on simplified diet 360 umol/L, p=0.42<br>Mild HPA (4 patients): data not included in statistical analysis<br>**Blood Phe Levels by Age:**<br>Age 2-9 years (12 patients): median blood phe level on traditional diet 200 | *Standardized meals were not used* |

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| | | | | umol/L, median phe on simplified diet 245 umol/L, p=0.183<br>Age 10-16 years (13 patients): median blood phe level on traditional diet 275 umol/L, median phe on simplified diet 406 umol/L, p=0.4993<br>Age >16 years (25 patients): median blood phe level on traditional diet 400 umol/L, median phe on simplified diet 362 umol/L, p=0.0001 | |

# CHAPTER 5: DISCUSSION

## Evidence Summary

1. Overall Summary Statement

Five studies were systematically reviewed using the criteria established by AND's Evidence Analysis Library. These five studies sought to identify the potential metabolic effects of allowing uncounted fruit and vegetable consumption has for people with PKU. Although the study designs and interventions differed between each study, they all came to the same general conclusion: allowing consumption of uncounted fruits and vegetables does not have a negative impact on the metabolic control of those with PKU.

2. Comparison Factors Statements

The studies reviewed sought to find ways to improve the quality of life for PKU patients by increasing dietary choices and making the PKU diet easier to follow. Their aim was to simplify the traditional PKU diet, while keeping metabolic control within recommended ranges. All five studies noted the challenge in maintaining the complex traditional PKU diet. They also discussed the risk of increasingly poor compliance and poor outcomes for this population, especially those in adolescence and beyond. All of the study sample sizes were small, which is not surprising as PKU is a rare genetic disorder and the number of patients seen by each clinic is likely limited.

MacDonald et al. (2003), Sweeney et al. (2011) and Zimmermann et al. (2012) divided patients into severity of PAH deficiency to find effects of diet changes on varying types of PKU. Rhode et al. (2012) and Rhode et al. (2014) only included participants with average blood phe levels <360 umol/L and did not differentiate between degree of PAH deficiency. These studies

were not blinded, which gives similar opportunity for bias across the studies. MacDonald et al. (2003) used standardized meals for a portion of their study, while the other four studies did not have any. By using standardized meals, the errors in self reporting or caregiver reporting diet recalls and the interpretation of diet records is decreased.

Diet records were kept in MacDonald et al. (2003), Rohde et al. (2012), and Rohde et al. (2014) studies with an increase in frequency of monitoring blood phe levels. Zimmermann et al. (2012) did not collect diet records, and continued to monitor blood phe levels at usually recommended frequencies based on age. Sweeney et al. (2011) collected diet records but did not publish the findings due to inaccuracy of these records. Synthetic protein formula intake was kept consistent throughout all five studies in an attempt to not confound the results due to changes in synthetic protein intake as well as changes in how phe intake from food was monitored.

The studies also differed in how to define what fruits or vegetables should not have to be counted. MacDonald et al. (2003) had three different phases which studied the metabolic effects of free foods with 0-50 milligrams phe per 100 grams, 51-75 milligrams phe per 100 grams, and 76-100 milligrams phe per 100 grams. Rohde et al. (2012) and Rohde et al. (2014) defined a free food as those containing less than 75 milligrams phe per 100 grams. Sweeney et al. (2011) had two phases with foods with less than 20 milligrams phe per 100 grams considered free in the first phase and foods with 40-50 milligrams phe per 100 grams considered free in the second phase. Zimmerman et al. (2012) defined free foods as those with less than 100 milligrams phe per 100 grams. These varied definitions of uncounted foods for the PKU diet make it difficult to make a clinical recommendation.

3. Methodological Statements

The study with the lowest study design classification is MacDonald et al. (2003), which is a 'Class C' non randomized crossover trial. They conducted a fifteen week three part study where the test period always followed the control period. The definition of an uncounted food changed with the phe content increasing per serving for each phase. The first phase defined free fruits and vegetables as 0-50 milligrams phe per 100 grams, they second phase the subjects ate at least one daily serving of fruits or vegetables with 51-75 milligrams phe per 100 grams and the third phase, which was optional, subjects consumed at least three servings per week of foods with 75-100 milligrams phe per 100 grams. Fifteen subjects completed phases one and two while only 12 completed phase three.

Zimmerman et al. (2012) conducted a 'Class B' retrospective cohort study. Likely due to the retrospective study design and length of time information was gathered, this study contained the largest subject size, 80 initial and 50 final subjects. Seventy three out of the 80 subjects enrolled in the study were initially on the traditional PKU diet for which all fruits and vegetables were weighed and measured to calculate exact milligram phe amount consumed while seven were on the simplified diet since birth. Only fifty subjects agreed to switch to the more simplified diet of allowing five servings of fruits and vegetables with less than 100 milligrams phe per 100 grams.

Rohde et al. (2012), Rohde et al. (2014) and Sweeney et al. (2011) designs are 'Class A' randomized crossover studies, the highest classification a study can achieve. Fourteen subjects in Rohde et al. (2012) started with a three day control period with all subjects following their traditional PKU diet, and were then randomized to either continue the traditional PKU diet or

consume free fruits and vegetables with less than 75 milligrams phe per 100 grams. Two weeks later the study groups switched to the other diet for another two weeks. Rohde et al. (2014) took the study a step further, looking at the long term implications of allowing free fruits and vegetables with less than 75 milligrams phe per 100 grams. Nineteen subjects continued to consume free fruits and vegetables for another 6 months, and sixteen subjects continued for 6 months after that for twelve months total collected data on the long term effects of metabolic control.

Sweeney et al (2011) had two study phases which evaluated uncounted fruits and vegetables as well as transitioning from counting 15 milligram phe exchanges to 50 milligram (one gram protein) exchanges. Eighteen patients entered phase one and were randomized to continue 15 milligrams phe exchange counting or to change to counting one gram protein to equal 50 milligrams phe. Phase one also allowed foods with less than 20 milligrams phe per serving to be consumed freely. After six months, the control group switched to the new method of counting grams of protein and data was collected for another 6 months. All subjects on phase two continued counting grams of protein and were further liberalized to allow fruits and vegetables with less than 50 milligrams phe per serving to be consumed freely. Eighteen subjects entered phase two, but only 14 subjects were included in analysis of results.

All of the studies except for Rohde et al. (2012) encountered increased subject dropout rate with further liberalization of the PKU diet. Some reasons the authors provided for these drop outs included lack of motivation to continue the studies, fear of the simplified diet, lack of blood phe levels, and comfort with following the traditional diet. Sweeney et al. (2011) was the only study to include a questionnaire to gain insight on the feelings of the subjects and their caregivers

towards the diet and management of their PKU, which also provides critical insight on improving feelings they have towards their diet management.

4. Outcome Impact Statements

When analyzing the results, Zimmerman et al. (2012) was the only study to divide the outcomes by severity of subjects' PKU as well as by subjects' age. This provides clinical insight to determine if the uncounted fruit and vegetable diet is more suitable for specific age groups or severity of PKU. Zimmerman et al. (2012) did not find a significant difference in blood phe levels when organizing the subjects by severity of PKU, but did find a significant improvement in blood phe levels for those over sixteen years of age when they were switched to the simplified diet ($p=0.0001$). Phase one of Sweeney et al. (2011) showed no significant change in blood phe levels between traditional and simplified diet, while four patients in phase 2 had significant improvement, nine showed no significant difference and one patient had significantly higher blood phe levels.

Total phe intake in the Rohde et al. (2012) study increased significantly ($p=0.037$) while blood phe levels remained stable ($p=0.76$). Frequency of blood phe levels above the normal range increased, but not significantly ($p=0.123$). Rohde et al. (2014) subjects had significantly increased phe tolerance ($p<0.001$) with mean blood phe levels staying in the recommended range and frequency of elevated blood phe levels decreasing slightly. Between weeks one and fifteen in MacDonald et al. (2003), natural protein increased significantly ($p<0.001$) with no significant changes in blood phe levels.

## Implications for Future Research

All of the studies concluded that consumption of uncounted fruits and vegetables at varying amounts did not compromise metabolic control and even improved control in some groups of people with PKU. These studies motivate future research to find opportunities to improve the quality of life of those with PKU by simplifying diet management and encouraging variety of foods consumed. These studies also encourage practitioners to develop creative solutions when providing the most effective dietary treatment for their patients with PKU. One diet regimen may not produce satisfactory results for all patients and families and safe alternatives should be considered to improve health outcomes.

Most of these studies noted the resistance of some patients and families to continue participation when the degree of uncounted foods increased. This provides insight to dietitians on the importance of educating families on the benefits of following a non-traditional PKU diet, but to also respect families' wishes if they decline this method of management. Dietitians may also decide to define uncounted foods as those with the smallest amount of phe per serving and slowly increase to allowing foods with 50 to 100 mg per serving to slowly incorporate uncounted foods into patient diets. This method may make the transition easier and less worrisome for some patients and families. To increase the number of subjects, future studies could consider incorporating multiple clinics that treat patients with PKU. Future studies should also consider conducting long term studies that could show the progression of outcomes of using a simplified diet through the patient's different life stages. This could help narrow age specific recommendations for this diet technique and could provide more evidence for what age it is appropriate to start this technique.

## Conclusion Statement

The effects of allowing the consumption of varying unlimited fruits and vegetables has been described in five studies. These studies differed in design and classification of uncounted fruits and vegetables, but all analyzed the effect of the change the PKU diet had on metabolic control for those with PKU. All five studies stated the same conclusion: allowing uncounted fruits and vegetables in the PKU diet does not worsen metabolic control in patients with PKU when compared to counting the milligrams of phe consumed by fruits and vegetables towards the daily phe intake goal.

