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Patients with Phenylketonuria Consumed Adequate Amounts of Calcium and Vitamin D

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PATIENTS WITH PHENYLKETONURIA CONSUMED ADEQUATE AMOUNTS OF CALCIUM AND VITAMIN D

by

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DEDICATION

This thesis is dedicated to my parents whom have nurtured, supported, loved, and believed in me. This is also dedicated to my sister and best friend, Norika Kuo. I am also dedicating this thesis to Shahram Jamshidi who always supports and encourages me and makes me realize my potential.

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ABSTRACT

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Objective: To determine if patients with PKU at the mid south clinic are getting the recommended amount of vitamin D and calcium in accordance with the Recommended Dietary Allowance (RDA).

Design: This was a retrospective study using exiting nutrition data which were from using the MetabolicPro software to analyze the 3-day food records collected between June 30, 2010 to February 29, 2012. The means of calcium and vitamin D intake were Calculated and compared with the subject's RDA. Descriptive analysis was used to Determine frequency, percentages, mean, and standard deviation. T-tests were conducted to compare calcium and vitamin D intake to the RDA recommendations based on age.

Subjects: The participants had a diagnosis of PKU, were between 0-21 years old, and had completed the 3-day food record (N=13).

Results: The patients are getting sufficient vitamin D and calcium when compared to the RDA respectively ($p=0.084$ and $p=0.626$).

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CHAPTER I

INTRODUCTION

Phenylketonuria (PKU) is an autosomal recessive inborn error of metabolism (IEM) which is caused by the mutation of phenylalanine hydroxylase (PAH) gene on chromosome 12q23.2 (1). The mutation of the gene causes deficiency of the PAH enzyme in the liver to convert phenylalanine (phe) to tyrosine (tyr) which causes elevated blood phe levels (1). Having untreated high levels of phe in the blood is associated with impairments of brain function, intellectual disability, and severe behavioral and psychiatric disturbances (2, 3).

Currently, neonates in the United States (US) are screened for PKU using a tool called tandem mass spectrometry (MS/MS) to analyze newborns' dried blood spots specimen. Each year, approximately four million neonates in the U.S. undergo newborn screening (4). The neonates who have a positive newborn screening test for PKU are referred to medical professionals to prevent further mental damage.

To avoid being intellectually disability, the diet should be started in the first week of life (5). Current treatment for PKU consists of dietary therapy and restricting natural protein to minimize phe intake (5). In addition to restricting natural protein sources, a special medical supplement is added to the diet to provide sufficient vitamins, minerals, and other essential amino acids while remaining low in phe (5, 6). The dietary treatment should be adhered to for life because elevated phe levels in adulthood are shown to increase depression and attention problems (5, 7).

The low natural protein diet limits the intake of meat, eggs, milk, dairy products, fruits, vegetables, grains and pasta due to the phenylalanine content in these foods (8).

Although the phe-restricted diet combined with medical supplements is effective in preventing intellectual disability, the diet has many burdens (9). The medical supplement is not palatable to many individuals which frequently causes non-compliance (5, 8). There are also social burdens associated with this diet. For example, children with PKU are taught to decline hamburgers and ice cream that may be offered at a friend's house in order to avoid a spike in their serum phe level. Children must be taught to avoid many common foods, such as milk, cheese, and yogurts which are high in calcium (8, 10). Having to avoid calcium rich food and/or being non-compliant can lead to other health risks for these individuals. There are many reports of suboptimal growth and specific nutrient deficiencies, such as calcium, iron, selenium, zinc, vitamin D, and vitamin B12 (5, 11-13). Studies have shown that patients with PKU have lower bone mineral density (BMD) and a greater risk of developing osteoporosis (3).

Approximately 99% of calcium in the human body is distributed in the skeleton, providing support for the body's structure. Absorption of calcium relies on vitamin D-dependent pathway which occurs in the proximal small intestine (14). If calcium intake is low, the body must compensate through hormone regulation to draw the calcium the skeleton, where most of the calcium is stored. The clinical implications of calcium deficiency are rickets, poor bone mass accrual, and poor peak bone mass (14). Since absorption of calcium is reliant on the vitamin D-dependent pathway, low intake of vitamin D negatively affects absorption of calcium in the body.

The aim of this study was to determine if patients with PKU ages between zero to 21 years old in the University of Tennessee Boling Center are getting the recommended daily amount of calcium and vitamin D in their diet.

