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Secondary Analyses of Dietary Data from PRISM Clinical Trials to Evaluate Safety and Efficacy of Pegvaliase for Adults with PKU.

By
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A DISSERTATION

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CERTIFICATE OF APPROVAL

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ABSTRACT

Background: Phenylketonuria (PKU) is a genetic disorder caused by a deficiency of phenylalanine hydroxylase leading to decreased conversion of phenylalanine (Phe) to tyrosine (Tyr). Pegvaliase™ (Palynziq™, BioMarin Pharmaceutical Inc., Novato, CA, USA), an injectable enzyme replacement therapy decreases plasma Phe by converting Phe intro trans-cinnamic acid and ammonia. This study aims to evaluate the changes in the dietary composition of participants with PKU on pegvaliase.

Methods: Adults with PKU with blood Phe concentration >600 µmol/L and who were naïve to pegvaliase™ treatment self-administered the drug subcutaneously following an induction, titration and maintenance schedule. Throughout the trial, all participants were instructed to maintain consistent total protein intake based on their usual intact protein and medical food intake established at baseline. Participants provided a 3-day food diary prior to each scheduled blood draw for plasma amino acids. Diet records were analyzed using Metabolic Pro® and mean total protein, intact protein and Phe intake were calculated. Participants were instructed to increase protein intake only when blood Phe was <30µmol/L. Each diet adjustments included an increase of 10g intact protein and a decrease of 5g protein equivalents from medical food for those consuming one.

Results: In 261 participants at baseline, mean (SD) blood Phe was 1232µmol/L (286), mean (SD) intake of intact protein and total protein was 38.4g/day (27.7) and 64.73g/day (32.2), respectively. With pegvaliase administration, there was a 3.83% increase in mean body weight at month 12 compared to baseline. Blood Phe decreased to a mean (SD) of -563µmol/L (528) at month 12. At month 12, total protein and intact protein increased by mean (SD) 4.7g/day (24.5) and 8.13g/day (24.13), respectively.

43% of participants (N=123) consumed a total protein intake between 0.4 – 0.8g/kg and 54% consumed an intact protein intake of ≤ 0.4g/kg. By month 12, 44% of participants consumed a total protein intake between 0.8 – 1.2g/kg and 57% of participants consumed an intact protein intake > 0.4g/kg.

41.37% of all participants experienced at least one hypophenylalaninemia (hypoPhe) event and the average duration of these events was 258 days. Mean intact protein intake increased more quickly over 12 months in participants who experienced hypoPhe (35.5g/day in participants who did not experienced hypoPhe vs 54.8g/day in participants who experienced hypoPhe). There was no significant correlation between change in blood Phe compared to change in pegvaliase dose, medical food protein or intact protein intake. However, there was a statistically significant negative correlation between change in blood Phe and total protein intake ($P=0.043$; $R^2= - 0.22$)

Conclusion: This secondary data analysis is the first study to extensively evaluate the dietary data collected during the Phase 3 PRISM clinical trial. Overall, long-term pegvaliase administration in adults with PKU led to an increase in total protein, intact protein and dietary Phe intake. By month 12, fewer participants (35%) consumed total protein below the dietary reference intake (DRI) of < 0.8g/kg of body weight. This study found that increasing intact protein intake in participants who experienced hypoPhe while on pegvaliase did not significantly change mean blood Phe concentration.

1. SPECIFIC AIMS

Phenylketonuria (PKU) is an autosomal recessive inborn error of amino acid metabolism caused by variants in the phenylalanine hydroxylase (PAH) gene. Reduced PAH activity results in an increase in plasma and cerebral phenylalanine (Phe) concentrations, which have a negative impact on neuropsychological function. Life-long treatment with a Phe-restricted diet is required to decrease and prevent excessive accumulation of Phe. A PKU diet involves limiting sources of dietary Phe and supplementing with Phe-free medical foods to ensure the optimal intake of all other essential amino acids, tyrosine, micronutrients, and kilocalories. Maintaining life-long diet treatment has been shown to prevent cognitive and executive function decline for adults with PKU. However, due to the complexity of the diet, studies have shown that adults often maintain a blood Phe concentration significantly above the target range of 120µmol/L to 360µmol/L, suggesting suboptimal adherence to this difficult diet.

In 2018, BioMarin Pharmaceutical Inc. received FDA approval to launch a new treatment option for adults with PKU called pegvaliase. This treatment is an injectable form of the enzyme phenylalanine ammonia lyase (PAL). Pegvaliase catalyzes the conversion of blood Phe to trans-cinnamic acid and ammonia, which are excreted in the urine. Recent publications from the phase 3 clinical trial demonstrates the efficacy of pegvaliase with most study participants achieving a blood Phe concentrations of ≤600 µmol/L. With this groundbreaking treatment, there is a need to modify the diet therapy for adults with PKU who are treated with pegvaliase.

The overall goal of this project is to evaluate the changes in dietary protein sources and dietary Phe intake of participants enrolled in the long-term phase 3 clinical

trial (PRISM) funded by BioMarin Pharmaceutical Inc. Using existing diet data collected in the PRISM trial, a retrospective analysis will be conducted to evaluate the changes in the participants' diets over time. This project will expand current pegvaliase research by correlating the type and amount of dietary protein and dietary Phe intake with blood Phe concentrations collected over the first 12 months of data collection. Additionally, this project will evaluate the dietary recommendations for participants who developed hypophenylalaninemia, defined as blood Phe <30µmol/L, during the trial. The specific aims of this project are:

Specific Aim 1: To evaluate changes in the intake of intact protein, medical food protein and dietary Phe during the first 12 months of administration of pegvaliase in the PRISM trial.

Hypothesis 1: We hypothesize that the administration of pegvaliase is associated with decreased blood Phe and medical food intake and an increase in total protein, intact protein, and dietary Phe intake of study participants.

Hypothesis 2: We hypothesize that after 12 months of pegvaliase administration, the intact protein intake of participants will increase, and a greater proportion will consume intact protein at or above the dietary reference intake (DRI) of 0.8 g protein/kg body weight for adults.

Specific Aim 2: To evaluate the trial's recommendations to increase intact protein and decrease medical food intake for participants who developed hypophenylalaninemia with pegvaliase administration.

Hypothesis 1: We hypothesize that the increase in intact protein intake, and thus dietary Phe, is positively correlated with the increase in blood Phe over time in those who developed hypophenylalaninemia.

Hypothesis 2: We hypothesize that the decrease in medical food intake is positively correlated with the increase in blood Phe over time in those who developed hypophenylalaninemia.

2. BACKGROUND

2.1 Brief history and Overview of PKU

Phenylketonuria (PKU) was identified 85 years ago by the Norwegian biochemist and physician Asbjorn Fölling.[1] This unexpected discovery was identified in two young children, ages 6 and 4 years, who had severe mental retardation. These two children were described as restless, with limited ability to speak, inability to focus, and required assistance to nourish themselves. Additionally, the parents of these two children indicated that their children's urine smelled peculiar. The testing of the children's urine samples for ketoacidosis led to Dr. Fölling's discovery of the substance phenylpyruvic acid. Dr. Fölling then postulated the association between the children's mental retardation and the phenylpyruvic acid found in urine.[1] Dr. Fölling then collected and tested 400 additional urine samples – those positive for phenylpyruvic acid were from children who shared similar characteristics including severe mental impairment, fair complexion, eczema, stooping figures and spastic gait.[1] Dr. Fölling also theorized that the presence of increased amounts of phenylpyruvic acid was caused by the inability to metabolize the amino acid phenylalanine (Phe).[1] In the same year, Dr. Lionel Penrose, an English geneticist, coined the term "Phenylketonuria" which persists to this day.[2]

In 1937, Dr. Penrose confirmed that feeding Phe increased the excretion of phenylpyruvic acid in those with PKU. In 1951, Dr. Horst Bickel in England introduced a low Phe diet as a method to treat a child who demonstrated characteristics of PKU, including fair hair, eczema, delayed mental development and several strange behaviors like headbanging, and constant moaning.[3,4] This child was placed on a low-Phe diet with a low Phe casein hydrolysate formula to compensate for the decreased dietary

protein intake. While on this diet, Dr. Bickel noted that phenylpyruvic acid was no longer excreted in the urine.[4] The child began to learn to stand and crawl with improved behavior and mood. In order to solidify his findings, Dr. Bickel reintroduced Phe back into the child's diet.[4] This re-introduction increased blood Phe, increased excretion of phenylpyruvic acid and the child began to deteriorate to her former self.[4] This was the first successful intervention in the management of PKU, and the use of Phe-free formula became the standard treatment for patients with PKU.

In 1953, Dr. George Jervis in the U.S. demonstrated that the defect of phenylalanine hydroxylase in the liver interferes with the normal conversion of phenylalanine to tyrosine.[3] In 1961, Dr. Robert Guthrie developed a bacterial inhibition assay to detect blood Phe concentrations in dried blood spots collected from the heels of newborn infants. The United States Children's Bureau provided funding and PKU screening was performed on 400,000 infants in 29 states.[5] Dr. Guthrie's work allowed for the diagnosis of PKU at birth, allowing early dietary intervention to prevent the severe consequences of untreated PKU[6].

Since the development of the "Guthrie test", more clinically effective and reliable methods of testing have been developed. Infants in most developed countries are screened for PKU within the first few days of life through national newborn screening programs. Tandem mass-spectrometry is now used to determine the concentration of amino acids in small quantities of blood from dried blood spots allowing for early identification of PKU and other inborn errors in metabolism. Immediate dietary intervention is protective against neurological damage in children with PKU.[7] The

prevalence of PKU varies among geographical and ethnic groups; according to the National Institute of Health, PKU occurs 1 in 10,000 to 15,000 newborns in the U.S.[8,9]

Disease severity of PKU can vary from mild to severe. Different forms of PKU have been described depending upon the enzyme defect, genotype, and clinical phenotype.[7,10] Hence, a classification blueprint (Table A) was developed in an effort to standardize the classification of various forms of hyperphenylalaninemia (HPA) and PKU.[11] Classical PKU is the most severe form of PKU with a blood Phe concentration greater than 1200µmol/L (20mg/dL) prior to initiation of treatment. Those with blood Phe concentrations remaining between 120 to 360µmol/L (2-6 mg/dL) are classified as mild HPA and treatment is not required. However, caution must be exercised when phenotyping PKU since blood Phe measurement is dependent upon other factors such as diet and time of blood sampling.[7,10,11]

Phe-Related Disorder	**Pre-Treatment Phe Concentrations (µmol/L)**
PAH Deficiency Requiring Treatment	
Classical PKU	>1200
Moderate PKU	900 – 1200
Mild PKU	600 – 900
Hyperphenylalaninemia	360 – 600
PAH Deficiency Not Requiring treatment	
Mild Hyperphenylalaninemia	120 – 360
Normal	50 – 110

Table A: Summary classification for Phe-related disorder.[11]

2.2 Biochemistry of PKU

L-Phenylalanine (Phe) is an essential amino acid and is the precursor for the synthesis of L-tyrosine (Tyr) required for the synthesis of the catecholamines, dopamine, norepinephrine and epinephrine.[12] This conversion of Phe to Tyr by phenylalanine hydroxylase (PAH) allows Phe to serve as the dietary precursor to Tyr, and is the first step of Phe catabolism in mammals.[13]

The conversion of Phe to Tyr by PAH is irreversible and PAH is mostly expressed in the liver and the kidneys.[14] The PAH system requires the cofactor tetrahydrobiopterin (BH4), and two regenerating enzymes pterin-4α-carbinolamine dehydrates (PCD) and dihydropteridine reductase (DHPR). In normal conditions, Phe is hydroxylated into Tyr at the C4 aromatic ring and the catabolism of Phe is regulated by the activity of the PAH enzyme. In this system, the cofactor BH4 is oxidized to an intermediate 4α-hydroxy-BH4 in the presence of oxygen, iron, and PAH. BH4 is then regenerated by PCD and DHPR. In this reaction, the rate-limiting step is the activity of PAH. The absence or decrease in PAH activity results in hypotyrosinemia and increased blood Phe concentrations[15,16] (Figure A).

PAH is composed of a central catalytic domain, a c-terminal oligomerization domain, and an N-terminal regulatory domain where each domain plays a different role. The catalytic domain contains the binding site for iron, cofactor, and enzyme while the N-terminal regulatory domain is where there is an increased affinity for the binding of phenylalanine. Several mechanisms are in place together as the regulation of PAH activity is tightly controlled. These mechanisms include the presence of cofactor BH4 and cAMP kinase dependent phosphorylation at Ser16.[16,17] Phe itself is the main

regulator where an increase in Phe concentration activates with positive cooperativity to the allosteric enzyme, PAH. While Phe activates PAH, the cofactor BH4 also functions as an inhibitor to the enzyme. This tightly controlled activation sequence of PAH is absent in many mutants of PAH associated with PKU.

The regeneration, regulation, and biosynthesis of BH4 includes six enzymes and eight cofactor dependent enzymes.[18] This synthesis of BH4 requires three enzymes: GTP cyclohydrolase I (GTPCH), 6-pyuvoiyl-tetrahydropterin synthase (PTPS), and sepiapterin reductase (SR).[18],[15] BH4 synthesis starts from guanosine triphosphate (GTP) and is carried out by GTPCH to form 7,8-dihydroneopterin triphosphate. In the presence of Mg^{2+} and Zn^{2+} the intermediate 7,8-dihydroneopterin triphosphate is converted into 6-pyrucoyl-tetrahydropterin which is catalyzed by PTPS. The final step of the synthesis of BH4 derivatives is the reductions a NADPH-dependent reduction of two side chains 6-pyrucoyl-tetrahydropterin involving SR.[15,18]

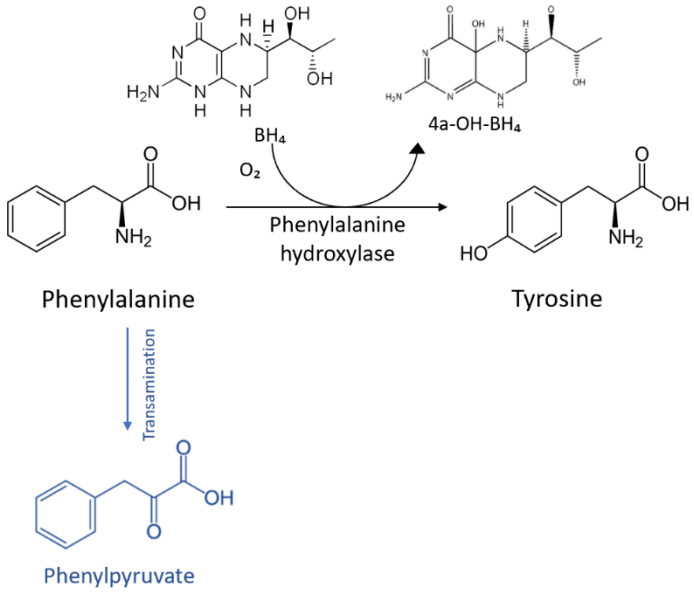


Figure A: Phenylalanine catabolism and normal PAH system (black). Abnormal metabolism in phenylketonuria (blue).

2.3 Characteristics of Late treated/ untreated PKU

Most neonates with PKU do not have a physical indication of PKU at birth, although a peculiar odor and infantile eczema can develop.[19] A retrospective cross-sectional survey completed in 1967 found that children with untreated PKU had poor growth and reduced head circumference.[20] The accumulation of blood Phe influences the blood brain barrier transport of other large neutral amino acids (LNAA), including tyrosine, threonine, histidine and tryptophan, which shares the same carrier as Phe. Hence, the increase in blood Phe concentration reduces the entry of other LNAA due to the carrier's higher affinity for Phe. Consequently, a secondary deficiency in LNAA can occur in the brain.[21,22] A reduction in tyrosine in the brain reduces the synthesis of catecholamines such as dopamine, norepinephrine, and epinephrine which potentially impacts various physiological and behavioral functions.[23] High concentrations of Phe are also thought to be directly detrimental to developing myelin and nervous tissue.[24] If treatment is not started or is delayed, those with PKU will develop severe mental retardation, cognitive decline and decreased intelligence secondary to HPA.