**Grade I: Good/Strong**

**Table 3.** Conclusion Grading Table (AND, 2012)

| Conclusion Grading Table | | | | | |
|---|---|---|---|---|---|
| Strength of Evidence Elements | Grade I: Good/Strong | Grade II: Fair | Grade III: Limited/Weak | Grade IV: Expert Opinion Only | Grade V: Grade Not Assignabl |
| Quality Scientific rigor/validity Considers design and execution | Studies of strong design for question Free from design flaws, bias and execution problems | Studies of strong design from question with minor methodological concerns, OR only studies of weaker study design for question | Studies of weak design for answering the question OR Inconclusive findings due to design flaws, bias, or execution problems | No studies available<br><br>Conclusion based on usual practice, expert consensus, clinical experience, opinion, or extrapolation from basic research | No evidence that pertains to question being addressed |
| Consistency Of findings across | Findings generally | Inconsistency among | Unexplained inconsistency | Conclusion supported | NA |

| | | | | | |
|---|---|---|---|---|---|
| studies | consistent in direction and size of effect or degree of association, and statistical significance with minor exceptions at most | results of studies with strong design, OR consistency with minor exceptions across studies of weaker design | among results from different studies OR single study unconfirmed by other studies | solely by statements or informed nutrition or medical commentators | |
| Quantity<br>Number of studies<br>Number of subjects in studies | One to several good quality studies<br><br>Large number of subjects studied<br><br>Studies with negative results have sufficiently large sample size for adequate statistical power | Several studies by independent investigators<br><br>Doubts about adequacy of sample size to avoid Type I and Type II error | Limited number of studies<br><br>Low number of subjects studied and/or inadequate sample size within studies | Unsubstantiated by published research studies | Relevant studies have not been done |
| Clinical impact<br>Importance of studied outcomes<br>Magnitude of effect | Studied outcome relates directly to the question<br><br>Size of effect is clinically meaningful | Some doubt about the statistical or clinical significance of the effect | Studied outcome is an intermediate outcome or surrogate for the true outcome of interest<br>OR | Objective data unavailable | Indicates area for future research |

| | | | | | |
|---|---|---|---|---|---|
| | Significant (statistical difference) is large | | Size of effect is small or lacks statistical and/or clinical significance | | |
| Generalizability To population of interest | Studied population, intervention and outcomes are free from serious doubts about generalizability | Minor doubts about generalizability | Serious doubts about generalizability due to narrow or different study population, intervention or outcomes studied | Generalizability limited to scope of experience | NA |

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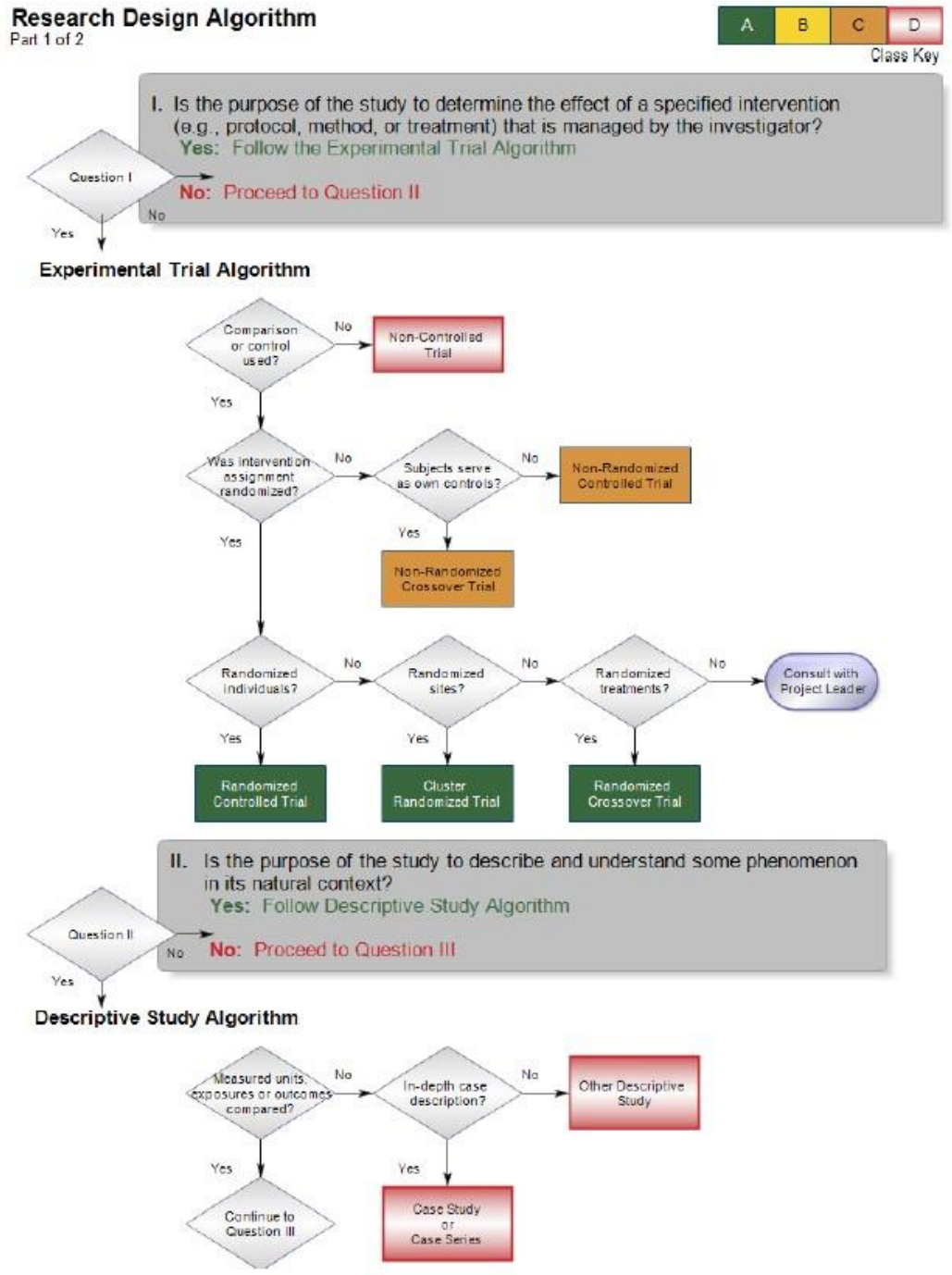
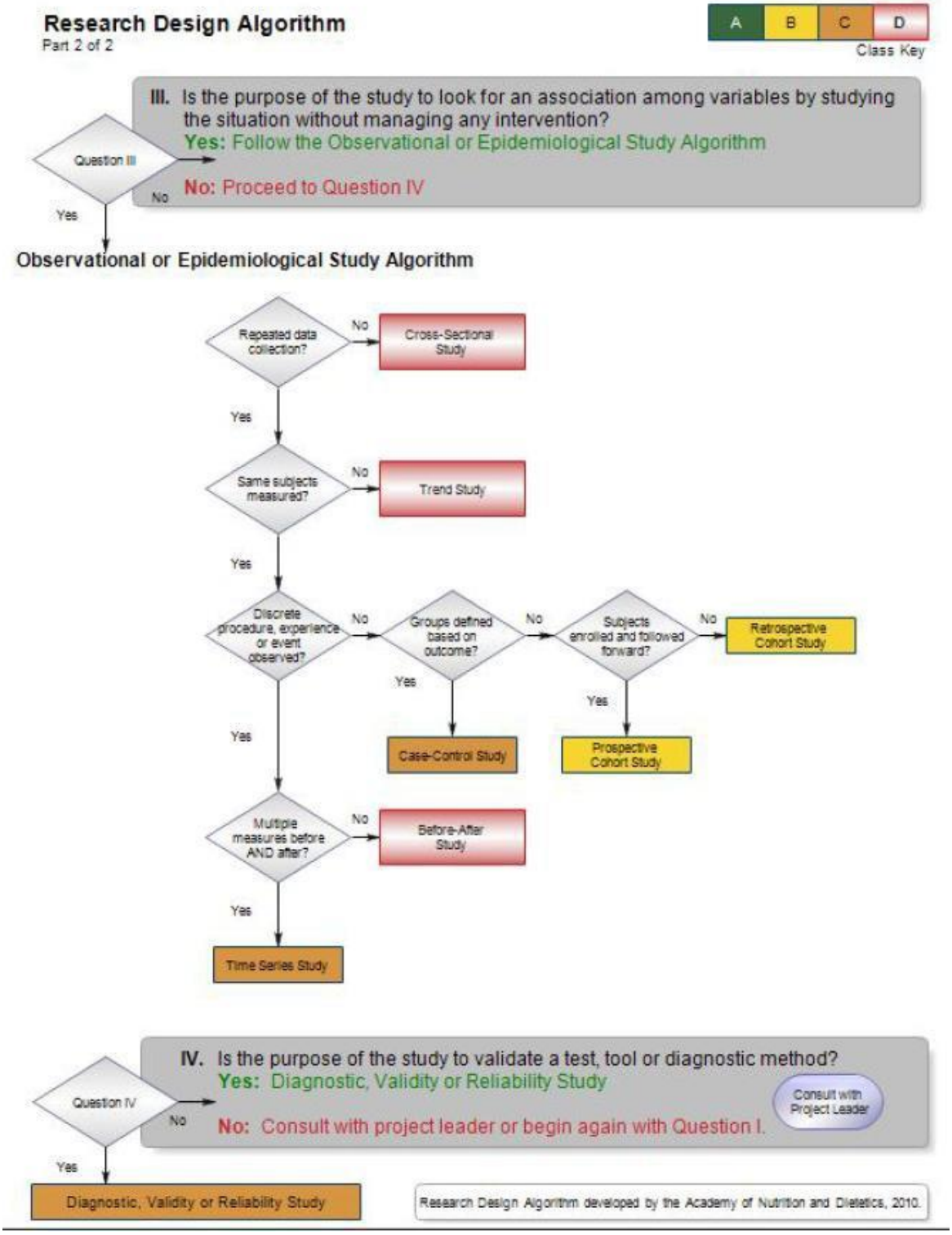
APPENDIX A:


Research Design Algorithm
Part 1 of 2
A
B
C
D
Class Key
I. Is the purpose of the study to determine the effect of a specified intervention (e.g., protocol, method, or treatment) that is managed by the investigator?
Yes: Follow the Experimental Trial Algorithm
Question I
No: Proceed to Question II
No
Yes
Experimental Trial Algorithm
Comparison or control used?
No
Non-Controlled Trial
Yes
Was intervention assignment randomized?
No
Subjects serve as own controls?
No
Non-Randomized Controlled Trial
Yes
Non-Randomized Crossover Trial
Yes
Randomized individuals?
No
Randomized sites?
No
Randomized treatments?
No
Consult with Project Leader
Yes
Randomized Controlled Trial
Yes
Cluster Randomized Trial
Yes
Randomized Crossover Trial
II. Is the purpose of the study to describe and understand some phenomenon in its natural context?
Yes: Follow Descriptive Study Algorithm
Question II
No
No: Proceed to Question III
Yes
Descriptive Study Algorithm
Measured units, exposures or outcomes compared?
No
In-depth case description?
No
Other Descriptive Study
Yes
Continue to Question III
Yes
Case Study or Case Series


Research Design Algorithm
Part 2 of 2
A
B
C
D
Class Key
III. Is the purpose of the study to look for an association among variables by studying the situation without managing any intervention?
Yes: Follow the Observational or Epidemiological Study Algorithm
No: Proceed to Question IV
Question III
No
Yes
Observational or Epidemiological Study Algorithm
Repeated data collection?
No
Cross-Sectional Study
Yes
Same subjects measured?
No
Trend Study
Yes
Discrete procedure, experience or event observed?
No
Groups defined based on outcome?
No
Subjects enrolled and followed forward?
No
Retrospective Cohort Study
Yes
Case-Control Study
Yes
Prospective Cohort Study
Yes
Multiple measures before AND after?
No
Before-After Study
Yes
Time Series Study
IV. Is the purpose of the study to validate a test, tool or diagnostic method?
Yes: Diagnostic, Validity or Reliability Study
No: Consult with project leader or begin again with Question I.
Consult with Project Leader
Question IV
No
Yes
Diagnostic, Validity or Reliability Study
Research Design Algorithm developed by the Academy of Nutrition and Dietetics, 2010.