CHAPTER 2

LITERATURE REVIEW

Inborn Errors of Metabolism (IEM)

The English physician, Sir Archibald Garrod, first introduced the concept of an "Inborn Errors of Metabolism" (IEM) (15) and described IEM as a lifetime disorder. The cause of these disorders is a blockage in the metabolic flow due to an impaired enzyme that creates accumulation in the precursor, and thus deficiency in metabolic products (15). Most inborn errors of metabolism are inherited from both parents through an autosomal, recessive gene; meaning both parents passed the defected gene to their children, even if both parents have no clinical symptoms. If both parents carry the gene, there is a 25% chance of having an affected offspring with each pregnancy (15). Phenylketonuria (PKU) is an IEMs.

Newborn Screening

Robert Guthrie developed the bloodspot screening, a technique to identify PKU in newborns in the early 1960's (16, 17). However, currently the tandem mass spectrometry (MS/MS) is used as a Newborn Screening tool, and has been used since the 1990's. In the United States, approximately 4 million neonates are screened each year (4). All states have screening programs in place to screen all newborn infants within 24-48 hours of life. However, each state has different screening requirements for which disorders must be screened (18, 19). Fortunately, all states must include PKU in their newborn screenings (18, 19).

Phenylketonuria (PKU)

PKU is an autosomal, recessive, inborn error of metabolism characterized by a deficiency in the liver enzyme phenylalanine hydroxylase (PHA) which causes the inability to convert an amino acid, phenylalanine (phe), to another amino acid, tyrosine (tyr) (1). Untreated patients with PKU have elevated phe which causes growth impairment and neurological damage with severe behavioral and psychiatric disturbances (2, 20). Other clinical signs include signs of pigment dilution, skin rash, and seizures (21). In current screening, if the plasma phe levels are > 1200 µmol/L (20mg/dL), it is classified as "Classic PKU". A range between 600-1200 µmol/L (10-20mg/dL) is considered as "Mild PKU", while a range between 120-599 µmol/L (2 < 10 mg/dL) is described as "hyperphenylalaninemias (HPA)". Normal individual usually have plasma phe levels of 48-109µmol/L (0.8–1.8 mg/dL) (21, 22).

Diet for Life

In the 1960s, children with PKU were identified through bloodspot screening, but the nutrition therapy was typically discontinued after childhood (around 6 years old) (7) A few years later, the researchers found that if patients continued the phe-restricted diet longer, they would have a better mental and physical outcome (7), but were still not certain how long the restricted diet should be consumed. Even though there were evidence of better outcome of in continuing the phe-restricted diet after childhood which led to a philosophy of "diet for life", health care professionals were not sure if the prolonged treatment was really necessary after adolescent. The rationale for discontinuing the diet during adolescence was that health care providers believed the brain was fully developed at that age, so the accumulation of serum phe would not cause

harm (7). In one study, fifty seven late-diagnosed patients with PKU from 3 months to 43 years of age (average at age 8) were followed from the Children's Hospital of Los Angeles. Among the 57 patients, 28 patients started on a phe-restricted diet continuously after their late diagnosis. After they continued the low- phe diet, the average IQ went from 44 (moderately retarded) and up to 100 (normal). Of the 28 patients who started the phe-restricted diet, there were 9 employed and 6 in college (23). This study showed that re-starting nutrition therapy to decrease serum phe levels, may improve neurological functioning (24). The nutrition therapy recommendations are to start therapy as early as possible and maintain the blood phe levels within the normal range, in order to demonstrate normal development (24). The National Institutes of Child Health and Human Development re-evaluated the patients in 1998 to assess patients with PKU to determine if their medical, nutritional, psychological, genetic and socio-economic status was adequate or impaired. Through this follow up study, researchers were able to look at the long term effects of discontinuing the phe-restricted diet (7). They found that discontinuing the phe-restricted diet in adolescence/adulthood increases the incidents of anxiety, depression, attention problems, and other issues later in life (5, 9, 25). The current recommendation is that patients with PKU start the diet therapy in the first weeks of life and continue for their lifetime (5).