2.3.1 Neurocognitive deficits in treated PKU patients

Severe neurological effects of PKU can be prevented via dietary management but this does not completely protect patients with PKU from neurocognitive dysfunction.[25,26] [27] Anderson et al completed magnetic resonance imaging in 32 children with PKU (ages 7 to 18 years) who started diet treatment within the first 3 weeks of life. In this study, varying degrees of white matter abnormalities were found in 81% of the participants who also demonstrated mild executive impairment and a decreased processing efficiency.[28] A meta-analysis of 40 studies found a correlation between blood

Phe concentrations and IQ in early treated patients with PKU; for each increase of 100 µmol/L of blood Phe during critical periods of childhood, there was a predicted average 1.3 – 3.1 decrease in IQ.[29] Weglage et al. found IQ within the normal range in early treated adults with PKU; however, the range of IQ was significantly lower than that of their healthy counterparts. Similar to the study by Anderson et al, this study also found that 96% of adults with PKU had abnormalities in white matter suggesting that early-treated patients with PKU can have a decrease in intellectual performance compared to the general population.[28,30]

In addition to negative effects on neurocognitive function, PKU can also have an impact on psychosocial behavior. Executive function deficits have been reported in both adults and children with early treated PKU. Executive function includes skills such as sustaining attention, organizing, planning, impulse control and the ability to stay focused on a task.[31] Other neurocognitive deficits in this population include an increased incidence of attention deficit hyperactivity disorder (ADHD). It has been suggested that low dopamine concentration in the brain could possibly link PKU and ADHD.[31,32] Children and young adults of PKU also tend to perform less well academically. A study of 37 early treated children with PKU found lower than average academic performance compared to controls matched for age, sex and educational level. Those with PKU also demonstrated less self-reliance than their matched controls.[33]

Gassio et al found similar results as children with PKU demonstrated poorer fine motor and executive functions, and an increase in school problems compared to controls.[34] Hence, individuals with PKU require support from a multidisciplinary team that includes a dietitian, metabolic physician or nurse and a social worker. Treatment for

neonates with PKU should start as soon as possible to promote optimal physical growth, neurocognitive skills and psychosocial behaviors.

2.4 Genotype and Phenotype Relationship

PKU is an autosomal recessive disorder caused by variation in both alleles that encodes for the PAH enzyme located chromosome 12. For the disease to develop, two copies of the abnormal gene must be present. As of July, 2019 1149 variants have been reported.[35] Several of these variations can cause complete nullification of PAH enzyme function while others are associated with some residual enzyme activity. For example, the variant p.R252W encodes for only 1% of residual PAH activity while the variant p.R261Q encodes for 44% of residual activity.[36] The heterogeneity of disease is demonstrated in a study whereby 686 patients with PAH deficiency and their PAH gene variations were genotyped and phenotype. Participants were first phenotyped according to Phe tolerance and blood Phe concentrations. Participant's genotypes were used to predict phenotype into four categories of classical PKU, moderate PKU, mild PKU and mild hyperphenylalaninemia.

This study demonstrated only an 80% match between observed phenotype and predicted phenotype.[37] Hence, to some extent, PKU genotype can predict phenotype outcomes.[37] A meta-analysis analyzed genotype-phenotype relationship and found that variations of the PAH genotype are predictive of PKU phenotype but some predictions made were inconsistent.[38] Variation analysis in newborns can be an important tool in refining the diagnosis and implementing dietary therapy, but its limitations need to be considered.

2.5 General Principles of the PKU Diet

The primary goal of medical nutrition therapy for PKU is to correct and maintain blood Phe concentrations within the target range of 120 to 360 µmol/L.[39] Other goals of the Phe-restricted diet include supporting normal neurocognitive function, physical growth and health maintenance. Many studies have demonstrated that the Phe-restricted diet cannot be relaxed, even after normal growth and development has been achieved.[40-42] Hence, current recommendations suggest that nutrition therapy be maintained into adulthood.[43,44] The conventional diet therapy for PKU is life-long restriction of natural protein intake in combination with a synthetic amino acid based medical food. An individualized dietary prescription for those with classical PKU eliminates high dietary Phe sources such as meats, eggs, dairy products, beans, nuts, grains and the artificial sweetener aspartame. Limited quantities of low protein intact protein sources is necessary to meet Phe needs for growth and protein maintenance but excessive intake of Phe can cause elevated blood Phe. Thus, amino acid supplementation from medical foods is needed to meet needs for tyrosine and essential amino acids. Tyr becomes a conditionally essential amino acid in patients with PKU due to their lack of ability to synthesize Tyr.[44-46]

Individuals with PKU who have little PAH activity have a high dependency on medical foods and low protein foods.[10,39,47] Medical foods defined in section 5(b)(3)under the Orphan Drug Act, is “a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by

medical evaluation".[48] Medical foods cannot be purchased from retail outlets and most of these products also provide a full complement of macro- and micronutrients except the offending nutrient (Phe).

To assure adequate energy, but low Phe intake, foods modified to be low in protein are included in the diet. Modified low-protein foods are formulated to contain less than 1g of protein per serving and include baked goods, pasta, rice and meat and cheese substitutes. As modified low-protein foods mimic foods commonly consumed by a healthy individual, their use not only decrease intact protein intake but also increase the variety of the diet to help normalizes the highly restricted diet.[10] Those with mild to moderate HPA, may not require as much medical food in order to maintain optimal nutrition and blood Phe concentrations.[39]

Age	PKU Protein[a] (g/kg/day)	Protein[b] (g/kg/day)
0 to < 3 months	2.5 – 3.0	1.52
3 to < 6 months	2.0 – 3.0	1.52
6 to < 9 months	2.0 – 2.5	1.2
9 to <12 months	2.0 – 2.5	1.2
1 to < 4 years	1.5 – 2.1	1.1
> 4 years to adulthood	120% - 140% RDA for age	0.80 - 0.95

Table B: Comparison of recommended protein intake for individuals with PKU compared to the recommended dietary allowance for protein by age for the general population.
[a] Protein recommendation for individuals with PKU consuming Phe-free amino acid medical food as part of their protein sources.[47]
[b] Protein recommendations for the average healthy individual by age. Obtained from Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acid (2005).[49]

The minimum protein intake recommendations established by the Institute of Medicine for healthy adults is 0.8 g/kg of body weight/day.[49] In contrast, recommendations for protein for adults with PKU are higher (Table B). Higher protein recommendations for individuals with PKU are required because of the less efficient utilization of amino acids compared to intact protein. The digestion and absorption of

natural protein is slower than synthetic amino acids.[50] There is a consensus that individuals with PKU should consume medical foods throughout the day to maintain more stable blood Phe concentrations.[39] Phe-free amino acid medical foods consumed only once or twice per day reduces the efficacy of utilization of synthetic amino acids.[51] Thus, the recommendation for protein intake for individuals with PKU is between 120-140% of the DRI to compensate for this difference (Table B). For those with PKU, needs for other macro- and micronutrients do not differ from those of the average healthy population and are estimated using DRI recommendations.

As the conventional PKU diet is highly restrictive, individuals are at increased risk for vitamin and mineral deficiencies. The risk of vitamin B12 deficiency increases in diets which are predominantly plant based.[52] In those with PKU, vitamin B12 deficiency is primarily reported in adolescents or adults with decreased intake of Phe-free medical foods as well as those who stopped or relaxed the Phe-restricted diet.[53,54] A study of 31 adults with PKU who were no longer on their prescribed diet found that two thirds of the participants had low serum concentrations of cobalamin and holotransocobalmin and increased plasma methylmalonic acid suggesting early vitamin B12 deficiency.[54]

Iron deficiency is also common in children with PKU, despite therapy with iron supplemented Phe-free medical foods.[25,53] Arnold et al found 10 of 28 children with PKU who were consuming medical formula with iron intake above the RDA had marginal ferritin concentrations without overt anemia. The authors also hypothesized that in the presence of iron and protein deficiency, hematopoiesis is altered. Low hematocrit and mean red blood cell count were found in children with PKU, even though the children had adequate iron intake.[55] Another study of 37 children who had mean protein and iron

intake greater than RDA for age found an abnormal iron status biomarker in 14 of the children.[56] Additionally, increased fractures and reduced bone mineral density has been reported in patients with PKU.[53,57,58] The etiology of reduced bone mineral density is unclear but individuals with PKU who have poor diet adherence could be potentially at risk for deficient intake of protein, calcium and vitamin D, as well as various trace elements.[59]

Since individuals with PKU have an inability to convert Phe into tyrosine, tyrosine becomes a conditionally essential amino acid. Individuals with PKU have lower fasting plasma tyrosine which could impact neuropsychological outcomes.[60] However, a Cochrane review of six studies indicated that tyrosine supplementation increases plasma tyrosine but does not improve neuropsychological performance or quality of life.[61]

2.4.1 Initiation of Phe-restricted diet during infancy

Diet therapy for PKU is initiated after positive confirmatory testing following a positive newborn screen with elevated blood Phe. Depending on the initial Phe level, a "washout" period may be used to quickly reduce blood Phe concentrations. During this period, dietary Phe is completely removed from the diet by stopping feeds of breast milk or regular infant formula and providing only Phe-free medical food. Once the blood Phe concentration is estimated to be close or within treatment range (120 to 360µmol/L), limited Phe is reintroduce into the diet from limited quantities of a standard infant formula or breast milk with adlib intake of Phe-free medical food to assure adequate nutrition to promote optimal growth. Frequent monitoring and diet adjustments are required to determine the infant's Phe tolerance.[10,39,43]

2.4.2 Phe-restricted diet during childhood

Van Spronsen et al found a clear correlation between Phe tolerance at ages 2,3 and 5 years with Phe tolerance at 10 years of age suggesting a consistent Phe tolerance from 2 years of age onwards.[62] Phe tolerance is defined as the amount of Phe that a patient with PKU can tolerate without blood Phe concentrations increasing above the high end of the target range. Phe tolerance is influenced by various factors including the amount of residual PAH enzymatic activity.[44] The average Phe tolerance for those with classical PKU is usually between 200 and 500 mg dietary Phe/day.

2.4.3 Monitoring

In order to adjust dietary Phe and medical food intake, blood Phe is used as the primary biomarker in monitoring adherence in individuals with PKU. The most common way to monitor Phe is to collect a blood spot on filter paper at home. Ideally, blood spots are collected at the same time of day, between 2 and 3 hours after a meal and not immediately after consumption of medical food.[39] Currently, there isn't a universal protocol for frequency of blood collection, but blood Phe is monitored more frequently during infancy, early childhood and pregnancy. Plasma tyrosine is another biomarker that is routinely measured in individuals with PKU and is often measured simultaneously on dried blood spots. Hypotyrosinemia has been noted in this population, especially in those with inadequate intake of a tyrosine-supplemented medical food. Tyrosine concentrations should be maintained between 50 and 100µmol/L.[63] In addition to blood Phe and Tyr monitoring, frequent anthropometric measures of weight, height and head circumference are used to assess growth. Reduced growth can suggest Phe, protein and/or insufficient energy intake. Evaluation of dietary records also plays a vital role in

the management of PKU to help assess adherence to dietary Phe restriction and ensure adequate energy and protein intake.[11]

2.4.4 Adherence to the PKU diet

Individuals with PKU face many barriers and challenges such as difficulty in food preparation, lack of access to treatment and family dysfunction making adherence to the restrictive diet treatment difficult, especially for adolescents and adults.[10,11,44,64] Walter et al found that the occurrence of elevated Phe concentrations increase with age: 30% of children younger than age 4 had samples with blood Phe concentration above the recommended range, but this number increased to 80% in those between ages of 15 to 19 years. This study concluded that adolescents and young adults have generally poor overall compliance to the PKU diet.[65] 77% of adults with PKU in the United States between the ages of 25 and 45 years are not routinely followed by a metabolic clinic.[66] In a 2012 survey conducted across clinics in the US, only about 41% of estimated adults with PKU were actively being treated. In this study, adherence was defined by comparing target blood Phe recommendations to patients' average blood Phe. The survey found most adults had a blood Phe concentration of >360µmol/L. Additionally, adults with PKU did not demonstrate better adherence in clinics with a relaxed blood Phe recommendation of 600µmol/L.[67]

2.5 Sapropterin Dihydrochloride (Kuvan®)

Sapropterin Dihydrochloride (Kuvan®), an oral active synthetic form of the naturally occurring cofactor BH4, was approved by the Food Drug and Administration (FDA) in 2007. Kuvan® functions by enhancing and stimulating residual PAH enzyme

activity which increases the oxidation of Phe to reduce blood Phe concentrations low and increase tyrosine production.[68,69] In a study of 29 PKU patients who were offered Kuvan®, only 18 (62%) patients were considered responders. Responders were defined as those showing at least a 30% reduction in blood Phe concentration compared to baseline.[70]

In the phase III randomized placebo-controlled study, 89 PKU patients who had relaxed or stopped diet therapy were randomized to either receive Kuvan® or placebo tablets. Blood Phe concentration was measured after 6 weeks of treatment to determine the efficacy of Kuvan®. At week 6, participants blood Phe concentration decreased by 50% or more in 13 of 41 patients who received Kuvan® with an overall mean decrease of -235.9µmol/L in blood Phe concentration.[71] In another study performed in 2015, 206 PKU patients were randomized to receive either Kuvan® or placebo – mean blood Phe concentrations were higher in the placebo group compared to those who received Kuvan®.[72] Kuvan®, in conjunction with conventional diet therapy, can improve neurocognitive outcomes as it results in greater stability of blood Phe concentrations in those responding to this medication.[73,74]

2.6 Pegvaliase: an enzyme substitution therapy

The standard therapy for PKU is a lifelong Phe-restricted diet. The diet's strict regimen requires consistency and becomes burdensome for individuals with PKU, especially adolescents and adults. Additionally, the use of BH4 therapy benefits only part of the PKU population and it is more common for those with milder forms of PKU to respond to this medication.[75,76] Non-responders to BH4 could benefit most from alternative therapies, such as enzyme substitution therapy. Phenylalanine ammonia

lyase (PAL), an enzyme that is not found in mammals, was suggested as a therapeutic agent for PKU more than two decades ago [77]. PAL catalyzes the conversion of Phe to *t*-cinnamic acid and ammonia. With PAL treatment, an estimated 3g of t-cinnamic acid and ammonia is produced/day which is harmless to individuals with PKU since these metabolites are metabolized by the liver and excreted in urine.[78-80]

There are several challenges in the replacement of the native enzyme PAH. PAH is an unstable enzyme, sensitive to degradation as well as potential immunogenicity in individuals who lack the functional enzyme.[80] PAL is a "foreign" protein developed from recombinant *Anabaena variabilis* a species of blue green algae. PAL lowers blood Phe concentrations in PKU mouse models, but efforts to ensure long-term sustainable reductions of blood Phe concentrations was thwarted as PAL was neutralized by proteolysis.[81] Addition of polyethylene glycol (PEG) not only prevents PAL from protease degradation but also masks PAL from the host's immune system leading to decrease in immunogenicity and other adverse events[82].

The approval for pegvaliase (Palynziq™, Biomarin Pharmaceutical Inc.); an injectable form of therapy to treat adults with PKU is groundbreaking. Human trials for pegvaliase (initially called PegPAL) started in March of 2008. Phase 1 studies demonstrated that pegvaliase administered subcutaneously is effective in reducing blood Phe concentrations and is safe in adults with PKU.[83] Phase 2 studies determined the dosing regimen for pegvaliase and also demonstrated consistent reductions in blood Phe concentrations.[84] The phase 3 long-term clinical trial program (PRISM) supported the efficacy of pegvaliase as an alternative treatment for adults with PKU. During this study initiated in 2013, 261 adults with PKU with blood Phe >600 µmol at baseline were

enrolled. From baseline to month 12, mean blood Phe concentrations decreased by 51.1% and continued to decrease over time.[85] More than half of the participants were able to achieve blood Phe ≤120 µmol/L, which are concentrations of Phe that are comparable to those with mild HPA who do not require diet treatment.

Additionally, with long-term pegvaliase treatment, improvement in Attention Deficit Hyperactivity Disorder Rating Scale-IV (ADHD RS-IV) scores indicated improvement in attention as well as mood. In an eight-week randomized discontinuation trial (PRISM, Part 2), 86 participants who were already receiving pegvaliase were randomized 2:1 to either continue pegvaliase or give placebo injections. At baseline, mean blood Phe for all participants was 503.9 µmol/L. Blood Phe of participants who were randomized to remain on pegvaliase remained stable compared to participants who received the placebo. Mean blood Phe increased to 1647.1µmol/L and 1273.1µmol/L in participants receiving placebo that corresponded to either 20mg/day and 40mg/day dosing of pegvaliase.[86] Thus, the randomized discontinuation trial confirmed the efficacy of pegvaliase to decrease blood Phe concentrations in adults with PKU.