APPENDIX B:

Evidence Worksheet for Primary Research Article

| | |
|---|---|
| **Citation:** | MacDonald, A., Rylance, G., Davies, P., Asplin, D., Hall, S.K., Booth, I. W. (2003). Free use of fruits and vegetables in phenylketonuria. *Journal of Inherited Metabolic Disease, 26*, 327-338. |
| **Study design:** | Non Randomized Crossover Trial |
| **Study Class (A,B,C,D):** | C |
| **Research Quality Rating:** | Positive (+) |
| *Purpose/Population Studied/Practice Studied* | |
| **Research purpose:** | This study aimed to evaluate the effect of the free use of fruits and vegetables containing 51-100 mg phe per 100g on biochemical control of children with PKU. |
| **Inclusion criteria:** | Maintenance of at least 70% plasma phe within recommended ranges for 6 months before entering the study<br>Over 1 year of age,<br>Ability to eat fruits and vegetables with 50-75 mg/ 100g or 76-100mg/g,<br>Parental or own ability to take skin puncture blood specimens at home. |
| **Exclusion criteria:** | Factors that opposed the inclusion criteria |
| **Recruitment:** | Patients seen at Birmingham clinic |
| **Blinding used:** | Researchers who handled blood specimens and blood phe results were blinded<br>Patients and families were not told blood phe levels until the end of the study |
| **Description of study protocol:** | Three part 15 week study, part three optional<br>Part 1: weeks 1-3 patients ate freely fruits and vegetables only containing 0-50mg/100g. Twice daily blood phe concentrations were collected the last three days of weeks 1 and 3. Standardized meals were given on blood sampling days.<br>Part 2: weeks 4-8 patients ate at least one daily portion of fruits and vegetables containing 51-75mg/100g. Twice daily blood phe concentrations were collected the last three days of weeks 6 and 8. The same standardized meals from Part 1 were given on blood sampling days, but with at least one portion of fruits and |

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| | vegetables containing phe 51-75 mg/100 g added to each meal.<br>Part 3: Optional. Weeks 9-15 patients ate at least three portions per week of vegetables containing 76-100 mg phe per 100g. Twice daily blood phe concentrations were collected the last three days of weeks 11, 13, and 15. The same standardized meals from Part 1 were given on blood sampling days, but with at least one portion of vegetables containing phe 76-100 mg/100 g added to each meal. When possible they also continued to eat fruits and vegetables with 51-75 mg phe per 100 g. |
| **Intervention:** | Parents or subjects took heel or thumb skin puncture blood specimens into heparinized tubes at home, then mailed to the hospital.<br>Plasma phe was measured by HPLC.<br>Throughout the study, fruits and vegetables containing 51-75 mg and 76-100 mg phe per 100 gram were recorded.<br>synthetic protein formula was consumed at consistent times on blood sampling days.<br>All foods and drinks were measured with a gram scale on blood sampling days. Nutrition analysis was calculated using Microdiet computer program, Phe analysis of fruits and vegetables was based on data from Leatherhead Food Research Association and Laboratory of the Government Chemist in London. |
| **Statistical analysis:** | Repeated measures analysis of variance were applies to look for changes in time and to compare differences between each part of the study.<br>Summary results are presented on the original scale as medians and ranges. Paired t tests were used to compare differences in nutrient intake between weeks 1-3 and 4-8 as well as between 1-3 and 9-15.<br>$P < 0.005$ for protein intake increased significantly between weeks 1-3 and 4-8. |
| **Timing of measurements:** | Plasma phe was taken for three consecutive days at the end of weeks 1, 3, 6, 8, 11, 13 and 15.<br>Blood was drawn before breakfast and before evening meal. |
| **Dependent variables:** | Metabolic control as defined by plasma phe concentrations |

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| **Independent variables:** | Amount of varying free fruits and vegetables consumed |
| **Control Variables:** | synthetic protein formula intake remained unchanged throughout |

| | |
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| | study<br>Mg phe/100g of fruits and vegetables were controlled through each stage of the study<br>Timing of fasting for blood draws<br>Phe intake was kept consistent from other foods throughout all 3 stages |
| **Initial n:** | 15 (13 girls, 2 boys) |
| **Final n:** (attrition) | 15 (13 girls, 2 boys) for phase 1 and phase 2<br>12 (10 girls, 2 boys) for phase 3 |
| **Age:** | Mean 6 years old, range 1-24 years |
| **Ethnicity**: | 14 Caucasian, 1 afro-Caribbean |
| **Other relevant demographics:** | All had moderate to severe PKU<br>Median phe intake of 50 mg phe exchanges was 6 (300 mg phe), range 5 (250 mg phe) to 16 (800 mg phe)<br>synthetic protein formula was consumed by all subjects |
| **Anthropometrics:** | None noted |
| **Location:** | The Children's Hospital, Birmingham, UK |
| **Summary of Results:** | Free use of fruits and vegetables containing 51-75 mg/100 g and 76-100 mg/100g did not adversely affect plasma phenylalanine control.<br>Repeated measures analysis of variance did not show any significant changes in plasma phe within or between the three parts of the study<br>Part 2: extra median intake of 54 mg (range 30-138 mg) phe on days of dietary assessment. Significant increase in natural protein ($p<0.005$), energy ($p<0.001$), and carbohydrate ($P<0.005$) intake between weeks 1-3 and 4-8.<br>Part 3: extra median intake of 39 mg (range 13-143mg) per day.<br>Overall, natural protein intake provided by all free foods in excess of prescribed phe increased from a median of 36% between weeks 1 and 3 to 63% between weeks 4 and 8 and weeks 9 and 15.<br>Between weeks 1-15, natural protein intake increased significantly ($p<0.005$), carbohydrate intake only increase slightly and fat intake remained unchanged.<br>Patients did not decrease intake of other foods to compensate for the extra volume from fruits and vegetables. |
| *Author's Conclusions* | |

| | |
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| **Author conclusion:** | This study demonstrated that it is safe to not restrict intake of fruits and vegetables whose phe content is 51-75 mg/100g in children with PKU. More research is needed in order to recommend unlimited fruits and vegetables in 76-100 mg/100g range. |
| **Reviewer comments:** | *Limitations:*<br>*This was an open study, not blinded except for analyzing blood phe levels and reporting levels to patients and families.*<br>*Non-randomized trial, test period always followed the control period.*<br>*Small sample size*<br>*Short term*<br>*Strengths:*<br>*Prospective study*<br>*Participants were comparable*<br>*Standardized meals were used* |

**Quality Criteria Checklist: Primary Research**

| **RELEVANCE QUESTIONS** | | | | | |
|---|---|---|---|---|---|
| **Citation:** MacDonald, A., Rylance, G., Davies, P., Asplin, D., Hall, S.K., Booth, I. W. (2003). Free use of fruits and vegetables in phenylketonuria. *Journal of Inherited Metabolic Disease, 26,* 327-338. | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies) | 1 | x | | | |
| 2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about? | 2 | x | | | |
| 3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice? | 3 | x | | | |
| 4. Is the intervention or procedure feasible (NA for some epidemiological studies)? | 4 | x | | | |

| | | | | | |
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| *If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.* | | | | | |
| **VALIDITY QUESTIONS** | | | | | |
| **1. Was the research question clearly stated? YES** | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified? | 1.1 | x | | | |
| 1.2 Was the outcome(s) (dependent variable(s)) clearly indicated? | 1.2 | x | | | |
| 1.3 Were the target population and setting specified? | 1.3 | x | | | |
| **2. Was the selection of study subjects / patients free from bias? YES** | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 2.1 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study? | 2.1 | x | | | |
| 2.2 Were criteria applied equally to all study groups? | 2.2 | x | | | |
| 2.3 Were health, demographics, and other characteristics of subjects described? | 2.3 | x | | | |
| 2.4 Were the subjects /patients in a representative sample of the relevant population? | 2.4 | x | | | |
| **3. Were study groups comparable? YES** | | **YES** | **NC** | **UNCLEAR** | **NA** |
| 3.1 Was the method of assigning subjects / patients to groups described | 3.1 | | | | x |

| | | | | | |
|---|---|---|---|---|---|
| and unbiased? (Method of randomization identified if RCT) | | | | | |
| 3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline? | 3.2 | x | | | |
| 3.3 Were concurrent controls used? (Concurrent preferred over historical controls.) | 3.3 | x | | | |
| 3.4 If cohort study or cross-sectional study were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis? | 3.4 | x | | | |
| 3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies. | 3.5 | | | | x |
| 3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")? | 3.6 | | | | x |
| **4. Was method of handling <u>withdrawals</u> described? YES** | | **YES** | **NO** | **UNCI** | **NA** |
| 4.1 Were follow up methods described and the same for all groups? | 4.1 | **x** | | | |
| 4.2 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80 %.) | 4.2 | **x** | | | |
| 4.3 Were all enrolled subjects/patients (in the original sample) accounted for? | 4.3 | **x** | | | |
| 4.4 Were reasons for withdrawals similar across groups? | 4.4 | **x** | | | |
| 4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study? | 4.5 | | | | **x** |
| 5. **Was <u>blinding</u> used to prevent introduction of bias? YES** | | **YES** | **NO** | **UNCI** | **NA** |