Nutrition Therapy for PKU

The Nutrition Care Manual from the Academy of Nutrition and Dietetics (AND) recommends specific nutrition therapy for PKU, which includes a semisynthetic, phe-free and tyrosine-supplemented medical supplement/formula (24). In addition, small amounts of natural protein are added to the meal plan. All natural protein contains phenylalanine,

so all high protein foods are excluded from the diet. Meat, milk, cheese, eggs, legumes, and nuts are all excluded from the phe-restricted diet (24). Dietary treatment for PKU can be complicated. Since the body cannot synthesize the phe, an essential amino acid, phe cannot be completely eliminated from the diet. In the meantime, high phe ingestion can cause negative effects on the individuals. The specific range of recommendation daily phenylalanine and tyrosine intake for patients with PKU is listed (Table 1) (21). The recommendation for daily phe intake for patients with PKU is much lower than what non-PKU individuals' usual daily phe intake. For example, patients with PKU age 7 to 11 years old, the recommended phe intake is 220-500mg. Without carefully planning for the food, it is easily to over ingest phe because regular foods contain high amount of phe. For example, a slice of commonly prepared white bread contains 120 mg phe, 1 cup of mashed potato has 174 mg phe, 1 oz cheddar cheese comprised of 372 mg phe, and 2 oz turkey breast contains 447 mg phe (Appendix A).

The Medical Research Council Working Party on Phenylketonuria provides the recommendation specifically for a total amino acid intake; for children under 2 years old is 3g/ kg body weight (BW)/day and for children over 2 years old is 2g/kg BW/day (21). The protein requirements for PKU patients are slightly higher than individuals without PKU. While most common protein foods are high in phe, getting protein through medical formula become crucial for patient with PKU. Unfortunately, the medical formula of synthetic amino acids is not palatable to most people (10), thus the long term phe-restricted diet could cause eating disorders. By using Eating Attitudes Test-26 (EAT-26), the study found 7 out of 30 subjects displayed symptoms of disordered eating (8). Patients with PKU must follow a strict diet for their lifetime, with only small amounts of

meat or milk products. While following this diet, it is possible that they are not getting enough vitamins and minerals to meet the United States Dietary Reference Intake-Recommended Dietary Allowance (RDA) (26).

Table1. Range of Recommended Daily Phenylalanine for patients with PKU (21).

Age	Phe (mg)
0<3 months	130-430
3-6 months	135-400
6-9 months	145-370
9<12 months	135-330
1-4 yrs old	200-320
4-7 yrs old	200-400
7< 11 yrs old	220-500
11<19 yrs old	220-1000
Adult	220-1100

Calcium and Vitamin D Intake

Having to avoid foods with high calcium can lead to other health risks for these individuals; calcium is a crucial component in the diet necessary for healthy bones. Calcium absorption is dependent on vitamin D sufficiency and dietary calcium intake (14). Approximately 99% of calcium in humans is distributed in the bone to support body structure. The remaining 1% is present in the cell, and extracellular fluid. When serum calcium is low in the body, $1,25(OH)_2D$ is up-regulated to increase absorption in the small intestine through increasing Parathyroid hormone (PTH). When serum calcium is high, PTH activity decreases which will increase calcium absorption in to the bone. The serum calcium level has a strong correlation with dietary calcium intake. Therefore, lack of dietary calcium could potentially cause rickets in childhood, inadequate bone mass-for-ages, and osteoporosis (14, 27).

Several studies have shown a defect in bone mineralization in children, adolescents, and adults with PKU (28, 29). A study conducted in Athens, Greece in 1998, found that 22 out of 48 participants showed severe osteopenia (below 2 standard deviations) The researchers suggested that poor peak bone mass is possibly due to the dietary deficiency in protein, calcium and vitamin D. Another study conducted in Israel in 2006 also found patients continuing the diet met RDAs for calcium, while only 3 out of 14 non diet-adherent patients met the RDA for calcium. (27). This non-adherence to the dietary management may have negative consequences to the bone metabolism that increase the risk of failure to thrive in children and poor bone mineralization in adolescents (27).

Dietary Reference Intakes –Recommend Dietary Allowance (RDA) for Calcium and Vitamin D

Currently, there are differences in the calcium requirement for different age groups according to the RDA (14).The RDA for calcium: 0- 6 months old is 200 mg/day, 6-12 months old is 260 mg/day, 1- 3 years old is 700 mg/day, 4-8 years old is 1000 mg/day, 9-13 years old is 1300 mg/day, 14-18 years old is 1300 mg/day, 19-30 years old is 1000 mg/day (30). The RDA for vitamin D for age 0-6 months old is 10 mcg/day, 6-12 months old is 10 mcg/day, and 1-30 years old is 15 mcg/day (30) (see Appendix B).