3. METHODOLOGY

3.1 Study participants

Pegvaliase-naïve adults over 18 years of age who were in generally good health with blood Phe > 600 µmol/L at screening were eligible to participate in this Phase 3 clinical trial. Adults who previously received pegvaliase in the Phase 2 (PAL-003) clinical trial were also eligible. Participants (or participant-designated caregivers) were required to meet the predefined self-administration criteria to qualify for the study. This predefined criterion included demonstrating working knowledge of signs and symptoms of a hypersensitivity reaction and what to do if a hypersensitivity reaction is suspected. Eligible participants (or caregivers) were trained to self-administer the study drug.

Adults using Kuvan® as part of their routine treatment were eligible for participation if this medication was discontinued. Females of childbearing potential were screened for pregnancy and were excluded if currently pregnant, planning to become pregnant or breastfeeding. Additionally, potential participants were required to have documentation from a dietitian confirming the ability to adhere to their current diet. Other important exclusion criteria included those who were on medications (except participants who were on a medication to treat any psychiatric disorder for ≥8 week), immunosuppressive therapy and/or had increased concentrations of biomarkers such as alanine amino transferase and creatine. See Appendix A for detailed exclusion and inclusion criteria. Approximately 261 participant's data from the Phase 3 PRISM trial were used for this secondary dietary data analyses.

3.2 Overall Study Design

In PRISM 1 (an open-label, multicenter, parallel-group phase 3 trial), pegvaliase dose was delivered via an induction, titration and maintenance dosing schedule. Pegvaliase-naïve adults who were enrolled into PRISM 1 were randomized 1:1 to titrate pegvaliase injections to a maintenance dose of 20 mg/day or 40 mg/day. Participants started with an induction dose of 2.5 mg/week for the first four weeks followed by periodic increases in dose and dose frequency to reach their respective maintenance dose of either 20 mg/day or 40 mg/day. Once at a maintenance dose, participants were then eligible to participate in PRISM 2, a four-part, phase 3 trial to continue evaluation of safety and efficacy of pegvaliase administration.

Part 1 of PRISM 2 was an open-label period to establish the eligibility of participants for entry into Part 2 of PRISM 2. Part 2, a randomized discontinuation trial, was designed to compare blood Phe in participants treated with pegvaliase and those treated with placebo. Due to hypersensitivity adverse events associated with the initiation of pegvaliase, Part 2 was only open to participants who were already receiving pegvaliase. Participants in Part 2 were subsequently transferred to Part 3, an open-label period to assess pharmacodynamics (PD) and pharmacokinetics (PK) of pegvaliase. Finally, Part 4 of PRISM 2 was an open-label extension to assess the long-term outcomes of pegvaliase administration. Participants who completed PRISM 1 with a dose other than 20mg/day or 40mg/day were only eligible for Part 4 of PRISM 2 (Figure B). Additional details of PRISM 2 (Part 1, Part 2, Part 3, and Part 4) are described below.

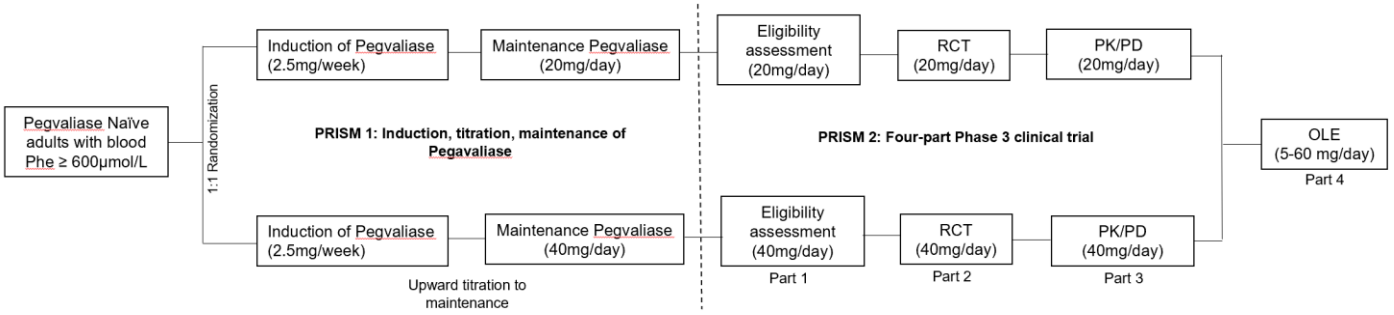


Figure B: The overall design of Phase 3 PRISM clinical trial. Pegvaliase was administered with an induction, titration, and maintenance schedule (PRISM 1). Part 1 of PRISM 2 assessed eligibility of participants to enter Part 2, the randomized discontinuation trial (RCT). Participants who completed Part 2 progressed to Part 3 to assess pharmacokinetics (PK) and pharmacodynamic (PD) of pegvaliase. Participants from PRISM 1 and 2 transitioned to Part 4, an open label extension (OLE) to assess long-term outcomes.

PART 1: Open-Label Blood Phe Assessment

Part 1 (up to 13 weeks) was an open-label period designed to establish eligibility for participation in Part 2. During Part 1, participants continued with the pegvaliase dose regimen of 20 mg/day or 40 mg/day. Any previous premedication regimen was continued in Part 1 per investigator determination. Blood Phe was assessed every 2 weeks to determine if a mean blood Phe reduction of ≥ 20% (based on two consecutive assessments) from baseline had been achieved. Once Part 2 eligibility had been established, no further blood Phe assessments were performed for Part 1 of the study. Blood Phe assessments were continued when the participants moved to Part 2 of the study and Part 4 if participants were not eligible for Part 2. Participants remained in Part 1 until blood Phe reduction of ≥ 20% from baseline was achieved. If participants were unable to achieve a blood Phe reduction ≥ 20% after 13 weeks in Part 1, they transitioned to Part 4, the long-term, open-label extension.

PART 2: Randomized, Double-Blind Discontinuation

Part 2 (8 weeks) was a randomized, double-blind, placebo-controlled, four-arm, discontinuation design to compare blood Phe levels in participants treated with

pegvaliase to those treated with a matching placebo. Enrollment for Part 2 closed when approximately 85 participants were randomized into Part 2. All other participants were enrolled into Part 4 of the study. At week 1, 4, and 8 of Part 2, plasma Phe, 3-day dietary diary, neurocognitive assessments (Profiles of Mood Survey (POMS) and Attention Deficit Hyperactivity Disorder Rating Survey (ADHD-RS)) were collected.

PART 3: Pharmacokinetic/Pharmacodynamic (PK/PD) Assessment

Only participants who completed Part 2 entered Part 3 (6 weeks) to assess safety with immunogenicity and PK/PD studies. Plasma for PK/PD, blood Phe and tyrosine were collected at week 1, 4 and 5. A 3-day dietary diary was collected only on during week 4 of Part 3.

PART 4: Open-Label, Long-Term Extension

Part 4 (approximately 274 weeks) was an open-label extension designed to evaluate long-term efficacy and safety and to provide long-term access to pegvaliase. During Part 4, pegvaliase was administered in prefilled syringes rather than manually filled syringes utilized prior to Part 4. In Part 4, dose increases to 60 mg/day were allowed per investigator discretion in consultation with the sponsor's medical monitor, provided the participant received a combined total of > 52 weeks of pegvaliase administration with a minimum of 8 weeks of 40 mg/day dosing. Blood Phe, tyrosine and 3-day diet diary were collected monthly while neurocognitive assessments (POMS and ADHD-RS) were performed every two months until the completion of study.

3.3 <u>Dietary Protocol</u>

A participant's ability to maintain a consistent diet was essential for the success of the study to ensure that the efficacy and safety end points were attributable to study

treatment rather than to changes in dietary protein intake. Before a participant could begin PRISM-1, a dietitian from each study site evaluated his/her dietary intake and documented approval if the participant was felt to be able to maintain a consistent diet for the duration of the study. Throughout the study, all participants were instructed to take a tyrosine supplement of 500 mg three times per day with meals. Participants were instructed not to change their dietary intake during the study, especially during Part 2.

Participants were provided food diaries to record all food sources, beverages and medical foods consumed for 3 consecutive days prior to Day 1 of PRISM 1 to establish baseline Phe and protein intake. 3-day food diaries were collected prior to each study clinic visit after baseline was established. At all study visits, a dietitian reviewed the food diaries with the participant to provide additional details and assess adherence to the diet protocol. A nutrient analysis software program (Metabolic Pro®) was used to assess total energy, protein (intact and medical food sources), Phe, tyrosine and the percentage of daily recommended intake (DRI) for protein, Phe, tyrosine, vitamins, and minerals. Protein from Phe-free medical foods and from intact foods (any other food sources containing Phe) were collectively referred to as total protein.

Participants were required to maintain a total dietary protein intake that was consistent with their baseline intake for the entire duration of the study. A consistent protein intake was one with minimal variation and defined as maintaining intake of intact protein ≤ ±25% from baseline and maintaining intake of medical food protein ≤ ±25% from baseline. When intake of intact protein was determined to be ≥ ±10% and/or medical food protein was determined to be ≥ ±10% from baseline, additional counselling was provided to modify intake of intact and/or medical food protein. If intact protein

and/or medical food protein was ≥ ±25% from baseline, further corrective actions were implemented. When diet adjustments were necessary, participants were counseled to resume their baseline diet by modifying intake of intact protein and/or medical food and an additional 3-day diet record and blood Phe was requested in 2 weeks. If the participant demonstrated ≥ ±25% change in intake of intact protein and/or medical food protein at the next scheduled study visit (after 4 weeks), the medical monitor was notified, and further actions related to non-adherence with consistent dietary protein was discussed.

If blood Phe levels decreased to ≤ 30µmol/L and it was determined that the participant was consuming less than his/her DRI for intact protein (Table C), the participant was instructed by the site dietitian to increase intact protein intake by 10g and decrease medical food protein intake by 5g. If a participant was consuming more than the DRI, but less than 2x the DRI, for intact protein, participants were instructed to increase intact protein intake by 10% and decrease medical food protein intake by 5g. Any adjustments made to medical and/or intact protein intakes then served as the new reference baseline for dietary protein intake for future dietary assessments. However, if a participant was consuming ≥2x the DRI for intact protein, he/she was instructed to maintain their current intact and medical food protein intake. It is important to note that pegvaliase dose could be lowered per investigator's discretion if participant a with blood Phe ≤30µmol/L consumed ≥2x the DRI for intact protein.

Sex	Age	DRI of Protein of an Average Healthy Individual (g/day)
Men	18	52[a]
	≥19	56[b]
Women	18	46[c]
	≥19	46[c]

Table C: Recommended DRI of protein intake by sex and age. DRI of protein is determined using the average weight of healthy individual.
[a] The average weight for healthy sedentary male 65kg
[b] The average weight for a healthy sedentary male was 70kg
[c] The average weight for a healthy sedentary female was 57.5kg

3.4 Data and Statistical Analysis

Dietary data collected from participants in PRISM 1 and PRISM 2 between May, 2013 and February, 2018 was included in this secondary data analysis. Statistical analysis was performed by a statistician at BioMarin Pharmaceutical Inc. The average of each 3-day food diary from each study visit was used to portray a more accurate depiction of participants' daily food intake.

Dietary data included 3-day mean intake of total calories, total protein (protein from medical food + intact protein), protein from medical food, intact protein, and dietary Phe. Dietary data were reviewed exhaustively to remove outliers to ensure strength of dietary data. To determine outliers, an estimate of dietary Phe was calculated from each participant's three-day average intake of intact protein. The commonly used ratio to estimate dietary Phe intake is 1g of intact protein provides 30 to 50 mg of dietary Phe. However, to remove outliers, a wider range of estimated Phe in intact protein was used: 20 mg to 65 mg Phe/g intact protein. Using the intact protein value from Metabolic Pro analysis, 1g of intact protein was multiplied by a lower bound of 20 mg and an upper bound of 65 mg of dietary Phe/g protein. This estimated Phe intake was compared to the participant's dietary Phe intake determined by Metabolic Pro analysis.

If a participant's dietary Phe intake determined from Metabolic Pro did not fall within the estimated upper and lower limits for estimated dietary Phe calculated from intact protein, then further review was performed by reviewing dietary information from each day of the participant's individual food records. If dietary Phe from Metabolic Pro did not fall within the estimated range of dietary Phe data (20 – 65 mg/g protein) from any day in an individual's food record, then data for that individual day was removed and excluded from all analyses. If data from a day in an individual food record was removed, a new average was determined from the remaining days in the diet record and this value was incorporated in all future analyses.

3.4.1 Percent weight change from baseline over time

Participant's weight was collected and documented at every scheduled visit. In order to assess weight change over time, each participant's weight values were extensively reviewed. First, any weight change from baseline to month 12 greater than 9kg (20lbs) was further reviewed. Then, weight change was calculated between the most recent measured weight with the weight measured at the previous scheduled visit. Any change in weight greater than 9kg (20lb) between visits was considered an outlier. These individual weights were removed from the analysis.

3.4.2 Protein intake and DRI comparison

Total protein, medical food protein and intact protein were analyzed by calculating five different categories based on the DRI established by the Institute of Medicine.[49] The DRI is the recommended daily intake to meet the nutrient requirements of 98% of healthy individuals. For adults, the DRI for protein is 0.8 g protein/kg body weight.

For this study, only the first twelve months of food intake data was included with one month defined as 30.5 days. Body mass index (BMI) was calculated for all participants from the first height and weight measurements collected during the study. Adjusted body weight was calculated for participants with a BMI greater than 30kg/m^2. For these participants, baseline weight was replaced with adjusted body weight. Adjusted body weight was calculated with the following equation:

*[(actual body weight – ideal body weight) * 0.25 + ideal body weight]*

Ideal body weight was calculated based on sex using one of the following equations:

Sex	Equation: Ideal body weight
Men	*106lbs for the 1st 5 ft + 6 lbs for each additional inch*
	48kg for the first 152.4 cm + 1.1kg for each additional cm
Female	*100lbs for the 1st 5ft + 5lbs for each additional inch*
	45 kg first 152.4cm + 0.9 kg for each additional cm

For this study, 100% protein intake was defined as 0.8g protein per kg of actual or adjusted body weight. To categorize participants, protein intakes (total and intact protein) were calculated using the equation (A) at each selected timepoint and then categorized in one of the categories listed in Table D:

(A) $$\frac{3\ day\ average\ of\ daily\ protein\ intake\ (g)}{Baseline\ weight\ (kg)} = Protein\ category$$

Protein Intake Categories (g/kg body weight)	Protein Intake (Percent Equivalent)
Protein ≤ 0.4	Protein ≤ 50%
0.4 < Protein ≤ 0.8	50% < Protein ≤ 100%
0.8 < Protein ≤ 1.2	100% < Protein ≤ 150%
1.2 < Protein ≤ 1.6	150% < Protein ≤ 200%
Protein > 1.6	Protein > 200%

Table D: Protein intake categories and it's percent equivalent

Each participant's mean protein intake (total, medical food, intact) was determined at three separate time points: baseline, month 6 and month 12. Month 6 was approximately 26 weeks from baseline. For participants eligible for part 2 and 3 of the trial, month 6 was approximately the 5th week of part 3. For those who didn't complete part 2 and part 3, month 6 was approximately 13 weeks into Part 4. Month 12 was approximately 52 weeks from baseline and all participants were in Part 4 of PRISM-2 at that time point.

Additionally, change from baseline was calculated at month 6 and month 12 for the following variables:

1. Dietary Phe (mg)
2. Blood Phe (µmol/L)
3. Total protein intake (g)
4. Protein from medical food intake (g)
5. Intact protein intake (g)

Figure C indicates the subpopulation used in analyzing protein intake over time. 11 participants did not have any recorded total protein or intact protein intake or both at baseline and were excluded from analysis. 88 participants were excluded from month 6 analyses as there was no recorded protein intake data at month 6. Finally, an additional 61 participants were excluded from month 12 analyses as there was no recorded protein intake data at month 12.