| | | | | | |
|---|---|---|---|---|---|
| 5.1 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **as appropriate**? | 5.1 | **x** | | | |
| 5.2 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.) | 5.2 | **X** | | | |
| 5.3 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded? | 5.3 | **x** | | | |
| 5.4 In case control study, was case definition explicit and case ascertainment not influenced by exposure status? | 5.4 | | | | **x** |
| 5.5 In diagnostic study, were test results blinded to patient history and other test results? | 5.5 | | | | **x** |
| **6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described? YES** | | **YES** | **NC** | **UNCI** | **NA** |
| 6.1 In RCT or other intervention trial, were protocols described for all regimens studied? | 6.1 | x | | | |
| 6.2 In observational study, were interventions, study settings, and clinicians / provider described? | 6.2 | | | | x |
| 6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect? | 6.3 | x | | | |
| 6.4 Was the amount of exposure and, if relevant, subject / patient compliance measured? | 6.4 | x | | | |
| 6.5 Were co-interventions (e.g., ancillary treatments other therapies) described? | 6.5 | **X** | | | |
| 6.6 Were extra or unplanned treatments described? | 6.6 | | x | | |
| 6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups? | 6.7 | x | | | |
| 6.8 In diagnostic study, were details of test administration and replication sufficient? | 6.8 | | | | x |
| **7. Were outcomes clearly defined and the measurements valid and reliable? YES** | | **YES** | **NC** | **UN** | **NA** |

| | | | | | |
|---|---|---|---|---|---|
| | | | | **EAR** | |
| 7.1 Were primary and secondary endpoints described and relevant to the question? | 7.1 | x | | | |
| 7.2 Were nutrition measures appropriate to question and outcomes of concern? | 7.2 | x | | | |
| 7.3 Was the period of follow-up long enough for important outcome(s) to occur? | 7.3 | x | | | |
| 7.4 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures? | 7.4 | x | | | |
| 7.5 Was the measurement of effect at an appropriate level of precision? | 7.5 | x | | | |
| 7.6 Were other factors accounted for (measured) that could affect outcomes? | 7.6 | x | | | |
| 7.7 Were the measurements conducted consistently across groups? | 7.7 | x | | | |
| **8. Was the <u>statistical analysis</u> appropriate for the study design and type of outcome indicators? YES** | | **YES** | **NO** | **UNCI** | **NA** |
| 8.1 Were statistical analyses adequately described and the results reported appropriately? | 8.1 | x | | | |
| 8.2 Were correct statistical tests used and assumptions of test not violated? | 8.2 | x | | | |
| 8.3 Were statistics reported with levels of significance and/or confidence intervals? | 8.3 | x | | | |
| 8.4 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)? | 8.4 | X | | | |
| 8.5 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)? | 8.5 | **X** | | | |
| 8.6 Was clinical significance as well as statistical significance reported? | 8.6 | **x** | | | |
| 8.7 If negative findings, was a power calculation reported to address type 2 error? | 8.7 | | | | x |
| 9. **Are <u>conclusions</u> supported by results with biases and limitations taken into consideration? YES** | | | | | |
| 9.1 Is there a discussion of findings? | 9.1 | X | | | |

| | | | | | |
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| 9.2 Are biases and study limitations identified and discussed? | 9.2 | x | | | |
| **10. Is bias due to study's funding or sponsorship unlikely? YES** | | | | | |
| 10.1 Were sources of funding and investigators' affiliations described? | 10.1 | x | | | |
| 10.2 Was there no apparent conflict of interest? | 10.2 | x | | | |
| **SYMBOL** | | | | | |
| **MINUS/NEGATIVE (-)**<br>*If most (six or more) of the answers to the above validity questions are "no," the report should be designated with a minus (-) symbol on the Evidence Quality Worksheet.* | | | | | |
| **NEUTRAL (ø)**<br>*If the answers to validity criteria questions **2, 3, 6, and 7** do not indicate that the study is exceptionally strong, the report should be designated with a neutral* (ø) *symbol on the Evidence Quality Worksheet.* | | | | | |
| **PLUS/POSITIVE (+)**<br>*If most of the answers to the above validity questions are "Yes" including criteria **2, 3, 6, and 7 and at least one additional "yes"**,( the report should be designated with a plus symbol (+) on the Evidence Quality Worksheet.* | | | | | |

Evidence Worksheet for Primary Research Article

| | |
|---|---|
| **Citation:** | Sweeney, A.L., Roberts, R.M., Fletcher, J.M. (2011). Dietary Protein Counting as an Alternative Way of Maintaining Metabolic Control in Phenylketonuria. *Journal of Inherited Metabolic Disorder*, DOI 10.1007/8904. |
| **Study Design:** | Randomized Crossover Trial |
| **Study Class (A,B,C,D):** | A |
| **Research Quality Rating:** | Positive **(+)** |
| *Purpose/Population Studied/Practice Studied* | |
| **Research Purpose:** | To compare a gram protein exchange system (1 gram protein = 50 mg phe) with a unit exchange system (1 unit = 15 mg phe) and its effect on metabolic control measured by blood phe levels as well as acceptance from children and adolescents with PKU. |
| **Inclusion Criteria:** | Over 1 year of age<br>Patients treated for PKU at the Women's and Children's Hospital |

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|---|---|
| | Metabolic Unit |
| **Exclusion Criteria:** | <1 year of age<br>Patients not being treated for PKU at the Women's and Children's Hospital Metabolic Unit |
| **Recruitment:** | Those treated at the Women's and Children's Hospital Metabolic Unit |
| **Blinding Used:** | none |
| **Description of Study Protocol:** | Phase One: All subjects completed a baseline 3 day diet diary and PKU Diet Attitudes Questionnaire.<br>Subjects were then randomized into two groups: continue current phe exchange system (control group) or change to counting grams of protein (study group).<br>Foods with <20 mg phe/ serving were free, diet chart of free foods was provided.<br>Six months after initial appointment, questionnaires and diet diaries were completed for both groups.<br>The control group was then educated on counting grams of protein. Six months later, diet diaries and questionnaires were again collected.<br>Phase Two: Assessed the impact on blood phe levels of a further liberalization of uncounted foods.<br>Foods with 40-50 mg phe/serving were free but given a serving limit. Foods with >50 mg phe/ serving were counted at 0.5 g increments. Extensive diet chart of free foods was provided.<br>All subjects completed a baseline 3 day diet diary and PKU Diet Attitudes Questionnaire.<br>Six months later, 3 day diet diaries and questionnaires were again completed. |
| **Intervention:** | Blood phe levels were measured using weekly at home on filter paper and measured by tandem mass spectrometry.<br>A questionnaire was provided to evaluate the attitudes of young people with PKU and their parents on preparing foods, monitoring diet, collecting blood tests, variety, stress from diet, and quality of life.<br>3 day diet records were used to assess dietary and supplement intake but were not sufficient quality to formally report the results.<br>Frequency of clinic visits were unchanged as well as intake of synthetic protein formula. |

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| **Statistical Analysis:** | In phase 1 and 2, all blood test results and questionnaires were analyzed using a non-parametric statistic, the Wilcoxon Signed Rank Test. Statistical significance was set at $p<0.05$. |
| **Timing of Measurements:** | Blood phe was measured weekly at a time convenient to the family |
| **Dependent Variables:** | Metabolic control as measured by blood Phe concentration<br>Answers from PKU Diet Attitudes Questionnaire<br>Nutrient intake analyzed from three day diet records |

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| **Independent Variables:** | Definition of free fruits and vegetables (<20 mg phe/ serving versus <50 mg phe per serving)<br>Continue phe by exchange units or by grams of protein |
| **Control Variables:** | Patients were seen in clinic per usual clinic protocol<br>synthetic protein formula intake was unchanged during study phases |
| **Initial Number (n):** | Phase 1: 18 (13 female, 5 male)<br>Phase 2: 18 (13 female, 5 male) |
| **Final Number (n):** | Phase 1: 17<br>Phase 2: 18, data available for only 14 due to irregular blood phe levels and incomplete questionnaires. |
| **Age:** | Phase 1: median age of 10 years 1 month, range 2 years 5 months to 17 years 6 months<br>Phase 2: median age of 11 years 6 month, range 1 years 7 months to 20 years 3 months |
| **Ethnicity *(if given)*:** | Unknown |
| **Other Relevant Demographics:** | Phase 1: 16 had classic PKU phenotype, 2 had moderate PKU phenotype<br>Phase 2: 16 had classic PKU phenotype, 2 had moderate PKU phenotype |
| **Anthropometrics:** | unknown |
| **Location:** | Women's and Children's Hospital, Adelaide, Australia |
| **Summary of Results:** | Phase 1: Blood phe levels over 6 months were comparable to pre-study levels (mean phe pre 366 umol/L +/- 169, mean phe post change 388 umol/L +/- 160). Families and patients preferred the protein counting method per questionnaire. Patients reported significant increase in variety in diet using protein counting method.<br>Phase 2: four participants had a significant improvement on blood phe levels, nine showed no significant change and one participant's levels were significantly higher. All participants preferred the freer diet chart. |

| *Author's Conclusions* | |
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| **Author Conclusion:** | Protein exchanges (foods containing <50 mg phe/serving uncounted) are an alternative method of measuring Phe intake in the dietary management of PKU. |
| **Reviewer Comments:** | *Limitations:*<br>*Small sample size*<br>*No blinding took place*<br>*No standardized meals*<br>*No standardized timing for blood draws*<br><br>*Strengths:*<br>*Study groups were comparable*<br>*Randomization was used in determining control and study groups*<br>*Longer term study*<br>*Diet charts created in similar format to increase consistency of education materials*<br>*PKU attitudes questionnaire used* |

**Quality Criteria Checklist: Primary Research**

| **RELEVANCE QUESTIONS** | | | | | |
|---|---|---|---|---|---|
| **Citation:** Sweeney, A.L., Roberts, R.M., Fletcher, J.M. (2011). Dietary Protein Counting as an Alternative Way of Maintaining Metabolic Control in Phenylketonuria. *Journal of Inherited Metabolic Disorder*, DOI 10.1007/8904. | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies) | 1 | X | | | |
| 2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about? | 2 | X | | | |
| 3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice? | 3 | X | | | |
| 4. Is the intervention or procedure feasible (NA for some epidemiological studies)? | 4 | X | | | |

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| *If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.* | | | | | |
| **VALIDITY QUESTIONS** | | | | | |
| **1. Was the research question clearly stated? YES** | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified? | 1.1 | X | | | |
| 1.2 Was the outcome(s) (dependent variable(s)) clearly indicated? | 1.2 | X | | | |
| 1.3 Were the target population and setting specified? | 1.3 | X | | | |
| **2. Was the selection of study subjects / patients free from bias? YES** | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 2.1 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study? | 2.1 | X | | | |
| 2.2 Were criteria applied equally to all study groups? | 2.2 | X | | | |
| 2.3 Were health, demographics, and other characteristics of subjects described? | 2.3 | X | | | |
| 2.4 Were the subjects /patients in a representative sample of the relevant population? | 2.4 | X | | | |
| **3, Were study groups comparable? YES** | | **YES** | **NC** | **UNCLEAR** | **NA** |
| 3.1 Was the method of assigning subjects / patients to groups described | 3.1 | X | | | |