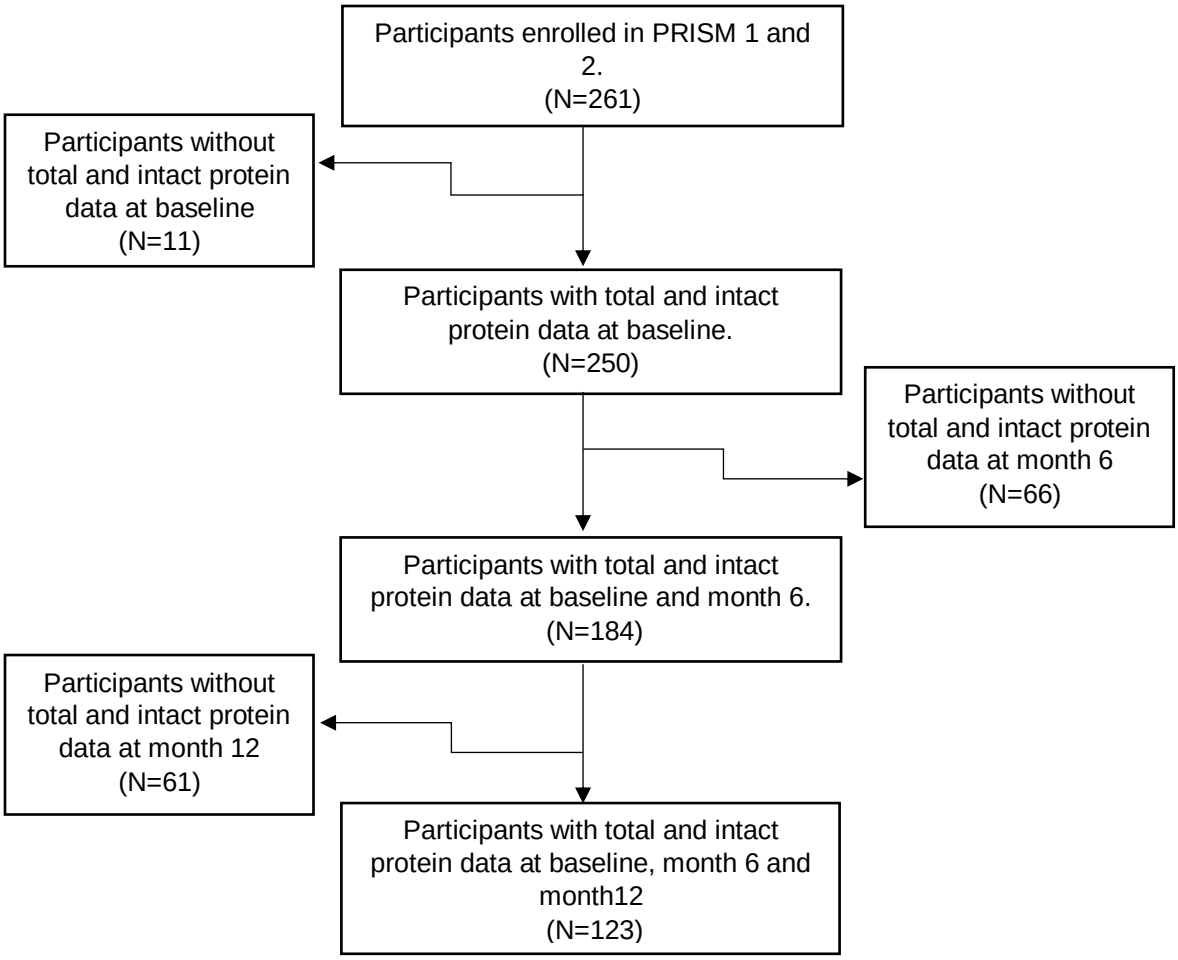


Figure C: Participant disposition for participant's with DRI at baseline, month 6 and month 12 (N=123)

3.4.3 <u>Hypophenylalaninemia analyses</u>

To investigate the relationship between dietary protein intake and hypophenylalanemia (hypoPhe), participants were first divided into two sub-populations: those who were diagnosed with hypoPhe and those who did not experience hypoPhe throughout the entire clinical trial. HypoPhe was defined as two consecutive blood Phe ≤ 30µmol/L at any point during the study. Baseline characteristics of these two sub-populations were compared and dietary intake trends over time were analyzed between the two groups. Table F1 categorizes participants by hypoPhe status. Participants who did not experience hypoPhe (N=152) and participants who experienced hypoPhe but did

not have a documented resolution at the time of data compilation (N=20) were excluded from the hypoPhe correlation and ancova analysis described below.

Participants by HypoPhe Status	**N = 261**
Participants who did not experienced hypoPhe	153
Participants who experienced hypoPhe	108
Table F1: Categorization of participants by hypoPhe status. HypoPhe is defined as ≥2 consecutive blood Phe ≤ 30µmol/L. Data-cut Feb 2018	

Data from participants who experienced hypoPhe during the trial was further categorized into three subcategories (Table E) and analyzed separately.

HypoPhe subcategories	**Definition**
Resolved Events[a]	**Hypophe Events with a documented resolution as of February 2018**
Single HypoPhe Events	Participants with only one resolved HypoPhe event as of February 2018
Multiple HypoPhe Events	Participants with more than one resolved HypoPhe event as of February 2018
Unresolved Events	**HypoPhe events without a documented resolution as of February 2018**
Unresolved hypophe Events	Participants whose most recent hypophe event did not have documentation of resolution as of February 2018
Table E: Definition of hypoPhe subcategories [a]Resolved events is defined as the first documentation of 2 consecutive blood Phe of > 30µmol/L	

To determine the correlation between blood Phe of participants who experienced hypoPhe and dietary intake, only data from participants with resolved hypoPhe events was used. Table F2 categorizes the number of participants who experienced hypoPhe (N=108) by type of hypoPhe event.

Type of HypoPhe event	**Number of events (N=108)**
1. Single hypoPhe event	32
2. Multiple hypoPhe events	56
3. Unresolved hypoPhe events	20

Table F2: Categorization of participants who experienced hypoPhe by type of HypoPhe event. HypoPhe is defined as ≥ 2 consecutive blood Phe ≤ 30µmol/L. “Single hypoPhe events” are participants experiencing one resolved hypoPhe event during the trial. “Multiple hypoPhe events” are participants with two occurrences of hypoPhe during the trial and both events with 2 consecutive blood Phe ≤ 30µmol/L. Unresolved events are participants with a hypophe event that did not resolve (blood Phe > 30µmol/L) before the Data-cut of Feb 2018

Participants with unresolved hypoPhe events were excluded from this analysis as we were unable to calculate change in intake from baseline. Change from baseline was calculated for the following variables:

1. Intact protein intake (g)
2. Protein from medical food intake (g)
3. Total protein Intake (g)
4. Dose of pegvaliase (mg/day) administered

Change from baseline of participants who experienced only one hypoPhe event and those who experienced multiple hypoPhe events were calculated differently. Figure D1 indicates how data was obtained to calculate change in intake for participants who experienced only one hypoPhe event. Change from baseline was calculated using data between the first of two consecutive hypoPhe measurements used to establish the hypoPhe diagnosis (blood Phe ≤ 30µmol/L) and the first measurement at the end of the hypoPhe event when blood Phe > 30µmol/L.

Figure D2 indicates how the data was used to calculate change in intake for participants who experienced more than one hypoPhe event. Change from baseline was calculated using data between the first hypoPhe event (first diagnosis of two consecutive blood Phe ≤ 30µmol/L) and the first blood Phe collected at the end of the most recent hypoPhe event when blood Phe was > 30µmol/L.

Figure D1

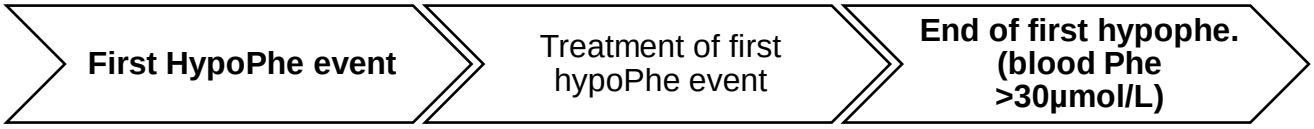
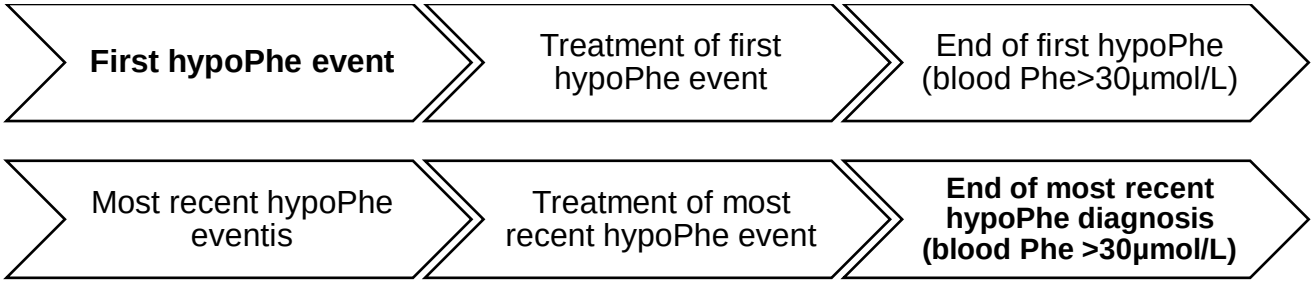


Figure D2



Correlation analyses was performed for four variables (pegvaliase dose, intact protein, medical food protein and total protein intake) and change in blood Phe. These four variables are change in protein intake from intact food, change in protein intake from medical food, change in total protein intake and change in pegvaliase dosage. A *p*-value of less than 0.05 is considered significant.

Ancova analysis was also performed for 88 of 108 participants who were included in either of the resolved hypoPhe subcategories. The Ancova analysis was used to determine the relationship between the change in intact protein intake, pegvaliase dose and blood Phe. For this analysis, participants who experienced hypoPhe were sub-categorized into two different categories: those who remained on a stable dose of pegvaliase throughout a hypoPhe event and those on an unstable dose of pegvaliase. Stable dose was defined as no change in pegvaliase dosage administered throughout a hypoPhe event while unstable dose was defined as any change in pegvaliase dosage administered at any time during a hypoPhe event.

4. RESULTS

4.1 Participants characteristics

The participant's characteristics are given in Table 1. At baseline, the mean (SD) age of the enrolled participants was 29.15 (8.75) years. Males and females were equally distributed. Of the 261 participants, 7 (2.69%) were Hispanic or Latino. Additionally, 95 of 260 participants (36.40%) were considered obese (BMI > 30) and 47.37% of those who were obese were males.

Total protein intake, which includes medical food and dietary intact protein sources, had a wide range at baseline between 3.57 – 263.53g/day. The majority of enrolled participants were taking some form of protein from medical food but only 41 participants (16.40%) were consuming greater than 75% of total protein intake from medical food.

4.2 Percent weight change over time

Participant's percent weight change over time increased (Figure 1). At month 0, the mean (SE) weight was 80.47kg (1.28). At month 4, month 12 and month 24 mean (SE) weight was 82.87kg (1.54), 82.30kg (2.18) and 87.29kg (1.14) respectively. There was a 3.83% increase in weight by month 12 and a 7.62% increase in weight from baseline to month 24.

4.3 Trends in protein intake and blood phenylalanine concentrations

Overall, participants' total protein intake increased slightly while blood Phe decreased over the course of 52 months of pegvaliase administration as shown in

whisker plots comparing intakes of total protein (Figure 2), intact protein (Figure 3), medical protein (Figure 4) and dietary Phe (Figure 5) with blood Phe over time.

Blood Phe trends: The mean (SD) of blood Phe decreased over time. The concentration of blood Phe at month 0, month 12 and month 24 was 1217.09µmol/L (369.07), 563.63µmol/L (528.15) and 425.06µmol/L (532.58), respectively. Mean (SD) of blood Phe reached the target range (120µmol/L - 360µmol/L) at 320.65µmol/L (299.80) by month 28. There was a change of mean (SD) in blood Phe of -954.74µmol/L (516.33) from month 0 to month 28.

Protein and dietary Phe intake:

Total protein: At month 0, the mean (SD) total protein intake was 65.66g (29.97). The mean (SD) total protein intake at month 12, month 24 and month 36 was 71.44g (24.96), 75.19g (25.19) and 77.05g (26.41), respectively, with an increase in change of mean (SD) of 7.91 from month 0 to month 24 (Figure 2). Participant's total protein intake increased while blood Phe decreased over time. The mean (SD) blood Phe decreased by -847.14µmol/L (517.54) from month 0 to month 36.

Intact protein: The mean (SD) intake of intact protein was 38.93g (27.31) at month 0, 60.24g (27.19) at month 24 and 70.34g (28.87) at month 36 (Figure 3) showing a mean (SD) increase from month 0 to month 36 of 28.86g (32.85). Participant's intact protein intake increased while blood Phe continued to decrease over time; blood Phe decreased by a mean (SD) of -847.14µmol/L (517.54) at month 36 compared to month 0.

Medical food: Intake of protein from medical food and blood Phe decreased over time (Figure 4). The mean (SD) of protein from medical food at month 0 and month 24 was 26.73g (27.02) and 14.95g (23.36), respectively. The mean (SD) of protein from medical food intake was lowest at month 36 (mean (SD) = 6.72g (16.75)), with an overall decrease in mean (SD) of medical food intake of -19.81g (23.74) by month 36.

Dietary Phe Intake: Dietary Phe intake increased over time while blood Phe decreased (Figure 5). The mean (SD) of dietary Phe intake at month 0, month 12, month 24, and month 36 was 1709.48mg (1146.85), 2129.35mg (1311.01), 2697.83mg (1222.07), and 3241.18mg (1361.19) respectively. Blood Phe decreased by a mean (SD) of -847.14µmol/L (517.54) by month 36.

4.4 Comparison of protein intake by DRI categories

At baseline, month 6 and month 12, there was an overall decrease in blood Phe with increased intake of total protein, intact protein, and dietary Phe over a period of 12 months (Table 2).

***Change from baseline to month 6*:** Blood Phe decreased by a mean (SE) of -473.15µmol/L (49.78) from baseline to month 6. Total protein and intact protein intake increased by 2.11g/d and 5.43g/d, respectively. Corresponding to the increase in intact protein, dietary Phe intake increased by 232.42mg/d by month 6.

***Change from baseline to month 12*:** Blood Phe decreased by a mean (SE) of -584.25µmol/L (51.84) from baseline to month 12 with a lower mean blood Phe at month 12 compared to month 6. Total protein intake increased by 4.2g/d from baseline to

month 12. Additionally, intact protein and dietary Phe intake increased by 8.86g/d and 460.20mg/d respectively.

Changes in DRI for total protein: Most participants had a total protein intake between 0.4 – 0.8g/kg body weight at baseline (43.09% of participants) and at month 6 (39.84% of participants) (Table 3). Comparing month 12 to baseline, an additional 10 participants increased total protein intake to 0.8 – 1.2g/kg and an additional 7 participants increased total protein to 1.2 – 1.6g/kg body weight.

Change in DRI for Intact Protein: At baseline, month 6 and month 12, most participants were consuming ≤0.4g protein/kg body weight from intact protein. However, the number of participants who consumed ≤0.4g intact protein/kg body weight decreased over the 12 months from 54.47% at baseline to 39.84% at 12 months.

4.5 Hypophenylalaninemia

Participant characteristics by hypoPhe status

108 of 261 participants (41.37%) experienced at least one hypoPhe event throughout the duration of this study. HypoPhe is defined as 2 consecutive blood Phe levels of ≤ 30µmol/L. There are no significant differences in baseline characteristics of participants who experienced hypoPhe compared to those who did not experience hypoPhe (Table 5). The distribution of age, race, ethnicity and sex was similar between the two sub-groups. The mean (SD) weight for those who experienced hypoPhe was 78.18kg (19.53) and weight for those who did not experienced any hypoPhe was 82.16kg (21.32). The proportion of participants with a BMI greater than 30kg/m^{-2} was

slightly higher in those who did not experience hypoPhe (39.22%) compared to those who experienced hypoPhe (32.41%).

Protein Intake by hypoPhe status

Total protein intake increased slightly in both participants who experienced hypoPhe and those who did not (Figure 6). For participants who did not experience hypoPhe, mean (SD) of total protein intake was 63.98g (31.33) at month 0, 66.36g (24.72) at month 12 and 71.45g (29.00) at month 24. For participants who did experience at least one hypoPhe event, mean (SD) total protein intake was 68.10g/d (27.85) at month 0, 75.15g/d (24.62) at month 12 and 77.90g/d (21.93) at month 24.

However, the increase in intact protein intake over time was greater in participants who experienced hypoPhe compared to participants who did not experience hypoPhe (Figure 7). At month 0, mean intact protein intake for those who did not developed hypoPhe was 37.78g/d and those who did experienced hypoPhe had a mean intake of 48.33g/d compared to those who did. Intact protein intake differed at month 12 in participants who experienced hypoPhe compared to participants who did not experience hypoPhe, with mean intact protein intake of 54.89g/d compared to 35.49g/d, respectively. The mean change of intact protein intake in participants who experienced hypoPhe was higher (6.56g/d) compared to those who did not experienced hypoPhe (-2.29g/d). The greatest mean intact protein intake was 75.55g/d at month 36 for participants who experienced hypoPhe.

Duration of hypoPhe

The total number of hypoPhe events (from individuals with resolved single

hypoPhe events and those with resolved multiple hypoPhe events) was 195, experienced in 88 participants. The length of time participants experienced hypoPhe varied greatly between 34 days to 1408 days. The average duration of hypoPhe events was 258.43 days as 117 of 195 (60%) of the hypoPhe events lasted for more than 120 days (Table 5).

Protein intake from intact food by type of HypoPhe events

The quarterly distribution of protein intake from intact food increased over time, regardless of type of hypoPhe event (single or multiple events) (Figure 8). At month 0, intact protein was higher in participants who experienced only a single hypoPhe event compared to participants who experienced multiple hypoPhe events. The baseline mean (SD) intake of intact protein of those who experienced a single hypoPhe event was 52.84g/d (33.82) compared to 36.08g/d (22.96) for participants who experienced multiple hypoPhe events. However, at month 16, mean (SD) intake of intact protein was similar between the two groups: 60.10g/d (27.33) for those who experienced a single hypoPhe event and 60.26g/d (29.55) for those who experienced multiple hypoPhe events).

Correlation analysis of resolved hypoPhe events

There wasn't a significant correlation between the change in blood Phe compared to pegvaliase dose, medical food protein intake and intact protein intake in participants who experienced hypoPhe (Figure 9A, 9C and 9D). However, there was a weak but significant negative correlation between change in total protein intake and change in blood Phe in participants with hypoPhe ($P = 0.043$; $R^2 = -0.22$).

Ancova Analysis for participants with resolved hypoPhe:

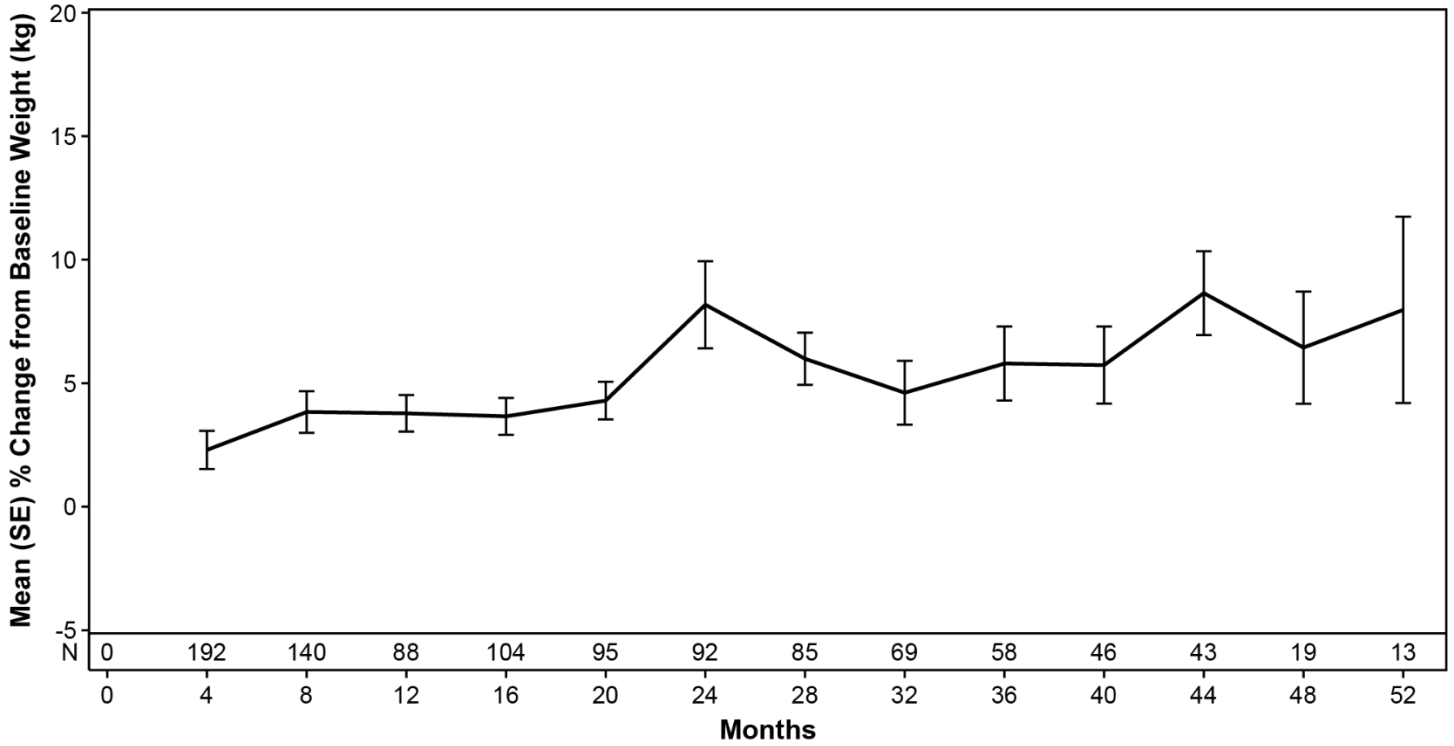
There wasn't a significant difference in the intake of intact protein between participants with stable or unstable doses of pegvaliase who experienced resolved hypoPhe events (Table 6). Additionally, there wasn't a significant difference in intake of intact protein between the two groups after adjusting for blood Phe (Appendix D).

4.6 List of Tables and Figures

Table 1: Baseline Characteristics of Enrolled Participants in PRISM Clinical Trial	Participants (N = 261)
Age at enrollment, years	
Mean (SD, SE)	29.15 (8.75, 0.54)
Median (min,max)	28.00 (16.00, 55.00)
Sex	
Female, N (%)	130 (49.81)
Ethnicity	
Not Hispanic or Latino, N (%)	253 (97.31)
Weight, kg	
Mean (SD, SE)	80.51 (20.66, 1.28)
Median (Min, Max)	77.20 (41.50, 139.20)
Height, cm	
Mean (SD, SE)	168.08 (9.45, 0.59)
Median (min, max)	167.60 (143.50, 192.00)
Body mass index, kg/m^2	N=260
Mean (SD, SE)	28.43 (6.74, 0.42)
Median (min, max)	27.73 (17.10, 47.33)
<18.5, N (%)	8 (3.07)
18.5 – 29.9, N (%)	157 (60.16)
≥30, N	95 (36.4)
Missing, N (%)	1 (0.40)
BMI ≥30kg/m^2	
Female, N (%)	50 (52.63)
Baseline blood phenylalanine, µmol/L	
Mean (SD, SE)	1232.71 (286.36, 23.92)
Median (min, max)	1221.00 (285.00, 2330.00)
Total protein intake, g/day	N = 250
Mean (SD, SE)	64.73 (32.15, 2.03)
Median (min, max)	62.59 (3.57, 263.53)
Intact protein intake, g/day	N = 250
Mean (SD, SE)	38.43 (27.75, 1.76)
Median (min, max)	29.82 (3.57, 155.50)
Medical protein intake, g/day	N = 250
Mean (SD, SE)	26.30 (18.51, 1.80)
Median (min, max)	16.77 (0.00, 120.00)
Receiving protein from medical food, N (%)	149 (57.09)
Patients on restricted diet, N (%)	41 (16.40)
Sample size indicates the number of participants that had data available for specific baseline characteristic. Sample size was indicated if data was not available for all participants. Baseline was defined as the first measurement available for each variable. Total protein intake includes medical food and dietary intact protein sources. Protein intakes were calculated as the daily average intake over 3 days. Restricted diet is defined as >75% of protein from medical food SD: standard deviation. SE: standard error; Data cut Feb 2018	

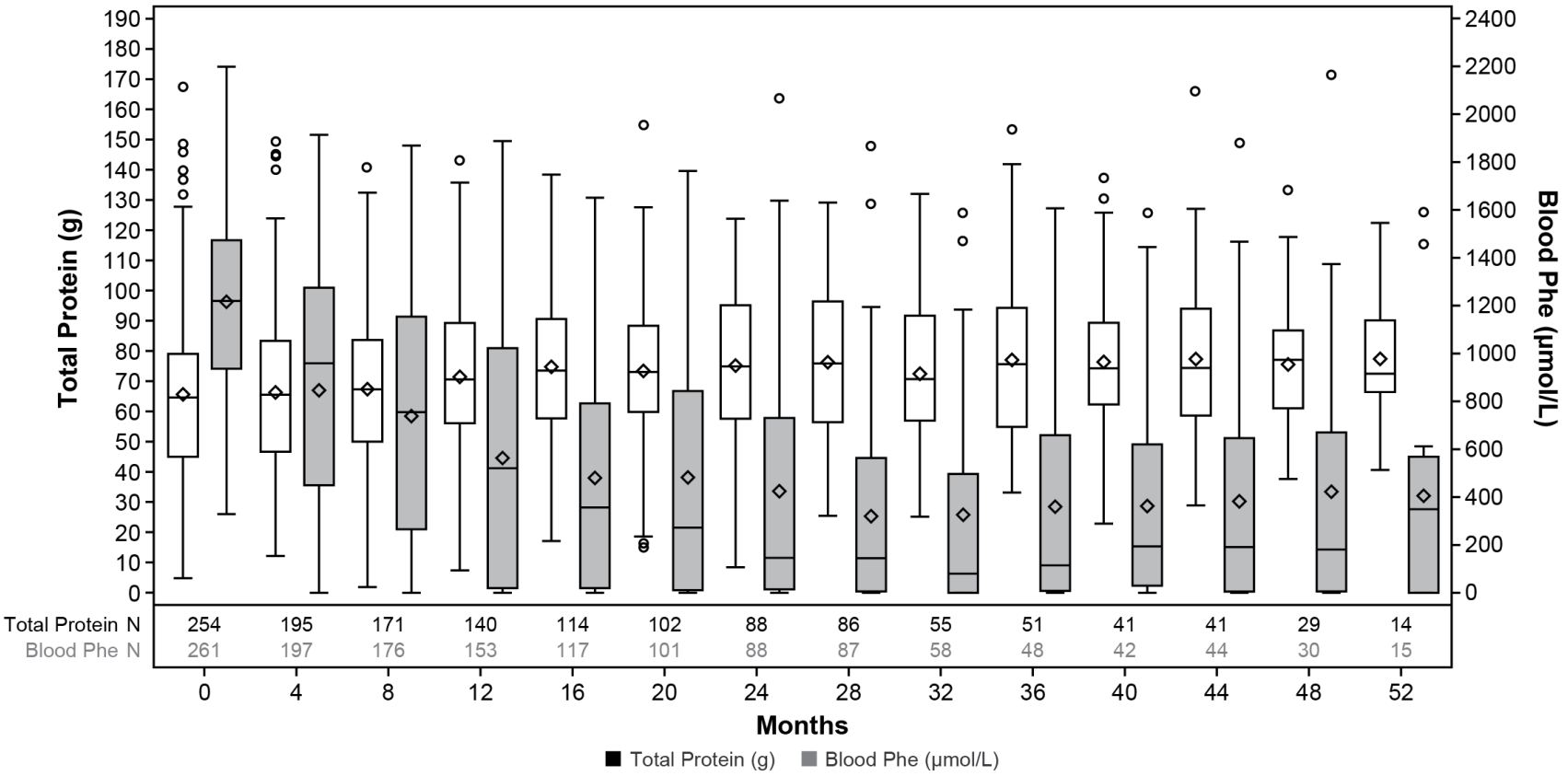
4.6 List of Tables and Figures

Figure 1: Percent weight change over time.



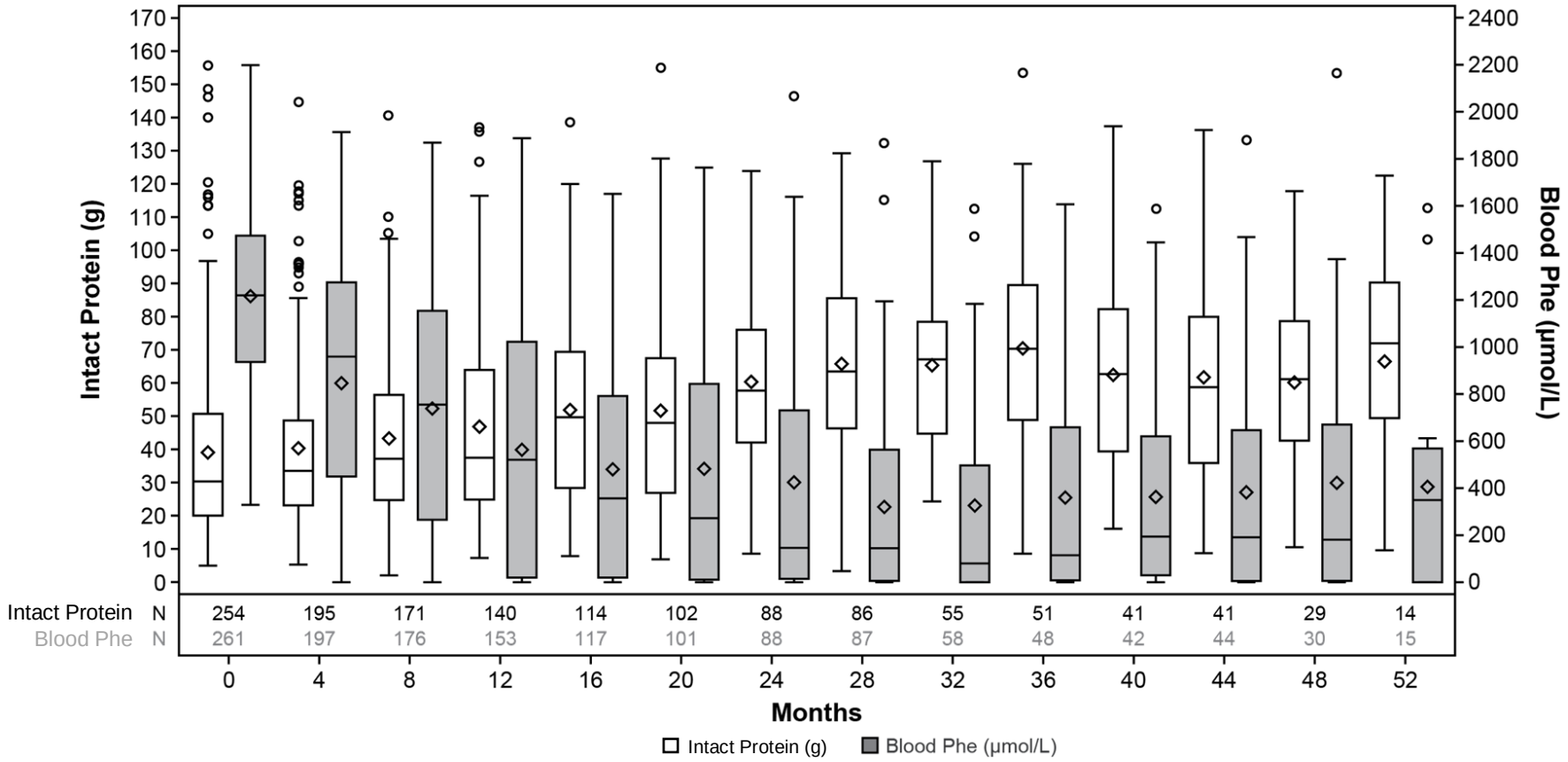
Percent weight (kg) change from baseline were plotted quarterly where 1 month was 30.5 days. Month 0 ≠ baseline. Quarterly was defined as 4 months. Data cut Feb 2018.

Figure 2: Quarterly distribution of total protein intake (g) and blood Phe (µmol/L) over time.