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| and unbiased? | | | | | |
| 3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline? | 3.2 | X | | | |
| 3.3 Were concurrent controls used? (Concurrent preferred over historical controls.) | 3.3 | X | | | |
| 3.4 If cohort study or cross-sectional study were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis? | 3.4 | X | | | |
| 3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies. | 3.5 | | | | X |
| 3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")? | 3.6 | | | | X |
| **4. Was method of handling <u>withdrawals</u> described? YES** | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 4.1 Were follow up methods described and the same for all groups? | 4.1 | X | | | |
| 4.2 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80 %.) | 4.2 | X | | | |
| 4.3 Were all enrolled subjects/patients (in the original sample) accounted for? | 4.3 | X | | | |
| 4.4 Were reasons for withdrawals similar across groups? | 4.4 | X | | | |
| 4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study? | 4.5 | | | | X |
| 5. **Was <u>blinding</u> used to prevent introduction of bias? NO** | | **YES** | **NO** | **UNCLEAR** | **NA** |

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| 5.1 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **as appropriate**? | 5.1 | X | | | |
| 5.2 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.) | 5.2 | | | X | |
| 5.3 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded? | 5.3 | | | X | |
| 5.4 In case control study, was case definition explicit and case ascertainment not influenced by exposure status? | 5.4 | | | | X |
| 5.5 In diagnostic study, were test results blinded to patient history and other test results? | 5.5 | | | | X |
| **6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described? YES** | | **YES** | **N C** | **U N C I** | **NA** |
| 6.1 In RCT or other intervention trial, were protocols described for all regimens studied? | 6.1 | X | | | |
| 6.2 In observational study, were interventions, study settings, and clinicians / provider described? | 6.2 | | | | X |
| 6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect? | 6.3 | X | | | |
| 6.4 Was the amount of exposure and, if relevant, subject / patient compliance measured? | 6.4 | X | | | |
| 6.5 Were co-interventions (e.g., ancillary treatments other therapies) described? | 6.5 | X | | | |
| 6.6 Were extra or unplanned treatments described? | 6.6 | | X | | |
| 6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups? | 6.7 | X | | | |
| 6.8 In diagnostic study, were details of test administration and replication sufficient? | 6.8 | | | | X |
| **7. Were outcomes clearly defined and the measurements valid and reliable? YES** | | **YES** | **N C** | **U N C** | **NA** |

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| 7.1 Were primary and secondary endpoints described and relevant to the question? | 7.1 | X | | | |
| 7.2 Were nutrition measures appropriate to question and outcomes of concern? | 7.2 | X | | | |
| 7.3 Was the period of follow-up long enough for important outcome(s) to occur? | 7.3 | X | | | |
| 7.4 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures? | 7.4 | X | | | |
| 7.5 Was the measurement of effect at an appropriate level of precision? | 7.5 | X | | | |
| 7.6 Were other factors accounted for (measured) that could affect outcomes? | 7.6 | X | | | |
| 7.7 Were the measurements conducted consistently across groups? | 7.7 | X | | | |
| **8. Was the <u>statistical analysis</u> appropriate for the study design and type of outcome indicators? YES** | | **YES** | **NO** | **UNCI** | **NA** |
| 8.1 Were statistical analyses adequately described and the results reported appropriately? | 8.1 | X | | | |
| 8.2 Were correct statistical tests used and assumptions of test not violated? | 8.2 | X | | | |
| 8.3 Were statistics reported with levels of significance and/or confidence intervals? | 8.3 | X | | | |
| 8.4 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)? | 8.4 | X | | | |
| 8.5 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)? | 8.5 | X | | | |
| 8.6 Was clinical significance as well as statistical significance reported? | 8.6 | X | | | |
| 8.7 If negative findings, was a power calculation reported to address type 2 error? | 8.7 | | | | X |
| 9. **Are <u>conclusions</u> supported by results with biases and limitations taken into consideration? YES** | | **YES** | **NO** | **UN** | **NA** |

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| | | | | **EAR** | |
| 9.1 Is there a discussion of findings? | 9.1 | X | | | |
| 9.2 Are biases and study limitations identified and discussed? | 9.2 | X | | | |
| **10. Is bias due to study's <u>funding or sponsorship</u> unlikely? YES** | | **YES** | **NO** | **UNCI** | **NA** |
| 10.1 Were sources of funding and investigators' affiliations described? | 10.1 | X | | | |
| 10.2 Was there no apparent conflict of interest? | 10.2 | X | | | |
| **SYMBOL** | | | | | |
| **MINUS/NEGATIVE (-)**<br>*If most (six or more) of the answers to the above validity questions are "no," the report should be designated with a minus (-) symbol on the Evidence Quality Worksheet.* | | | | | |
| **NEUTRAL (ø)**<br>*If the answers to validity criteria questions **2, 3, 6, and 7** do not indicate that the study is exceptionally strong, the report should be designated with a neutral (ø) symbol on the Evidence Quality Worksheet.* | | | | | |
| **PLUS/POSITIVE (+)**<br>*If most of the answers to the above validity questions are "Yes" including criteria **2, 3, 6, and 7 and at least one additional "yes",( the report should be designated with a plus symbol (+) on the Evidence Quality Worksheet.* | | | | | |

Evidence Worksheet for Primary Research Article

| | |
|---|---|
| **Citation:** | Rohde, C., Mutze, U., Weigel, J.F.W., Ceglarek, U., Thiery, J., Kiess, W., Beblo, S. (2012). Unrestricted consumption of fruits and vegetables in phenylketonuria: no major impact on metabolic control. *European Journal of Clinical Nutrition, 66,* 633-638. |
| **Study Design:** | Randomized Cross over study |
| **Study Class (A,B,C,D):** | A |
| **Research Quality Rating:** | Positive **(+)** |
| *Purpose/Population Studied/Practice Studied* | |
| **Research Purpose:** | To asses if free consumption of fruits and vegetables containing less than 75 mg phe per 100 g affects metabolic control in children with PKU. |
| **Inclusion Criteria:** | Diagnosis of PKU<br>Age 2-10 years<br>Blood phe <360 umol/L |
| **Exclusion Criteria:** | Patients with additional diseases and abnormal neurological exam |
| **Recruitment:** | Those treated at the University of Leipzig Genetics |
| **Blinding Used:** | Blinding was not used |
| **Description of Study Protocol:** | Day 1-3: all patients followed classic PKU treatment, recorded daily diet records and obtained daily dried blood phe concentrations.<br>Day 4-18: Patents are randomized into one of two groups, free fruit and vegetable consumption (those with less than 75 mg phe per 100 g) or restricted fruit and vegetable consumption. Daily dried blood he levels obtained, diet records recorded on days 5, 6, 15, 16, and 17.<br>Day 19-32: Patients switched to opposite group. Daily dried blood he levels obtained, diet records recorded on days 19, 20, 29, 30, 31. |
| **Intervention:** | Foods and drinks measured on scale for diet records<br>Hartman guideline used for calculating nutrient intake<br>Blood phe was collected on filter paper daily, tandem mass spectrometry was used for results<br>If a patient was ill, blood sampling and diet records were halted until they had recovered<br>synthetic protein formula intake remained consistent throughout study to match prescribed intake |

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| **Statistical Analysis:** | Data from each period were averaged for analysis<br>MANOVA was used to analyze longitudinal changes from the three periods<br>Time= within subject factor with three levels (enter, phase 1, phase 2)<br>Group= between subject factor with two levels (study phase 1-2 or 2-1)<br>A normal level of $P<0.05$ was regarded statistically significant<br>Data was described as the mean plus or minus standard deviation |
| **Timing of Measurements:** | Blood Phe concentration at baseline and daily between 0700-0900 after overnight fast<br>Diet records on days 5, 6, 15, 16, 17 and 19, 20, 29, 30, 31 |
| **Dependent Variables:** | Metabolic control as measured by blood Phe concentration |

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| **Independent Variables:** | Consumption of fruits and vegetables |
| **Control Variables:** | Data from each period were averaged to reduce number of variables<br>Fasting was the same for all blood draws<br>synthetic protein formula intake was unchanged during study phases |
| **Initial Number (n):** | 16 |
| **Final Number (n):** | 14 (8 female, 6 male) |
| **Age:** | Range 2-10, mean 5.7 years |
| **Ethnicity *(if given)*:** | Unknown |
| **Other Relevant Demographics:** | Patients were regularly followed in clinic<br>Phe tolerance 335 mg plus or minus 48 mg<br>Dried blood phe concentrations 230 umol/l, plus or minus 63 |
| **Anthropometrics:** | Mean BMI 15.5 kg/m2, plus or minus 1.4 |
| **Location:** | Germany |
| **Summary of Results:** | Total phe intake increased significantly: P= 0.037, average increase of 58 mg per day, 18% increase<br>Average blood phe levels remained stable: P= 0.76, restricted group 246 umol/l +/- 140, unrestricted: 243 umol/L, +/- 137<br>Frequency of blood levels above ideal range increased slightly but not significantly: P=0.123, restricted 30%, unrestricted 37%<br>Study sequence had no effect on dietary or laboratory variables<br>No influence on average dried blood tyrosine levels |
| *Author's Conclusions* | |
| **Author Conclusion:** | Unrestricted consumption of fruits and vegetables (those with less than 75 mg phe per 100 g) in the PKU diet did not compromise short term metabolic control. |
| **Reviewer Comments:** | *Limitations:*<br>*Small sample size*<br>*No blinding took place*<br>*The study was only 2 weeks long, question if sufficient time to change dietary habits or determine impact on metabolic control*<br>*Parents might have been keeping their children on a more strict diet during the trial, blood phe levels may have been lower than usual because of this*<br>*No standardized meals*<br><br>*Strengths:*<br>*Study groups were comparable*<br>*Randomization was used in determining study groups and order of study phases*<br>*Real life setting* |

*Frequency of blood phe concentration and diet records*

**Quality Criteria Checklist: Primary Research**

| **RELEVANCE QUESTIONS** | | | | | |
|---|---|---|---|---|---|
| **Citation:** Rohde, C., Mutze, U., Weigel, J.F.W., Ceglarek, U., Thiery, J., Kiess, W., Beblo, S. (2012). Unrestricted consumption of fruits and vegetables in phenylketonuria: no major impact on metabolic control. *European Journal of Clinical Nutrition, 66*, 633-638. | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies) | 1 | X | | | |
| 2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about? | 2 | X | | | |
| 3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice? | 3 | X | | | |
| 4. Is the intervention or procedure feasible (NA for some epidemiological studies)? | 4 | X | | | |
| *If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.* | | | | | |
| **VALIDITY QUESTIONS** | | | | | |
| **3. Was the research question clearly stated? Yes** | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified? | 1.1 | X | | | |
| 1.2 Was the outcome(s) (dependent variable(s)) clearly indicated? | 1.2 | X | | | |
| 1.3 Were the target population and setting specified? | 1.3 | X | | | |
| **4. Was the selection of study subjects / patients free from bias? YES** | | **YE** | **NO** | **UN** | **NA** |