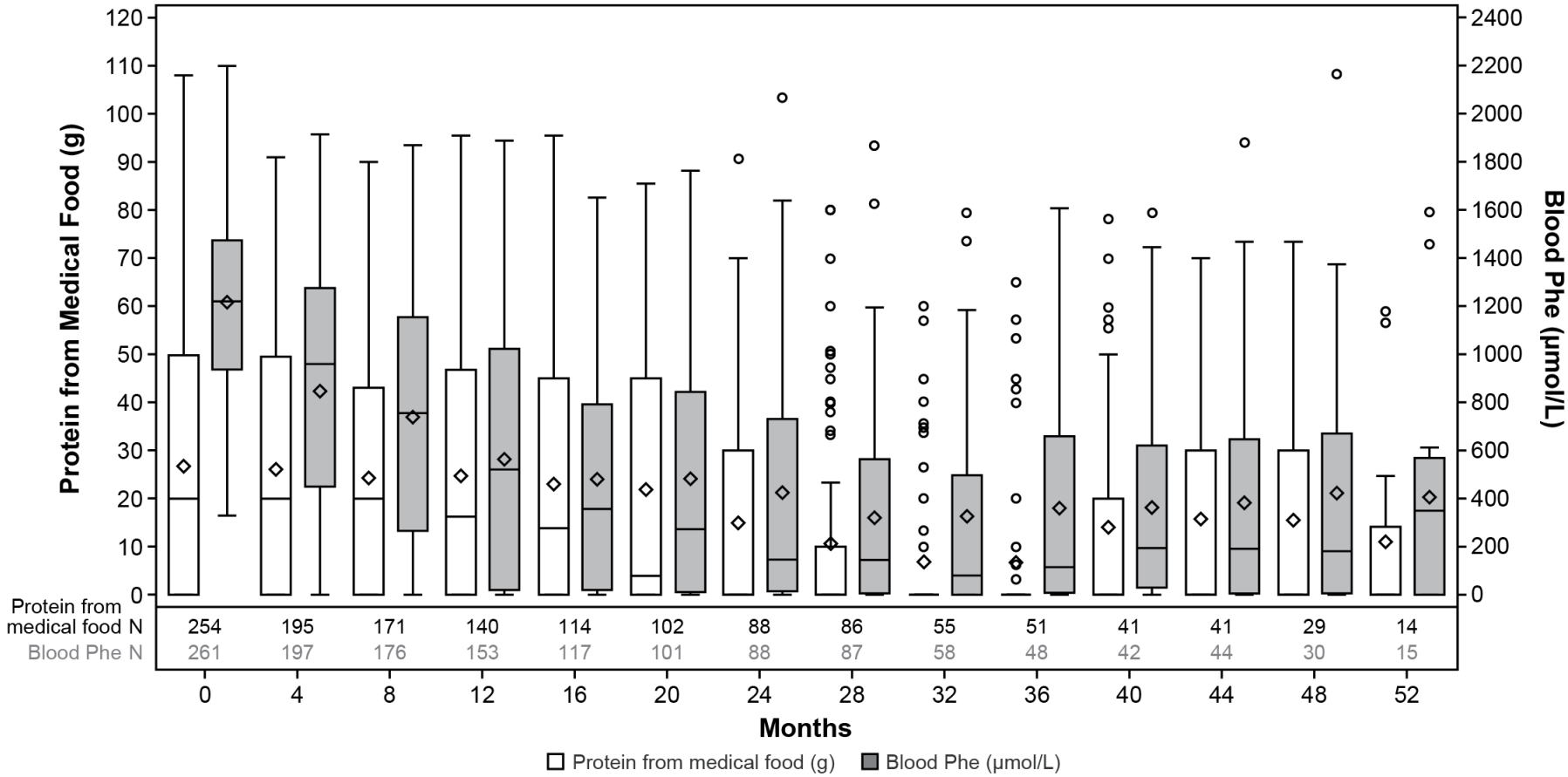
Whisker plots were plotted quarterly where 1 month was 30.5 days. Month 0 ≠ baseline. Quarterly was defined as 4 months. Plotted values were calculated from average protein intake over 3 days. Plot captures minimum, 25th percentile, median (horizontal line), 75th percentile, and maximum values; ◊ represent mean; ○ outliers; Data cut Feb 2018

Figure 3: Quarterly distribution of intact protein intake (g) and blood Phe ((µmol/L) over time.



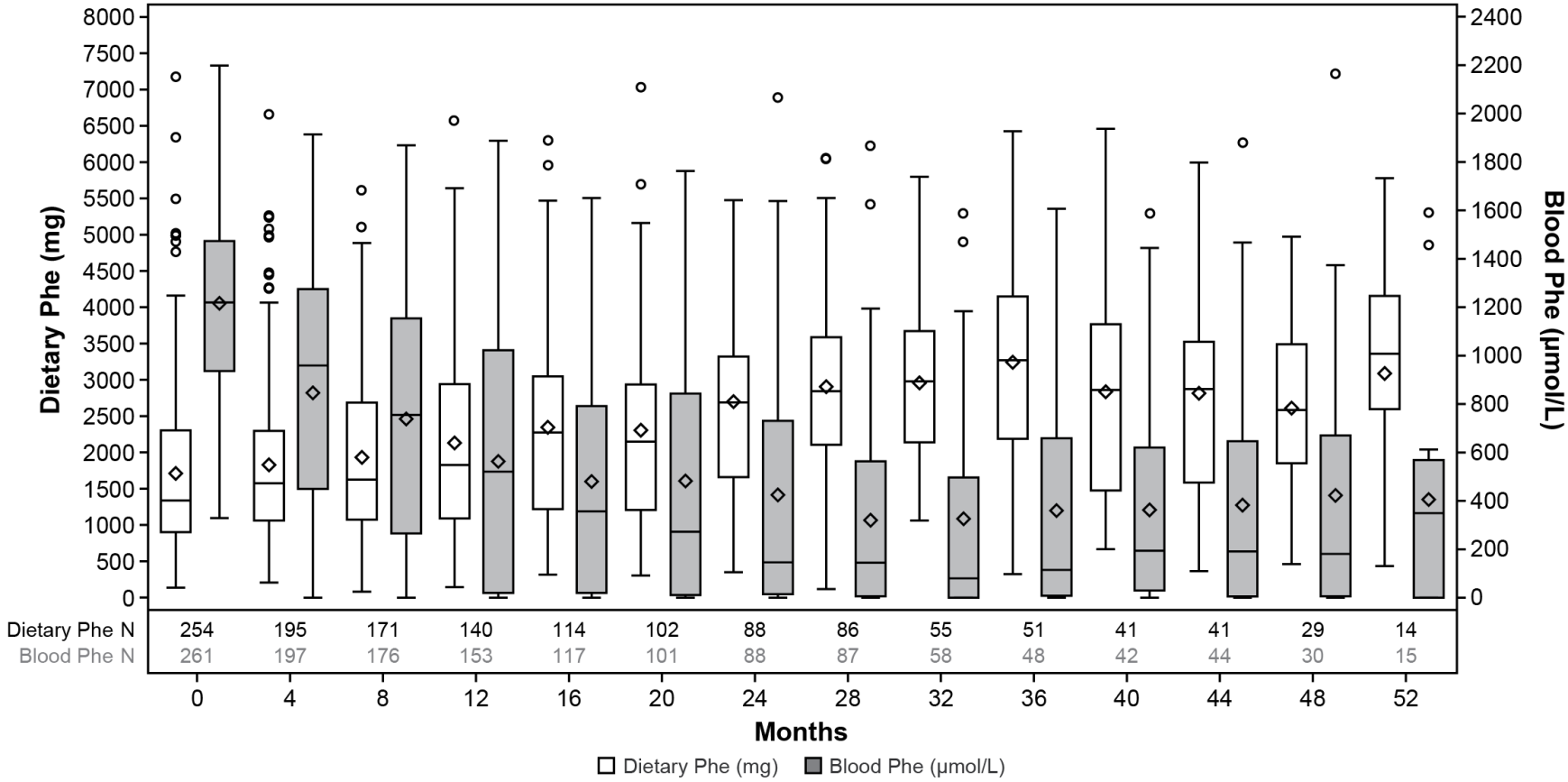
Whisker plots were plotted quarterly where 1 month was 30.5 days. Month 0 ≠ baseline. Quarterly was defined as 4 months. Plotted values were calculated from average protein intake over 3 days. Plot captures minimum, 25th percentile, median (horizontal line), 75th percentile, and maximum values; ◊ represent mean; ○ outliers; Data cut Feb 2018

Figure 4: Quarterly distribution of protein from medical food (g) and blood Phe (µmol/L) over time.



Whisker plots were plotted quarterly where 1 month was 30.5 days. Month 0 ≠ baseline. Quarterly was defined as 4 months. Plotted values were calculated from average protein intake over 3 days. Plot captures minimum, 25th percentile, median (horizontal line), 75th percentile, and maximum values; ◊ represent mean; ○ outliers; Data cut Feb 2018

Figure 5: Quarterly distribution of dietary Phe (mg) and blood Phe (µmol/L) over time.



Whisker plots were plotted quarterly where 1 month was 30.5 days. Month 0 ≠ baseline. Quarterly was defined as 4 months. Plotted values were calculated from average protein intake over 3 days. Plot captures minimum, 25th percentile, median (horizontal line), 75th percentile, and maximum values; ◊ represent mean; ○ outliers; Data cut Feb 2018

Table 2: Participants' characteristics at baseline, month 6 and month 12 (N=123).

Variables	Baseline	Month 6	Month 12
Blood Phe, µmol/L			
Mean (SE) Median (min, max) Q1,Q3	1205.50 (32.94) 1196.00 (483.00, 2229) 934.00, 1486.00	732.36 (47.71) 723.00 (0.00, 1818.00) 193.00, 1160.00	621.26 (48.31) 605.00 (0.00, 1889.00) 0.00, 1889.00
Total Protein, g/day			
Mean (SE) Median (min, max) Q1, Q3	67.61 (2.69) 64.60 (8.80, 180.80) 49.90, 82.20	69.72 (2.52) 66.20 (4.20, 143.10) 22.70, 54.40	71.81 (2.35) 71.4 (7.40, 143.30) 55.30, 91.30
Intact Protein, g/day			
Mean (SE) Median (min, max) Q1, Q3	36.87 (2.56) 27.30 (4.20, 155.30) 16.90, 49.60	42.30 (2.40) 34.80 (4.20, 143.10) 22.70, 54.40	45.73 (2.68) 35.70 (7.10, 137.00) 22.20, 61.80
Dietary Phe, mg/day			
Mean (SE) Median (min, max) Q1, Q3	1632.46 (109.42) 1205.30 (140.00, 6249.00) 737.00, 2253.70	1864.88 (103.44) 1573.30 (184.30, 5778.30) 1022.00, 2488.30	2092.65 (121.09) 1762.00 142.00, 6582.00
Only included participants with available data at baseline, month 6 and month 12 (30.5 days/month). Baseline is defined as any first available data collected for all variables. Participants with multiple protein, dietary Phe, blood Phe records were calculated as an average. Protein intakes were calculated as the daily over intake over 3 days. Data-cut Feb 2018			

Table 3: DRI of total and intact protein intake (N=123).

Protein Categories (g/kg of body weight)	Baseline, N (%)	Month 6, N (%)	Month 12, N (%)
	Total Protein Intake		
Protein ≤ 0.4	11 (8.94)	9 (7.32)	10 (8.13)
0.4 < Protein ≤ 0.8	53 (43.09)	49 (39.84)	33 (26.83)
0.8 < Protein ≤ 1.2	44 (35.77)	39 (31.71)	54 (43.9)
1.2 < Protein ≤ 1.6	8 (6.5)	17 (13.82)	15 (12.2)
Protein ≥ 1.6	7 (5.69)	9 (7.32)	11 (8.94)
	Intact Protein Intake		
Protein ≤ 0.4	67 (54.47)	51 (41.46)	49 (39.84)
0.4 < Protein ≤ 0.8	40 (32.52)	47 (38.21)	43 (34.96)
0.8 < Protein ≤ 1.2	14 (11.38)	21 (17.07)	22 (17.89)
1.2 < Protein ≤ 1.6	2 (1.63)	3 (2.44)	6 (4.88)
Protein ≥ 1.6	0	1 (0.81)	3 (2.44)

Only included participants with available data at baseline, month 6, month 12 (30.5days/month) with baseline BMI. Baseline was defined as any first available data collected for all variables. Participants with multiple protein records as an average. Protein intakes were calculated as the daily average intake over 3 days. DRI for participants with BMI < 30kg/m^2 = protein (g)/ weight at baseline (kg). DRI for participants with BMI ≥ 30kg/m^2 = protein (g)/ adjusted body weight (kg). Data cut Feb 2018.

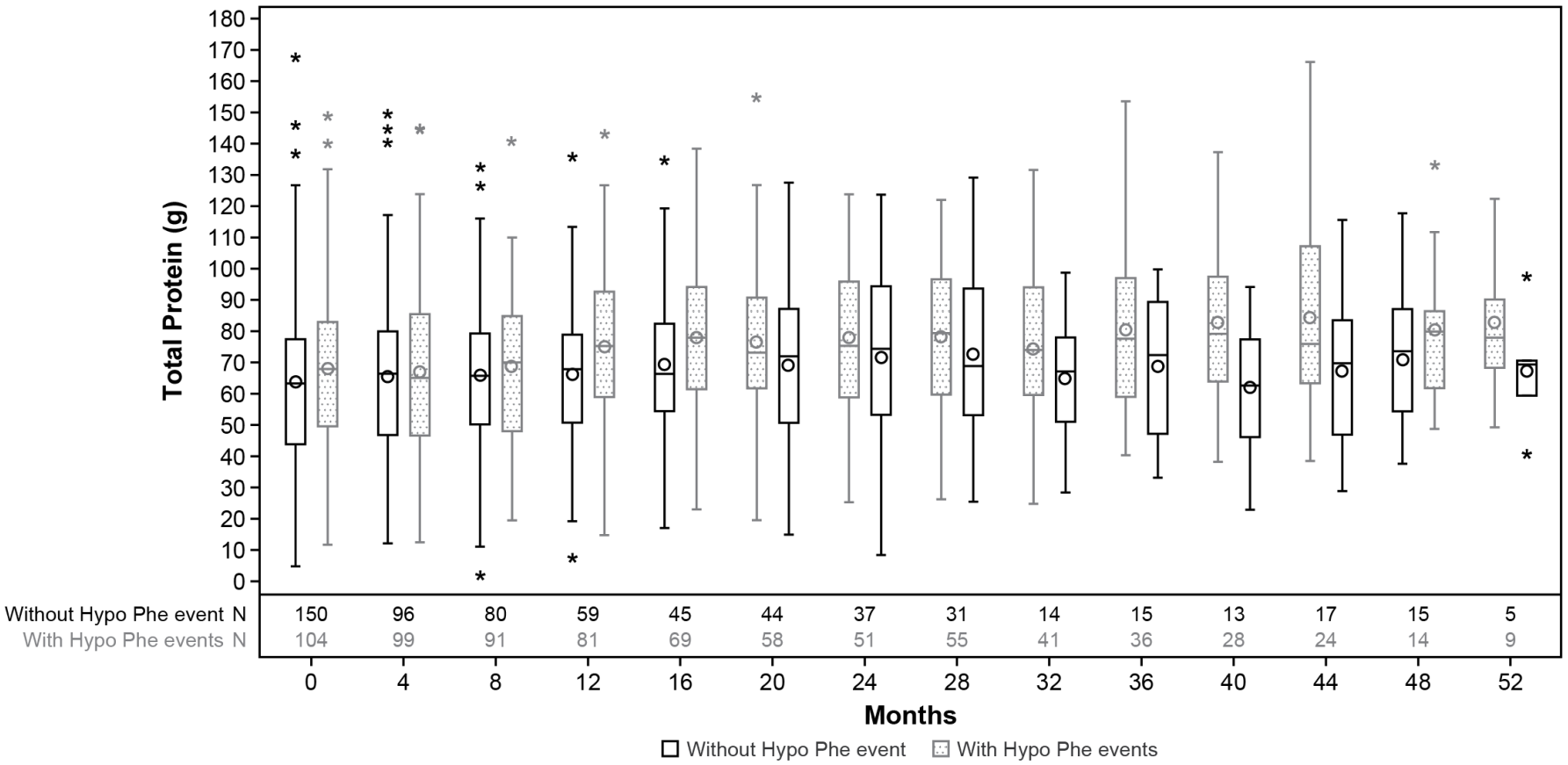
Table 4: Participant characteristics by hypoPhe status.