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| | | **S** | | **CLEAR** | |
| 2.2 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study? | 2.1 | X | | | |
| 2.3 Were criteria applied equally to all study groups? | 2.2 | X | | | |
| 2.4 Were health, demographics, and other characteristics of subjects described? | 2.3 | X | | | |
| 2.4 Were the subjects /patients in a representative sample of the relevant population? | 2.4 | X | | | |
| **3, Were study groups comparable? YES** | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 3.1 Was the method of assigning subjects / patients to groups described and unbiased?<br>Patients were randomized, but method was not stated. | 3.1X | | | | |
| 3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline? | 3.2 | X | | | |
| 3.3 Were concurrent controls used? (Concurrent preferred over historical controls.) | 3.3 | X | | | |
| 3.4 If cohort study or cross-sectional study were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis? | 3.4 | X | | | |
| 3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies. | 3.5X | | | | |
| 3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")? | 3.6X | | | | |
| **8. Was method of handling withdrawals described? YES** | | **YE** | **NO** | **UN** | **NA** |

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| | | **S** | | **CLEAR** | |
| 4.1 Were follow up methods described and the same for all groups? | 4.1 | X | | | |
| 4.3 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80 %.) | 4.2 | X | | | |
| 4.3 Were all enrolled subjects/patients (in the original sample) accounted for? | 4.3 | X | | | |
| | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 4.5 Were reasons for withdrawals similar across groups? | 4.4 | | | X | |
| 4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study? | 4.5 | | | | X |
| 9. **Was blinding used to prevent introduction of bias? NO** | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 5.5 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **as appropriate**? | 5.1 | X | | | |
| 5.6 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.) | 5.2 | | X | | |
| 5.7 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded? | 5.3 | | | X | |
| 5.8 In case control study, was case definition explicit and case ascertainment not influenced by exposure status? | 5.4 | | | | X |
| 5.5 In diagnostic study, were test results blinded to patient history and other test results? | 5.5 | | | | X |

| **10. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described? YES** | | **YES** | **NO** | **UNCLEAR** | **NA** |
|---|---|---|---|---|---|
| 6.1 In RCT or other intervention trial, were protocols described for all regimens studied? | 6.1 | X | | | |
| 6.7 In observational study, were interventions, study settings, and clinicians / provider described? | 6.2 | | | | X |
| 6.8 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect? | 6.3 | X | | | |
| 6.9 Was the amount of exposure and, if relevant, subject / patient compliance measured? | 6.4 | X | | | |
| 6.10 Were co-interventions (e.g., ancillary treatments other therapies) described? | 6.5 | X | | | |
| 6.11 Were extra or unplanned treatments described? | 6.6 | | X | | |
| 6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups? | 6.7 | X | | | |
| 6.8 In diagnostic study, were details of test administration and replication sufficient? | 6.8 | | | | X |
| **11. Were outcomes clearly defined and the measurements valid and reliable? YES** | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 7.2 Were primary and secondary endpoints described and relevant to the question? | 7.1 | X | | | |
| 7.2 Were nutrition measures appropriate to question and outcomes of concern? | 7.2 | X | | | |
| 7.7 Was the period of follow-up long enough for important outcome(s) to occur? | 7.3 | X | | | |
| 7.8 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures? | 7.4 | X | | | |
| 7.9 Was the measurement of effect at an appropriate level of precision? | 7.5 | X | | | |
| 7.10 Were other factors accounted for (measured) that could affect outcomes? | 7.6 | X | | | |

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| 7.7 Were the measurements conducted consistently across groups? | 7.7 | X | | | |
| **11. Was the statistical analysis appropriate for the study design and type of outcome indicators? YES** | | **YES** | **NO** | **UNCI** | **NA** |
| 8.6 Were statistical analyses adequately described and the results reported appropriately? | 8.1 | X | | | |
| 8.7 Were correct statistical tests used and assumptions of test not violated? | 8.2 | X | | | |
| 8.8 Were statistics reported with levels of significance and/or confidence intervals? | 8.3 | X | | | |
| 8.9 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)? | 8.4 | X | | | |
| 8.10 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)? | 8.5 | X | | | |
| 8.6 Was clinical significance as well as statistical significance reported? | 8.6 | X | | | |
| 8.7 If negative findings, was a power calculation reported to address type 2 error? | 8.7 | | | | X |
| 12. **Are conclusions supported by results with biases and limitations taken into consideration? YES** | | **YES** | **NO** | **UNCI** | **NA** |
| 9.1 Is there a discussion of findings? | 9.1 | X | | | |
| 9.2 Are biases and study limitations identified and discussed? | 9.2 | X | | | |
| **13. Is bias due to study's funding or sponsorship unlikely? YES** | | **YES** | **NO** | **UNC** | **NA** |

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| | | | | **R** | |
| 10.1 Were sources of funding and investigators' affiliations described? | 10.1 | X | | | |
| 10.2 Was there no apparent conflict of interest? | 10.2 | X | | | |
| **SYMBOL** | | | | | |
| **MINUS/NEGATIVE (-)**<br>*If most (six or more) of the answers to the above validity questions are "no," the report should be designated with a minus (-) symbol on the Evidence Quality Worksheet.* | | | | | |
| **NEUTRAL (ø)**<br>*If the answers to validity criteria questions **2, 3, 6, and 7** do not indicate that the study is exceptionally strong, the report should be designated with a neutral* (ø) *symbol on the Evidence Quality Worksheet.* | | | | | |
| **PLUS/POSITIVE (+)**<br>*If most of the answers to the above validity questions are "Yes" including criteria **2, 3, 6, and 7 and at least one additional "yes",(** the report should be designated with a plus symbol (+) on the Evidence Quality Worksheet.* | | | | | |
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Evidence Worksheet for Primary Research Article

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|---|---|
| **Citation:** | Rohde, C., Mutze, U., Schulz, S., Thiele, A.G., Ceglarek, U., Thiery, J., Mueller, A.S., Kiess, W., Beblo, S. (2014). Unrestricted fruits and vegetables in the PKU diet: a 1-year follow-up. *European Journal of Clinical Nutrition, 68,* 401-403. |
| **Study Design:** | Randomized Cross over study |
| **Study Class (A,B,C,D):** | A |
| **Research Quality Rating:** | Positive **(+)** |
| *Purpose/Population Studied/Practice Studied* | |
| **Research Purpose:** | To asses if free consumption of fruits and vegetables containing less than 75 mg phe per 100 g affects long term metabolic control in children with PKU. |
| **Inclusion Criteria:** | Diagnosis of PKU<br>Age 2-10 years<br>Blood phe <360 umol/L |

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| | Subjects who participated in the core trial (Phase 1 and Phase 2) |
| **Exclusion Criteria:** | Patients with additional diseases and abnormal neurological exam |
| **Recruitment:** | Those treated at the University of Leipzig Genetics |
| **Blinding Used:** | Blinding was not used |
| **Description of Study Protocol:** | After the core trial, remaining subjects continued the free fruit and vegetable diet for another 12 months.<br>Blood phe concentrations were collected every 2 to 4 weeks.<br>Daily diet records and daily blood phe concentrations were collected over a 3 day period at the end of 6 months (Phase 3) and at the end of 12 months (Phase 4). |
| **Intervention:** | Foods and drinks measured on scale for diet records<br>Hartman guideline used for calculating nutrient intake<br>Blood phe was collected on filter paper daily, tandem mass spectrometry was used for results<br>If a patient was ill, blood sampling and diet records were halted until they had recovered<br>synthetic protein formula intake remained consistent throughout study to match prescribed intake<br>No standardized meals were given |
| **Statistical Analysis:** | Patients completing at least three study phases were included in the analysis.<br>Data from each period were averaged for analysis.<br>The sequence of the core trial had no effect on the outcome. Data from the restricted phases were designated as Phase 1 and data from liberated consumption was designated as Phase 2.<br>Longitudinal changes were analyzed by Friedman test with 'time' as the within-subject factor.<br>If significant differences occurred, Wilcoxon test was used to compare individual study phases.<br>A normal level of $P<0.05$ was regarded statistically significant. |
| **Timing of Measurements:** | Blood Phe concentration at baseline and daily between 0700-0900 after overnight fast<br>Diet records collected on the last three days of Phase 3 and the last three days of Phase 4 |
| **Dependent Variables:** | Metabolic control as measured by blood Phe concentration |

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| **Independent Variables:** | Consumption of fruits and vegetables |

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| **Control Variables:** | Data from each period were averaged to reduce number of variables<br>Fasting was the same for all blood draws<br>synthetic protein formula intake was unchanged during study phases |
| **Initial Number (n):** | 25 |
| **Final Number (n):** | 19, but 3 did not complete the final Phase 4 |
| **Age:** | Range 2.6-6.8 years, mean 4.7years |
| **Ethnicity *(if given)*:** | Unknown |
| **Other Relevant Demographics:** | Patients were regularly followed in clinic<br>Mean phe tolerance 357 mg/day, ranging from 215-660 mg/day<br>Dried blood phe concentrations 230 umol/l, plus or minus 63 |
| **Anthropometrics:** | unknown |
| **Location:** | Germany |
| **Summary of Results:** | Mean blood phe concentrations remained within the recommended range (40-240 umol/L)<br>Frequency of blood phe concentrations decreased slightly but not significantly<br>The weight of fruits and vegetables consumed as well as the mg phe consumed from them remained stable, despite liberalization of fruits and vegetables<br>Phe tolerance increased significantly, $p<0.001$, by an average of 68 mg/day<br>Total protein intake increased in study phases 3 and 4 due to increase in synthetic protein formula to maintain total protein requirements |
| *Author's Conclusions* | |
| **Author Conclusion:** | Unrestricted consumption of fruits and vegetables (those with less than 75 mg phe per 100 g) in the PKU diet did not compromise long term metabolic control, despite an overall increase of phe tolerance by 68 mg/day. They hypothesized that there is poor phe utilization from fruits and vegetables, because of lower protein digestibility. People who consume a lot of fruits and vegetables have a seemingly higher phe tolerance, but it is actually due to the poor utilization of protein from these foods. |
| **Reviewer Comments:** | *Limitations:*<br>*Small sample size*<br>*No blinding took place*<br>*Parents might have been keeping their children on a more strict diet* |

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| | *during the diet record and blood phe monitoring days, blood phe levels may have been lower than usual because of this*<br>*No standardized meals*<br><br>*Strengths:*<br>*Study groups were comparable*<br>*Real life setting*<br>*Frequency of blood phe concentration and diet records were consistent* |

**Quality Criteria Checklist: Primary Research**

| **RELEVANCE QUESTIONS** | | | | | |
|---|---|---|---|---|---|
| **Citation:** Rohde, C., Mutze, U., Schulz, S., Thiele, A.G., Ceglarek, U., Thiery, J., Mueller, A.S., Kiess, W., Beblo, S. (2014). Unrestricted fruits and vegetables in the PKU diet: a 1-year follow-up. *European Journal of Clinical Nutrition*, *68*, 401-403. | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies) | 1 | X | | | |
| 2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about? | 2 | X | | | |
| 3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice? | 3 | X | | | |
| 4. Is the intervention or procedure feasible (NA for some epidemiological studies)? | 4 | X | | | |
| *If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.* | | | | | |
| **VALIDITY QUESTIONS** | | | | | |
| **5. Was the research question clearly stated? Yes** | | **YES** | **NO** | **L** | **NA** |

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| | | | | **EA** | |
| 1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified? | 1.1 | X | | | |
| 1.2 Was the outcome(s) (dependent variable(s)) clearly indicated? | 1.2 | X | | | |
| 1.3 Were the target population and setting specified? | 1.3 | X | | | |
| **6. Was the selection of study subjects / patients free from bias? YES** | | **YES** | **NO** | **UNCL** | **NA** |
| 2.3 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study? | 2.1 | X | | | |
| 2.4 Were criteria applied equally to all study groups? | 2.2 | X | | | |
| 2.5 Were health, demographics, and other characteristics of subjects described? | 2.3 | X | | | |
| 2.4 Were the subjects /patients in a representative sample of the relevant population? | 2.4 | X | | | |
| **3, Were study groups comparable? YES** | | **YES** | **NO** | **UNCL** | **NA** |
| 3.1 Was the method of assigning subjects / patients to groups described and unbiased?<br>Patients were randomized, but method was not stated. | 3.1 | | | X | |
| 3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline? | 3.2 | X | | | |
| 3.3 Were concurrent controls used? (Concurrent preferred over historical controls.) | 3.3 | X | | | |
| 3.4 If cohort study or cross-sectional study were groups comparable on important confounding factors and/or were preexisting differences | 3.4 | X | | | |

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| accounted for by using appropriate adjustments in statistical analysis? | | | | | |
| 3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies. | 3.5 | | | | X |
| 3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")? | 3.6 | | | | X |
| **12. Was method of handling <u>withdrawals</u> described? YES** | | **YES** | **NO** | **UNCI** | **NA** |
| 4.1 Were follow up methods described and the same for all groups? | 4.1 | X | | | |
| 4.4 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80 %.) | 4.2 | X | | | |
| 4.3 Were all enrolled subjects/patients (in the original sample) accounted for? | 4.3 | X | | | |
| | | **YES** | **NO** | **UNCI** | **NA** |
| 4.6 Were reasons for withdrawals similar across groups? | 4.4 | X | | | |
| 4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study? | 4.5 | | | | X |
| 13. **Was <u>blinding</u> used to prevent introduction of bias? NO** | | **YES** | **NO** | **UNCI** | **NA** |

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| 5.9 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **as appropriate**? | 5.1 | X | | | |
| 5.10 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.) | 5.2 | | X | | |
| 5.11 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded? | 5.3 | | | X | |
| 5.12 In case control study, was case definition explicit and case ascertainment not influenced by exposure status? | 5.4 | | | | X |
| 5.5 In diagnostic study, were test results blinded to patient history and other test results? | 5.5 | | | | X |
| **14. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described? YES** | | **YES** | **NO** | **UNCI** | **NA** |
| 6.1 In RCT or other intervention trial, were protocols described for all regimens studied? | 6.1 | X | | | |
| 6.12 In observational study, were interventions, study settings, and clinicians / provider described? | 6.2 | | | | X |
| 6.13 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect? | 6.3 | X | | | |
| 6.14 Was the amount of exposure and, if relevant, subject / patient compliance measured? | 6.4 | X | | | |
| 6.15 Were co-interventions (e.g., ancillary treatments other therapies) described? | 6.5 | X | | | |
| 6.16 Were extra or unplanned treatments described? | 6.6 | | X | | |
| 6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups? | 6.7 | X | | | |
| 6.8 In diagnostic study, were details of test administration and replication sufficient? | 6.8 | | | | X |
| **15. Were outcomes clearly defined and the measurements valid and reliable? YES** | | **YES** | **NO** | **UNCI** | **NA** |

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| 7.3 Were primary and secondary endpoints described and relevant to the question? | 7.1 | X | | | |
| 7.2 Were nutrition measures appropriate to question and outcomes of concern? | 7.2 | X | | | |
| 7.11 Was the period of follow-up long enough for important outcome(s) to occur? | 7.3 | X | | | |
| 7.12 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures? | 7.4 | X | | | |
| 7.13 Was the measurement of effect at an appropriate level of precision? | 7.5 | X | | | |
| 7.14 Were other factors accounted for (measured) that could affect outcomes? | 7.6 | X | | | |
| 7.7 Were the measurements conducted consistently across groups? | 7.7 | X | | | |
| **14. Was the <u>statistical analysis</u> appropriate for the study design and type of outcome indicators? YES** | | **YES** | **NO** | **UNCI** | **NA** |
| 8.11 Were statistical analyses adequately described and the results reported appropriately? | 8.1 | X | | | |
| 8.12 Were correct statistical tests used and assumptions of test not violated? | 8.2 | X | | | |
| 8.13 Were statistics reported with levels of significance and/or confidence intervals? | 8.3 | X | | | |
| 8.14 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)? | 8.4 | X | | | |
| 8.15 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)? | 8.5 | X | | | |
| 8.6 Was clinical significance as well as statistical significance reported? | 8.6 | X | | | |
| 8.7 If negative findings, was a power calculation reported to address type 2 error? | 8.7 | | | | X |
| 15. **Are <u>conclusions</u> supported by results with biases and limitations taken into consideration? YES** | | **YES** | **NO** | **UNCI** | **NA** |

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| | | | | **EAR** | |
| 9.1 Is there a discussion of findings? | 9.1 | X | | | |
| 9.2 Are biases and study limitations identified and discussed? | 9.2 | X | | | |
| **16. Is bias due to study's <u>funding or sponsorship</u> unlikely? YES** | | **YES** | **NO** | **UNCI** | **NA** |
| 10.1 Were sources of funding and investigators' affiliations described? | 10.1 | X | | | |
| 10.2 Was there no apparent conflict of interest? | 10.2 | X | | | |
| **SYMBOL** | | | | | |
| **MINUS/NEGATIVE (-)**<br>*If most (six or more) of the answers to the above validity questions are "no," the report should be designated with a minus (-) symbol on the Evidence Quality Worksheet.* | | | | | |
| **NEUTRAL (ø)**<br>*If the answers to validity criteria questions **2, 3, 6, and 7** do not indicate that the study is exceptionally strong, the report should be designated with a neutral* (ø) *symbol on the Evidence Quality Worksheet.* | | | | | |
| **PLUS/POSITIVE (+)**<br>*If most of the answers to the above validity questions are "Yes" including criteria **2, 3, 6, and 7 and at least one additional "yes"**,( the report should be designated with a plus symbol (+) on the Evidence Quality Worksheet.* | | | | | |
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Evidence Worksheet for Primary Research Article

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|---|---|
| **Citation:** | Zimmermann, M., Jacobs, P., Fingerhut, R., Torresani, T., Thöny, B., Blau, N. Baumgartner, M.R., Rohrbach, M. (2012). Positive effect of a simplified diet on blood phenylalanine control in different phenylketonuria variants, characterized by newborn BH4 loading test and PAH analysis. *Molecular Genetics Metabolism, 106*, 264-268. |

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| **Study design:** | Retrospective Cohort Study |
| **Study Class (A,B,C,D):** | B |
| **Research Quality Rating:** | Positive (+) |
| *Purpose/Population Studied/Practice Studied* | |
| **Research purpose:** | The aim of this study was to investigate the effect of the intake of fruits and vegetables containing less than 100 mg phe per 100 g weight, in quantities recommended by WHO, on the course and treatment control of the disease. |
| **Inclusion criteria:** | Patients diagnosed with PKU between 1955 and 2005<br>Patients who were regularly followed in clinic between 1992 and 2009 |
| **Exclusion criteria:** | Factors that opposed the inclusion criteria |
| **Recruitment:** | Patients seen at the University Children's Hospital Zurich |
| **Blinding used:** | none |
| **Description of study protocol:** | First months: advocate breastfeeding in combination with synthetic protein formula for first 6 months, followed by potatoes mixed with vegetables and fruits with monitoring blood phe levels every 3-7 days.<br>After weaning period: Encouraged 5 servings of fruits and vegetables with <100 mg phe per 100 g weight. Generally, all fruits and vegetables were recommended if consumed with variation. Special low protein foods could are eaten ad libitum.<br>The quantities of potatoes, rice and maize are fixed for a period of time; parents/patients estimate the fixed quantities and check these quantities with a scale periodically. Synthetic protein formula is the only component that needs to be weighed.<br>Quantity of synthetic protein formula, potatoes, rice and maize are adjusted based on blood phe concentrations. A lump sum of 25 mg phe for fruits, vegetables, and protein free foods was counted for toddlers, 50 mg for older children and adults. |
| **Intervention:** | BH4 loading test was performed to identify all BH4 responsive PKU patients with blood phe levels above 400 umol/L<br>PKU diagnosis and classification was primarily based on maximal blood phe concentration before starting treatment<br>Limitations of fruits and vegetables:<br>Only one portion of avocado, broccoli, Brussels sprouts, passion fruit, or kale per day. Potatoes, sweet corn, peas, and sprouts must be measured and counted |

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| **Statistical analysis:** | Statistical analysis was performed by Pearson's chi-square test |
| **Timing of measurements:** | Guthrie cards with dried blood spots were taken 1 hour after breakfast or dinner and sent to newborn screening center, analyzed with bacterial inhibition test until 2004, and afterwards by tandem mass spectrometry.<br>Frequency of blood phe levels was determined by age: 0-2 years every 1-2 weeks, 2- 10 years every 1-4 weeks, >10 years at least once per month |
| **Dependent variables:** | Metabolic control as defined by blood phe concentration |