Baseline Characteristics (N=261)	**HypoPhe (N=108)**	**Non-HypoPhe (N=153)**
Body mass index, kg/m^2	**N=107**	
Mean (SD, SE)	27.71 (6.26,0.61)	28.93 (7.04, 0.57)
Median (min, max)	27 (17.16, 46.70)	27.85 (17.10, 47.33)
<18.5, N (%)	4 (3.70)	4 (2.61)
18.5 – 29.9, N (%)	68 (62.96)	89 (58.17)
≥30, N (%)	35 (32.41)	60 (39.22)
Baseline blood Phe, µmol/L		
Mean (SD)	1248.82 (392.37)	1221.33 (382.94)
Median (min, max)	1274 (510, 2229)	1171 (285, 2330)
Total protein intake, (g)	**N=104**	**N=146**
Mean (SD)	68.81 (31.13)	61.82 (32.64)
Median (min, max)	20.05 (8.77, 180.77)	59.57 (3.57, 263.53)
Intact Protein intake, (g)	**N=104**	**N=146**
Mean (SD)	40.08 (28.46)	37.25 (27.27)
Median (min, max)	29.98 (5.07, 155.27)	29.55 (3.57, 155.5)
Protein from medical food intake, (g)	**N=104**	**N =146**
Mean (SD)	28.73 (29.49)	24.56 (27.76)
Median (min, max)	20 (0, 120)	13.33 (0, 115.5)
Dietary category at baseline, N (%)	**N=104**	**N=146**
On a restricted diet	15 (14.42)	26 (17.81)
Not on a restricted diet	89 (85.58)	120 (82.19)
HypoPhe is defined as ≥ 2 consecutive blood Phe ≤ 30µmol/L. Baseline is defined as first measurement available for each variable. Protein intakes were calculated as the daily average intake over 3 days. Sample size indicated if data not available for all participants. Restricted diet is defined as >75% of protein from medical food. SD, standard deviation. Data-cut Feb 2018.		

Table 5: Duration of hypoPhe

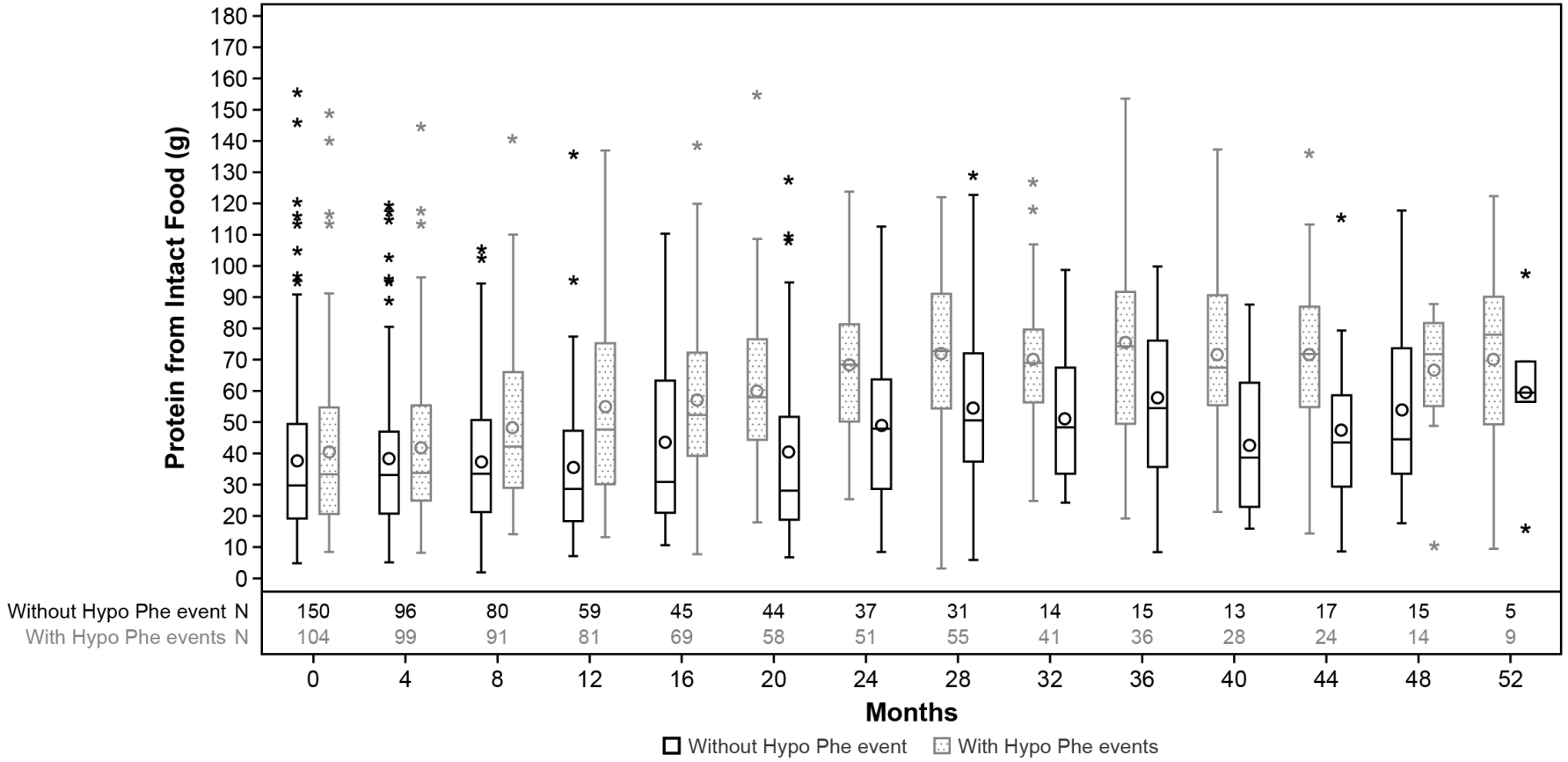
Total number of HypoPhe events	**N = 195**
Duration of hypoPhe events, Days	
Mean (SD)	258.43 (260.21)
Median (min, max)	165 (32, 1408)
Q1, Q3	97, 317
Duration of the number of hypoPhe events, N (%)	
≤60 days	19 (9.74)
>60 - ≤90 days	27 (13.85)
>90 - ≤120 days	32 (16.41)
>120 days	117 (60.00)
Hypophe is defined as ≥ 2 consecutive blood Phe ≤ 30µmol/L. Duration was calculated by using the start of the event, i.e. defined as the first blood Phe assessment day with ≤ 30µmol/L and the end of the event, i.e. the day prior to the first blood Phe assessment ≥ 30µmol/L. Data-cut Feb 2018.	

Figure 6: Quarterly distribution of total protein intake by participant's hypoPhe status over time.



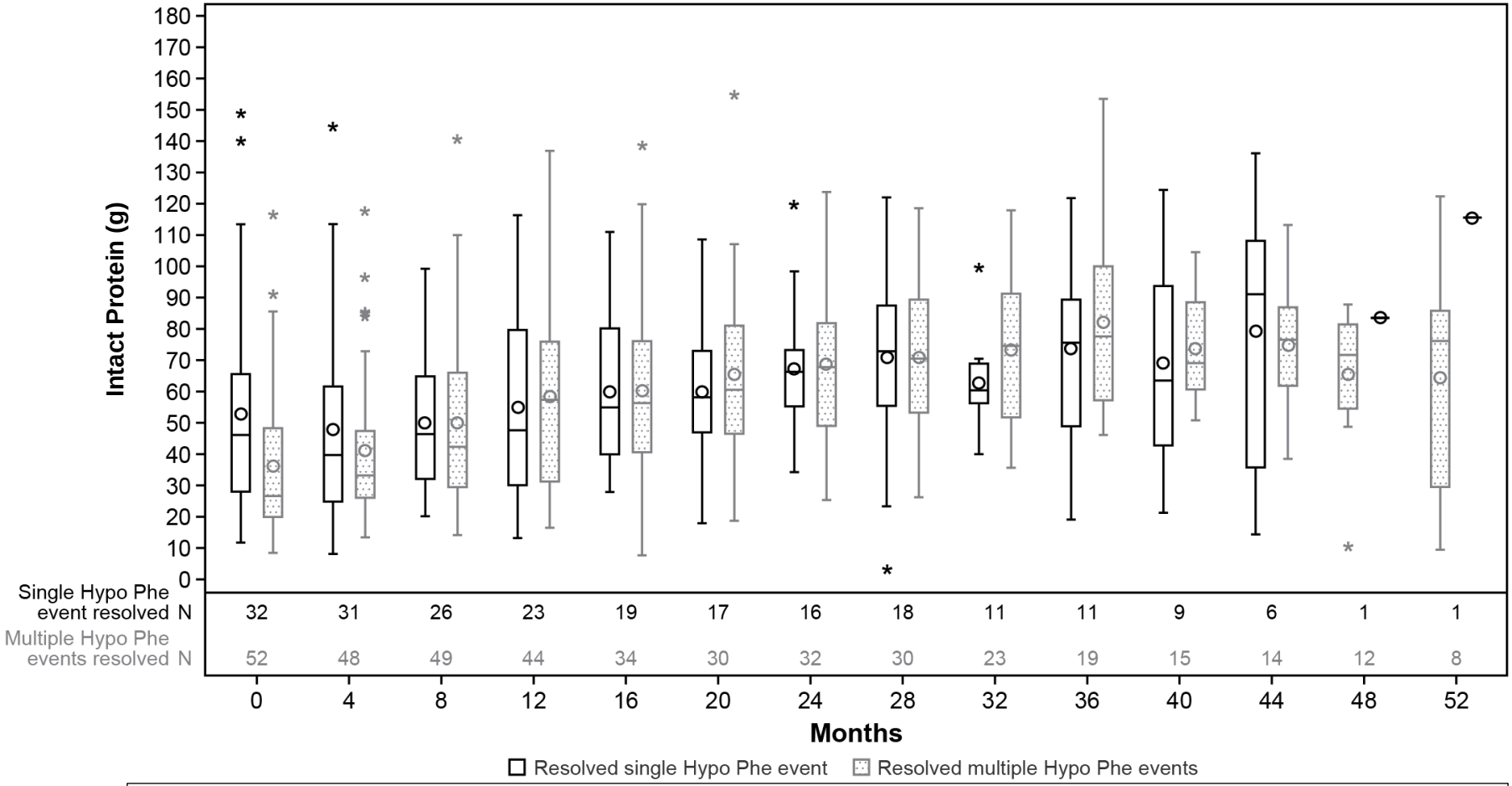
Whisker plots were plotted quarterly where 1 month was 30.5 days. Month 0 ≠ baseline. Quarterly was defined as 4 months. Plotted values were calculated from average protein intake over 3 days. Plot captures minimum, 25th percentile, median (horizontal line), 75th percentile, and maximum values; o represent mean; *outliers for participant with hypoPhe and without hypoPhe; Data cut Feb 2018

Figure 7: Quarterly distribution of intact protein intake (g) by participant's hypoPhe status.



Whisker plots were plotted quarterly where 1 month was 30.5 days. Month 0 ≠ baseline. Quarterly was defined as 4 months. Plotted values were calculated from average protein intake over 3 days. Plot captures minimum, 25th percentile, median (horizontal line), 75th percentile, and maximum values; ○ represent mean; * outliers for participant with hypophe and outliers for participant no hypophe; Data cut Feb 2018

Figure 8: Quarterly distribution of protein from intact food (g) by type of hypoPhe event in participants with hypoPhe only.



Whisker plots were plotted quarterly where 1 month was 30.5 days. Month 0 ≠ baseline. Quarterly was defined as 4 months. Plotted values were calculated from average protein intake over 3 days. Plot captures minimum, 25th percentile, median (horizontal line), 75th percentile, and maximum values; ○ represent mean; * outliers for participant with hypophe and outliers for participant no hypophe; Data cut Feb 2018

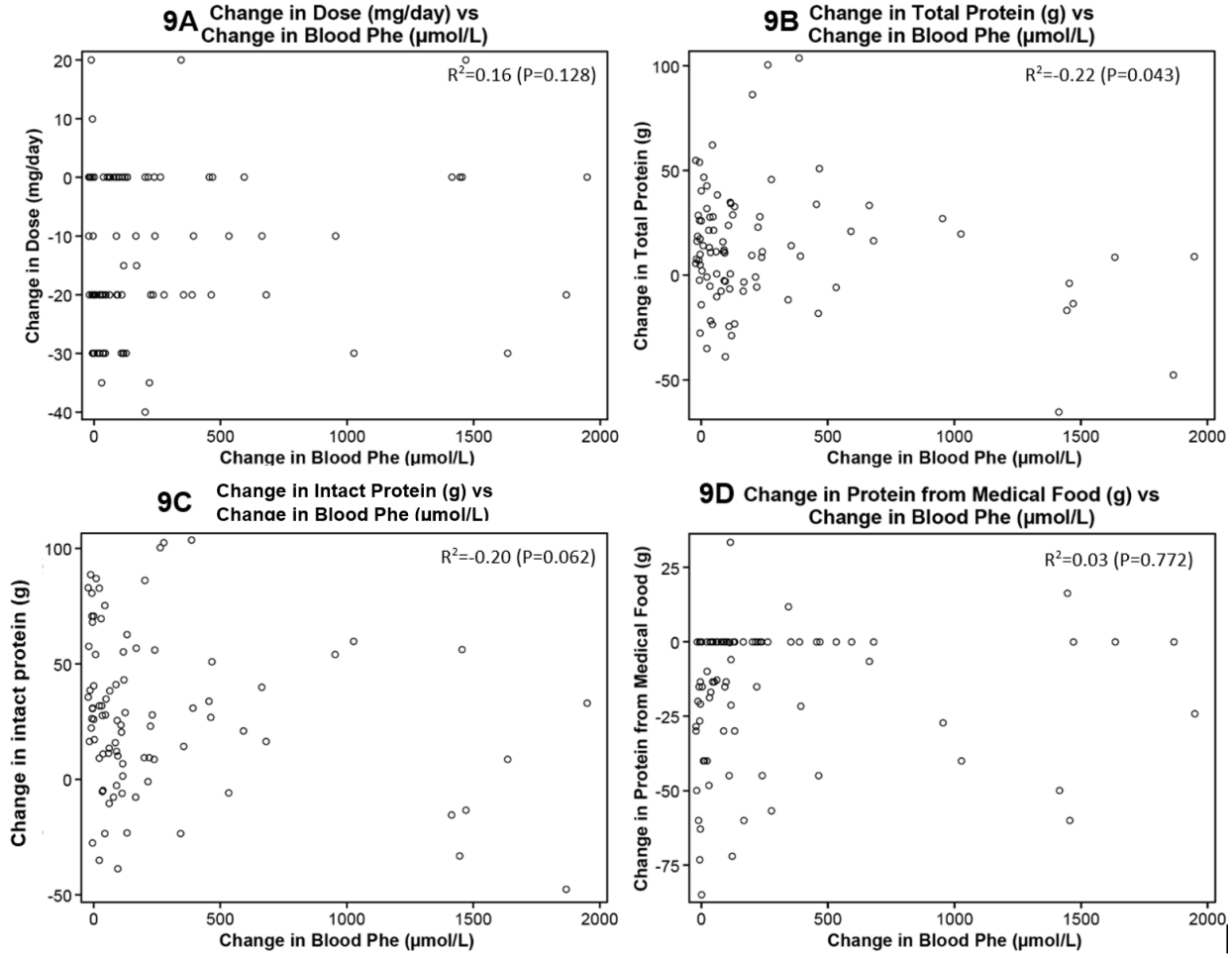


Figure 9A: Correlation between change in Pegvaliase dose (mg/day) and change in blood Phe (µmol/L).
Figure 9B: Correlation between change in total protein intake (g) and change in blood Phe (µmol/L).
Figure 9C: Correlation between change in protein from medical food (g) and change in blood Phe (µmol/L).
Figure 9D: Correlation between change in intact protein (g) and change in blood Phe (µmol/L).

Hypophe is defined as ≥ 2 consecutive blood Phe ≤ 30µmol/L. Change is calculated between end of most recent hyopPhe event (> 30µmol/L) and first day of hypoPhe (blood Phe ≤ 30µmol/L). Protein intakes were calculated as the daily average intake over 3 days. P <0.005 is significant. Data-cut Feb 2018

Table 6: Ancova analysis of participants with resolved hypoPhe only (N=88).