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| **Independent variables:** | Traditional calculation of phe intake for PKU diet versus simplified diet |
| **Control Variables:** | synthetic protein formula remained consistent and prescribed appropriate using recommendations for intake by age<br>Timing of fasting for blood draws |
| **Initial n:** | 80 (40 female, 40 male) |
| **Final n:** (attrition) | 50 |
| **Age:** | See 'Summary of Results' |
| **Ethnicity**: | Central European 57 subjects, Southeast Europe 9 subjects, Southern Europe 9 subjects, Lebanon 1 subject, Iran 2 subjects, 6 unknown origin |
| **Other relevant demographics:** | Classical PKU: 41 subjects<br>Moderate PKU: 16 subjects<br>Mild PKU: 10 subjects<br>Mild HPA: 13 subjects<br>Consanguinity was confirmed in 8 families<br>51 subjects had a 48 hour BH4 loading tests<br>32 subjects had molecular analysis of the PAH gene to identify disease causing mutation |
| **Anthropometrics:** | Information was not stated |
| **Location:** | The University Children's Hospital Zurich |
| **Summary of Results:** | 73 out of 80 patients were on traditional diet upon starting treatment at diagnosis<br>7 out of 80 were on the simplified diet since birth<br>50 patients chose to switch to the simplified diet, 23 refused and remained on the traditional diet (used to traditional diet, lack of |

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| | motivation to change)<br>**Blood Phe Levels by Severity of PKU:**<br>Classical PKU (26 patients): Median age 19 years, median blood phe level on traditional diet 275umol/L, median phe on simplified diet 355 umol/L, p=0.96<br>Moderate PKU (13 patients): Median age 10 years, median blood phe level on traditional diet 200umol/L, median phe on simplified diet 313 umol/L, p=0.06<br>Mild PKU (7 patients): Median age 16.5 years, median blood phe level on traditional diet 300 umol/L, median phe on simplified diet 360 umol/L, p=0.42<br>Mild HPA (4 patients): data not included in statistical analysis<br>**Blood phe Levels by Age:**<br>Age 2-9 years (12 patients): median blood phe level on traditional diet 200 umol/L, median phe on simplified diet 245 umol/L, p=0.183<br>Age 10-16 years (13 patients): median blood phe level on traditional diet 275 umol/L, median phe on simplified diet 406 umol/L, p=0.4993<br>Age >16 years (25 patients): median blood phe level on traditional diet 400 umol/L, median phe on simplified diet 362 umol/L, p=0.0001 |
| *Author's Conclusions* | |
| **Author conclusion:** | Not weighing intake of fruits and vegetables containing phe <100 mg per 100 grams had no destabilizing effect on the control of blood phe values in all PKU subgroups. 50 patients who switched to from the traditional restricted diet to the simplified diet reveal that blood phe levels were not negatively impacted by this liberalization. It is also demonstrated that this method is safe for patients <10 years of age. Severity of PKU and age at which diet was switched did not influence ability to allow fruits and vegetables containing phe <100 mg per 100 grams without measurement. |
| **Reviewer comments:** | *Limitations:*<br>*Small sample size*<br>*Possible misclassification of phenotypes due to classification of highest blood phe level before treatment*<br>*Possible bias from small sample size after switch to liberalized* |

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| | *diet*<br>*Lack of diet records, inability to quantify phe consumed after diet switch*<br>*No blinding was used*<br>*Standardized meals were not used*<br><br>*Strengths:*<br>*Classification of severity of PKU was described*<br>*Differences in median blood phe levels also described by patient age range*<br>*Participants were comparable* |

**Quality Criteria Checklist: Primary Research**

| **RELEVANCE QUESTIONS** | | | | | |
|---|---|---|---|---|---|
| **Citation:** Zimmermann, M., Jacobs, P., Fingerhut, R., Torresani, T., Thöny, B., Blau, N. Baumgartner, M.R., Rohrbach, M. (2012). Positive effect of a simplified diet on blood phenylalanine control in different phenylketonuria variants, characterized by newborn BH4 loading test and PAH analysis. *Molecular Genetics Metabolism, 106,* 264-268. | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies) | 1 | x | | | |
| 2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about? | 2 | x | | | |
| 3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice? | 3 | x | | | |
| 4. Is the intervention or procedure feasible (NA for some epidemiological studies)? | 4 | x | | | |
| *If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.* | | | | | |
| **VALIDITY QUESTIONS** | | | | | |
| **7. Was the <u>research question</u> clearly stated? YES** | | **YES** | **NO** | **UNC** | **NA** |

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| | | | | **LEAR** | |
| 1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified? | 1.1 | x | | | |
| 1.2 Was the outcome(s) (dependent variable(s)) clearly indicated? | 1.2 | x | | | |
| 1.3 Were the target population and setting specified? | 1.3 | x | | | |
| **8. Was the selection of study subjects / patients free from bias? YES** | | **YES** | **NO** | **UNCLEAR** | **NA** |
| 2.4 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study? | 2.1 | x | | | |
| 2.5 Were criteria applied equally to all study groups? | 2.2 | x | | | |
| 2.6 Were health, demographics, and other characteristics of subjects described? | 2.3 | x | | | |
| 2.4 Were the subjects /patients in a representative sample of the relevant population? | 2.4 | x | | | |
| **3. Were study groups comparable? YES** | | **YES** | **NO** | **UNCL** | **NA** |
| 3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT) | 3.1 | | | | x |
| 3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline? | 3.2 | x | | | |
| 3.3 Were concurrent controls used? (Concurrent preferred over historical controls.) | 3.3 | x | | | |
| 3.4 If cohort study or cross-sectional study were groups comparable on important confounding factors and/or were preexisting differences | 3.4 | x | | | |

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| accounted for by using appropriate adjustments in statistical analysis? | | | | | |
| 3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies. | 3.5 | | | | x |
| 3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")? | 3.6 | | | | x |
| **16. Was method of handling <u>withdrawals</u> described? YES** | | **YES** | **NO** | **UNCI** | **NA** |
| 4.1 Were follow up methods described and the same for all groups? | 4.1 | **x** | | | |
| 4.5 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80 %.) | 4.2 | **x** | | | |
| 4.3 Were all enrolled subjects/patients (in the original sample) accounted for? | 4.3 | **x** | | | |
| 4.7 Were reasons for withdrawals similar across groups? | 4.4 | **x** | | | |
| 4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study? | 4.5 | | | | **x** |
| 17. **Was <u>blinding</u> used to prevent introduction of bias? NO** | | **YES** | **NO** | **UNCI** | **NA** |
| 5.13 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **<u>as appropriate</u>**? | 5.1 | **x** | | | |
| 5.14 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.) | 5.2 | | **x** | | |
| 5.15 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded? | 5.3 | | **x** | | |

| | | | | | |
|---|---|---|---|---|---|
| 5.16 In case control study, was case definition explicit and case ascertainment not influenced by exposure status? | 5.4 | | | | **x** |
| 5.5 In diagnostic study, were test results blinded to patient history and other test results? | 5.5 | | | | **x** |
| **18. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described? YES** | | **YES** | **NC** | **UNCI** | **NA** |
| 6.1 In RCT or other intervention trial, were protocols described for all regimens studied? | 6.1 | x | | | |
| 6.17 In observational study, were interventions, study settings, and clinicians / provider described? | 6.2 | | | | x |
| 6.18 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect? | 6.3 | x | | | |
| 6.19 Was the amount of exposure and, if relevant, subject / patient compliance measured? | 6.4 | x | | | |
| 6.20 Were co-interventions (e.g., ancillary treatments other therapies) described? | 6.5 | **X** | | | |
| 6.21 Were extra or unplanned treatments described? | 6.6 | | x | | |
| 6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups? | 6.7 | x | | | |
| 6.8 In diagnostic study, were details of test administration and replication sufficient? | 6.8 | | | | x |
| **19. Were outcomes clearly defined and the measurements valid and reliable? YES** | | **YES** | **NC** | **UNCI** | **NA** |
| 7.4 Were primary and secondary endpoints described and relevant to the question? | 7.1 | x | | | |
| 7.2 Were nutrition measures appropriate to question and outcomes of concern? | 7.2 | x | | | |
| 7.15 Was the period of follow-up long enough for important outcome(s) to occur? | 7.3 | x | | | |

| | | | | | |
|---|---|---|---|---|---|
| 7.16 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures? | 7.4 | x | | | |
| 7.17 Was the measurement of effect at an appropriate level of precision? | 7.5 | x | | | |
| 7.18 Were other factors accounted for (measured) that could affect outcomes? | 7.6 | x | | | |
| 7.7 Were the measurements conducted consistently across groups? | 7.7 | x | | | |
| **17. Was the statistical analysis appropriate for the study design and type of outcome indicators? YES** | | **YES** | **NO** | **UNCI** | **NA** |
| 8.16 Were statistical analyses adequately described and the results reported appropriately? | 8.1 | x | | | |
| 8.17 Were correct statistical tests used and assumptions of test not violated? | 8.2 | x | | | |
| 8.18 Were statistics reported with levels of significance and/or confidence intervals? | 8.3 | x | | | |
| 8.19 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)? | 8.4 | | | | x |
| 8.20 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)? | 8.5 | | | **x** | |
| 8.6 Was clinical significance as well as statistical significance reported? | 8.6 | **x** | | | |
| 8.7 If negative findings, was a power calculation reported to address type 2 error? | 8.7 | | | | x |
| 18. **Are conclusions supported by results with biases and limitations taken into consideration? YES** | | | | | |
| 9.1 Is there a discussion of findings? | 9.1 | X | | | |
| 9.2 Are biases and study limitations identified and discussed? | 9.2 | x | | | |
| **19. Is bias due to study's funding or sponsorship unlikely? YES** | | | | | |
| 10.1 Were sources of funding and investigators' affiliations described? | 10.1 | x | | | |
| 10.2 Was there no apparent conflict of interest? | 10.2 | x | | | |
| **SYMBOL** | | | | | |
| **MINUS/NEGATIVE (-)** | | | | | |

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|---|
| *If most (six or more) of the answers to the above validity questions are "no," the report should be designated with a minus (-) symbol on the Evidence Quality Worksheet.* |
| **NEUTRAL (ø)**<br>*If the answers to validity criteria questions **2, 3, 6, and 7** do not indicate that the study is exceptionally strong, the report should be designated with a neutral* (ø) *symbol on the Evidence Quality Worksheet.* |
| **PLUS/POSITIVE (+)**<br>*If most of the answers to the above validity questions are "Yes" including criteria **2, 3, 6, and 7 and at least one additional "yes",( the report should be designated with a plus symbol (+) on the Evidence Quality Worksheet.* |