	Stable Dose (N = 29)	**Unstable Dose (N = 59)**
LS mean (SE)	25.30 (7.92)	28.39 (5.04)
95% CI of LS mean	(9.55, 41.06)	(18.37, 38.41)
LS mean difference (SE)		3.09 (10.47)
95% CI of mean difference		(-17.41, 23.71)
p-value		0.769
Stable dose is defined as same dose recorded throughout the duration of hypoPhe event; Only included participants where hypoPhe events resolved. HypoPhe is defined as ≥ 2 consecutive blood Phe ≤ 30µmol/L. Protein intakes were calculated as the daily average intake over 3 days. Data-cut Feb 2018.		

5. DISCUSSION

This secondary dietary data analysis is the first study to extensively evaluate the dietary data collected during the Phase 3 PRISM clinical trial for pegvaliase administration in adults with PKU. An initial limited analysis of the dietary data was published by Thomas et al. earlier this year.[85] Findings from this current study, which analyzed diet data collected through February 2018, corresponds to and supports this published data. Overall, our data verifies that long-term pegvaliase administration in adults with PKU leads to an increased intake of total protein, intact protein, and dietary Phe, while the intake of medical food decreases. Despite this increase in Phe intake, the overall mean blood Phe concentration decreased by 50.25% as the mean decrease from month 0 to month 12 of pegvaliase administration was -631.96 µmol/L.

Several studies have found that adults with PKU often have poor adherence to conventional diet therapy for PKU [64,65,87] resulting in elevation of blood Phe concentrations above the treatment range of 120 to 360 µmol/L. Long-term elevated blood Phe is known to cause neurocognitive difficulties and poor executive functioning in many individuals with PKU[30,87,88]. Self-management in adults with PKU is often compromised by their inability to organize and plan their diet on a daily basis. Hence, a perpetual cycle of poor diet adherence and high blood Phe results in poor quality of life.[89] Another significant factor impacting adherence for many adults is the lack of financial means to obtain coverage for medical foods and modified low protein products. Medical foods can be costly and, in the United States, coverage differs greatly by health insurance plans as well as by each state's coverage legislation.[90]

It is very difficult for those who have discontinued diet treatment to restart medical food and reduce intake of intact protein sources. A 2008 study by Bik-Multanowski et al recruited 53 young adults with PKU, ages 18 to 32 years, to resume the Phe-restricted diet by providing an extensive education and support curriculum. Of the 53 participants, only 11 managed to adhere to the diet for 3 months and only 10 participants were able to complete the entire 9 month study.[89] Poor or suboptimal adherence to the Phe-restricted diet also increases the risk for various nutritional deficiencies.[44,53,91] Additionally, qualitative studies often describe PKU as an "invisible disease" as early-treated adolescents and adults with PKU can appear to function normally in various life situations, but often report feeling isolated or stigmatized in social occasions, especially in the presence of food that is not allowed on their diet.[92-94] Preliminary evidence suggests that with pegvaliase treatment, adults with PKU can achieve improved cognitive function, which in turn may have a domino effect by improving and enhancing overall quality of nutrition, decreasing stigmatization of the disease and improving interactions in social settings.

***Overall effect of pegvaliase*:** Over the course of this study, participants were required to maintain a consistent protein intake with increases in intact protein allowed only when low phe concentrations (<30 umol/L) were measured. After 12 months of pegvaliase administration, the change in median intake of dietary Phe increased by 184 mg/day which was 46% greater than the change in median intake of 85 mg Phe/day during the initial 4 months of the study. This increase in dietary Phe corresponds to the overall change in diet composition with an increase in intact protein sources and a decrease in use of medical foods, although there was great variability in intake trends between

participants. By 12 months of pegvaliase administration, median intake of intact protein increased by 4.0g/day while mean blood Phe decreased from 1217.09 µmol/L to 563.53 µmol/L over this time. Adults enrolled in this clinical trial were not required to be on a Phe restricted diet (defined as consuming >75% of total protein from Phe-free medical food), but at baseline, approximately 60% of the participants reported consuming some form and amount of medical food compared to only 54% of participants reporting consuming medical food after 12 months of pegvaliase administration. Many participants in the phase 3 PRISM trial were able to achieve and maintain a blood Phe concentration within the treatment range of 120 – 360µmol/L and benefited from a liberalized diet and decreased dependence on Phe-free medical food.

***Comparison of protein intake to DRI recommendations*:** 52% of participants at baseline consumed suboptimal total protein, defined as less than 100% of the DRI for protein, or less than 0.8 g protein/kg body weight. However, after one year of pegvaliase administration only 35% of the participants had suboptimal total protein intake. Similarly, 55% of the participants at baseline had suboptimal intake of intact protein, defined as less than 50% of the DRI for protein or 0.4 g/kg, but after 12 months of pegvaliase administration, only 40% of the participants had suboptimal intake of intact protein sources. Since the conventional therapy for PKU includes the restriction of intact protein sources containing high amounts of Phe, this shift in dietary protein sources towards overall increased intake of intact protein and decreased dependency on medical food was demonstrated by a mean increase of 4.2 g total protein/day with reduced incidence of suboptimal intake of both total and intact protein when compared to the DRI.

Hypophenylalaninemia: This study is the first to evaluate protein intake of adults with PKU who experienced hypoPhe with administration of pegvaliase. HypoPhe was an adverse event defined as two consecutive blood Phe measurements less than 30 µmol/L. Approximately 41% (108 of 261 participants) experienced at least one hypoPhe event, lasting an average of 258 days. Evidence from conventional PKU diet therapy indicates that increasing intake of intact protein sources increases blood Phe concentrations. [42,95,96] Yet, observations from the pegvaliase trials finds that that there was not a significant correlation between the change in intake of either intact protein or medical food with blood Phe concentrations.

It is known that low blood Phe concentrations can precipitate severe side effects such as failure to thrive, weight loss, alopecia and prolonged diarrhea in infants.[97,98] Little research is available to document the effects of hypophe in older individuals since long-term exposure to very low concentrations of blood Phe is an unusual occurrence in individuals with PKU treated with conventional diet therapy since routine monitoring allows for increasing intake of intact protein sources when hypoPhe is detected. However, animal models demonstrate that effects of hypoPhe include weight loss, anemia, hypoproteinemia, dermatitis and scruffier coats.[97,99] Exocrine pancreatic atrophy and reduction in thymic mass have also been detected by histology studies of wild-type mice fed a low-Phe diet.[97] However, in the present study, weight loss was not noted and only 3% of the participants (N=285) on pegvaliase suffered from alopecia.

***Study Limitations*:** There were several limitations to this secondary analysis of diet data collected during the PRISM trial. Three-day food records were collected monthly and were used to ensure consistency of protein intake, as well as quantitate total

protein, intact protein, phenylalanine, energy and micronutrient intakes. It is a well-known complication from other nutrition studies that under-reporting food intake is a confounding factor, even with thorough review by trained professionals.[100,101] As the food records reflected only 3 days of every month, there was a risk for "self-monitoring" intake prior to each blood draw since participant's could alter their eating behavior to achieve the study's goals. However, recording diet intake is a routine procedure for individuals with PKU and familiarity with their clinic's dietitian likely reduced this concern. Additionally, to ensure the feasibility of recorded intake, various steps and considerations were taken in the process of data cleaning to ensure robust statistical measures, as detailed in the Methods section.

Another limitation to consider is the heterogeneity in clinical practice as this was a multi-center study. Despite a defined protocol for diet adjustments when hypoPhe was detected, there was possible heterogeneity in the approach that different health care providers took towards participants who experienced hypoPhe (Personal communication, Dr. Cary Harding, OHSU). In hindsight, greater increases in intact protein and decreases in medical food intake may have reduced the length of hypoPhe events. This observation will be employed in future pegvaliase clinical trials (Personal communication, Elaina Jurecki, Biomarin Pharmaceuticals).

***Study Strengths*:** A significant strength of this study is the large sample size (N=261), as most clinical studies are limited by the number of participants, given the relatively small population of early-treated adults with PKU. This large sample size allowed for greater generalizability of our conclusions about diet changes, despite the potential for participants to under-report their intake. All diet records were reviewed with study

participants by metabolic dietitians to detect potential inconsistencies and fill-in missing details. Additionally, to ensure consistent diet record analysis from several sites throughout the length of study, a dietitian was hired by BioMarin Pharmaceutical to enter all food record information and a standardized diet analysis program (Metabolic Pro™) was used to reduce analysis variability and ensure adherence to the study's nutrition protocol.

***Future study*:** Given the significant change in the consumption patterns of total protein, intact protein and medical food sources, there is a need to investigate other nutrition parameters collected during this clinical trial. Evaluation of the quality of the overall diet, type of intact protein sources consumed, micronutrient intake and the need for tyrosine supplementation with long-term pegvaliase administration would be helpful. In addition to collecting dietary intake information, future trials should evaluate biomarkers of protein nutriture and micronutrient status to better evaluate the nutrition status of adults with PKU on pegvaliase treatment. These assessments would be helpful to improve patient care and develop evidence-based guidelines for diet treatment with administration of pegvaliase. Presently, a somewhat more aggressive approach to diet changes has been recommended for those initiating treatment with commercially available pegvaliase (Palynziq).[85] Further investigation of long-term effects of hypoPhe is also warranted to determine if diet and/or dose adjustments can prevent or reduce the incidence of this presumed adverse event.

6. Conclusion

This research aimed to evaluate the changes in dietary composition (total protein, intact protein, medical food protein and dietary Phe) and provided initial insight into the potential of pegvaliase administration to normalize the diet for adults with PKU and help to decrease the burden of treatment of PKU as a disease. However, this study also emphasizes the challenges of preventing hypoPhe and improving dietary selection and nutritional intake. Additionally, this shift toward a more liberalized diet underscores the need to adjust nutrition education provided for adults with PKU treated with pegvaliase.

In conclusion, this study supported the first specific aim and hypothesis that long-term pegvaliase administration is associated with decrease blood Phe concentrations and increased intake of intact protein, total protein and dietary Phe. These changes in diet composition confirmed our hypothesis that the number of participants consuming intact protein above the DRI increased by month 12 of the study. However, this study's findings did not support the hypothesis for our second aim as there was not a significant correlation between increasing intact protein and decreasing medical food intake with blood Phe concentration in those experiencing hypophenylalaninemia.

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8. APPENDIX A

Inclusion Criteria

Individuals eligible to participate in this study met all the following criteria:

- Had completed a prior BMN 165 study (PAL-003 or 165-301) prior to screening
- Had a stable BMN 165 dose regimen for at least 14 days prior to screening
- Were at least 18 years of age and no older than 70 years of age at screening
- Had identified a competent person or persons who were > 18 years of age who would observe the participant during study drug administration and for a minimum of 1 hour following administration
- Was willing and provided written, signed informed consent after the nature of the study has been explained and prior to any research-related procedures
- Was willing and complied with all study procedures
- For females of childbearing potential, a negative pregnancy test at screening and willing to have additional pregnancy tests during the study.
- If sexually active, willing to use two acceptable methods of contraception during and for 4 weeks after the study.
- Had received documented approval from a study dietitian confirming that the participant could maintain their diet in accordance the dietary protocol.
- If applicable, maintained stable dose of medication for ADHD, depression, anxiety, or other psychiatric disorder for ≥8 weeks prior to enrollment and willing to maintain stable dose throughout study unless a change was medically indicated.

- Were in generally good health, as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and ECG tests at screening.

Exclusion Criteria

Individuals who met any of the following exclusion criteria were not eligible to participate in the study:

- Use of any investigational product (except BMN 165) or investigational medical device within 30 days prior to screening or requirement for any investigational agent prior to completion of all scheduled study assessments
- Used of any medication (except BMN 165) intended to treat PKU, including the use of large neutral amino acids, within 2 days prior to the administration of study drug (Day 1, first dose of BMN 165).
- Had known hypersensitivity to Dextran® or components of Dextran.
- Used or planned use of any injectable drugs containing PEG (except for BMN 165), including medroxyprogesterone injection, within 3 months prior to screening and during study participation
- Current use of levodopa
- Tested positive for HIV antibody, hepatitis B surface antigen, or hepatitis C antibody
- A history of organ transplantation or taking chronic immunosuppressive therapy
- A current or history (past 12 months) of substance abuse as defined by the American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders

- Current participation in the Kuvan registry study (PKU Demographics, Outcomes and Safety [PKUDOS]). Patients may discontinue the PKUDOS registry trial to allow enrollment in this study
- Pregnant or breastfeeding at screening or planning to become pregnant (self or partner) or breastfeed at any time during the study
- Concurrent disease or condition that would interfere with study participation or safety (e.g., history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
- Major surgery planned during the study period
- Any condition that, in the view of the investigator, places the participant at high risk of poor treatment compliance or terminating early from the study
- Poor treatment compliance or terminating early from the study
- Alanine aminotransferase (ALT) concentration at least 2 times the upper limit of normal
- Creatinine at least 1.5 times the upper limit of normal

APPENDIX B:

Participant's DRI at baseline (N=249)

At baseline, 44.98% of participants consumed an intact protein intake of ≤ 0.4g/kg of body weight.

Protein Categories (g/kg of body weight per day)	Baseline, N (%) (N=249)
Total Protein Intake	
Protein ≤ 0.4	26 (10.44)
0.4 < Protein ≤ 0.8	72 (38.92)
0.8 < Protein ≤ 1.2	100 (40.16)
1.2 < Protein ≤ 1.6	36 (14.46)
Protein > 1.6	15 (6.02)
Intact Protein Intake	
Protein ≤ 0.4	112 (44.98)
0.4 < Protein ≤ 0.8	86 (34.54)
0.8 < Protein ≤ 1.2	37 (14.86)
1.2 < Protein ≤ 1.6	10 (4.02)
Protein > 1.6	4 (1.61)
Only included participants with available data at baseline (30.5days/month) with baseline BMI. Baseline was defined as any first available data collected for all variables. Participants with multiple protein records as an average. Protein intakes were calculated as the daily average intake over 3 days. DRI for participants with BMI <30 kg/m^2 = protein (g)/ weight at baseline (kg). DRI for participants with 30 ≥ BMI = protein (g)/ adjusted body weight (kg). Data cut Feb 2018.	

APPENDIX C:

Participant's DRI at baseline and month 6 (N=181)

At month 6, majority of the subjects had a total protein intake (medical + intact) between 0.4-0.8 g/kg and intact protein intake ≤0.4 g/kg.

Protein Categories (g/kg of body weight)	Baseline, N (%)	Month 6, N (%)
Total Protein Intake		
Protein ≤ 0.4	23 (12.71)	15 (8.29)
0.4 < Protein ≤ 0.8	68 (37.57)	70 (38.67)
0.8 < Protein ≤ 1.2	61 (33.7)	59 (32.60)
1.2 < Protein ≤ 1.6	19 (10.50)	26 (14.36)
Protein > 1.6	10 (5.52)	11 (6.08)
Intact Protein Intake		
Protein ≤ 0.4	97 (53.59)	74 (40.88)
0.4 < Protein ≤ 0.8	59 (32.60)	70 (38.67)
0.8 < Protein ≤ 1.2	20 (11.05)	29 (16.02)
1.2 < Protein ≤ 1.6	4 (2.21)	7 (3.87)
Protein > 1.6	1 (0.55)	1 (0.55)

Only included participants with available data at baseline and month 6 (30.5days/month) with baseline BMI. Baseline was defined as any first available data collected for all variables. Participants with multiple protein records as an average. Protein intakes were calculated as the daily average intake over 3 days. DRI for participants with BMI <30 kg/m^2 = protein (g)/ weight at baseline (kg). DRI for participants with 30 ≥ BMI = protein (g)/ adjusted body weight (kg). Data cut Feb 2018.

APPENDIX D:

Ancova analysis with blood Phe adjustments

	Stable Dose (N = 29)	Unstable Dose (N = 59)
LS mean (SE)	25.26 (7.82)	28.41 (4.98)
95% CI of LS mean	(9.70, 40.82)	(18.51, 38.31)
LS mean difference (SE)		3.15 (10.34)
95% CI of mean difference		(-17.41, 23.71)
p-value		0.761
Stable dose is defined as same dose recorded throughout the duration of hypoPhe event; Only included participants where hypoPhe events resolved. HypoPhe is defined as ≥2 consecutive blood Phe <30µmol/L. Protein intakes were calculated as the daily average intake over 3 days. Data-cut Feb 2018.		