Besides dietary calcium, many factors influence calcium absorption, such as genetic, age group, physiological state, vitamin D, and the presence of a calcium binder in the diet (phosphate, oxalate, and phytate) (14, 31). Appropriate calcium intake plays a significant role in developing strong bones in children and adolescence. Therefore, this study will focus on dietary intake of calcium and vitamin D on patients with PKU.

CHAPTER 3

METHODOLOGY

Research Design

This was a retrospective cohort study investigating calcium and vitamin D intake in patients with classic PKU at the mid south clinic for inborn errors of metabolism. Since it is a retrospective study, it does not require inform consent from the patients (see Appendix B).

Population

Participants had a diagnosis of PKU, were between 0- 21 years old, and had completed the 3-day food record between June 30, 2010 to February 29, 2012. Thirteen patients with PKU met the criteria and participated in the study.

Instrument

The MetabolicPro is a web-based diet analysis tool, specially designed for metabolic nutritionists. Dietitians use this software to find out the specific nutrient intake the patient is receiving (i.e., number of calories, specific amino acids, vitamins and minerals, etc.) The MetabolicPro used in this study is owned and operated by Genetic Metabolic Dietitian International (32). Its terms and conditions state that the program should only be used for informational purposes. MetabolicPro does not intend to administer professional advice. Healthcare professionals should use proper judgment in assessing the analysis that is provided by the tool. Discretion should also be applied when determining which foods and the amount of foods are most comparable to the patient's 3 day food record. For an example of a MetabolicPro nutrition analysis, refer to Appendix A.

Procedure

The patients with PKU routinely visited the mid south clinic as their regular check up. When the patients visit the clinic, the patients were required to provide a 3-day food record at each visit with the dietitian. The 3-day food record included the amount of food and drink consumed during the 3 days prior to their clinic visit. If 3-day food records are not provided, the dietitians conducted a 24-hour recall with these patients. The data from the 3-day food records and 24-hour recall were then entered and analyzed using MetabolicPro. When the food that patients consumed was not listed in MetabolicPro, dietitians searched online using a variety of websites and made a decision on which source to use. The dietitians reviewed the nutrient intake sheet, which is a nutrient analysis result from MetabolicPro. The frequency of visiting was mainly dependent on the patient's age and serum phe levels. In this clinic, for patients with PKU, the adequate range of phe level is 2-6 mg/dL. If serum phe levels were not within the normal range according to the clinic, patients were be notified to consult with the dietitians. In general, the younger patients saw dietitians more often than the older patients, because maintaining the normal phe-levels is extremely crucial to younger children for normal brain development. In addition, younger children are physically growing rapidly dietitians have to make sure they are receiving adequate amount of protein to ensure optimal growth.

In this study, the author uses the existing data on calcium and vitamin D intake values based on the food records for each patient from their nutrition intake analysis sheet were compared the patients' calcium and vitamin D intake to the RDA recommendations within each appropriate age group.

Analysis

The analysis was conducted with SPSS version16.0 for Mac ("SPSS for Mac, Rel 16.0.1." 2007). Descriptive analysis was used to determine frequencies, percentages, means, and standard deviations. The t-test and compared the relationship between calcium and vitamin D intake compared to RDA recommendations based on age.

CHAPTER IV

RESULTS

Sample Characteristics

The total number of subjects included in the study with complete diet records and complete diet analysis using Metabolic Pro was 13. There were 9 boys and 4 girls in this study. Comparing the actual average intake of vitamin D and calcium versus RDA for each subject's RDA, we found that 9 out of 13 subjects (69%) did not meet the vitamin D requirement and 5 out of 13 (38%) did not meet the calcium intake requirement (Table 2).

In this study, means and standard deviations were calculated for vitamin D and calcium intake (Table 3).

Although calcium and vitamin D requirements are different for various age groups, a dependent t-test was conducted with all participants (Table 4). Separate t-tests were not conducted per age category because the sample size was too small. Results from the overall dependent t-test indicate vitamin D intake ($\bar{x}$ = 11.43; SD= 6.83) was not statistically significantly different from the RDA vitamin D requirement ($\bar{x}$ = 14.62, SD= 1.39) [t (12) = -1.625; p= 0.130]. In addition, calcium intake ($\bar{x}$= 1243.72; SD= 596.36) was not statistically significantly different from the calcium RDA requirement ($\bar{x}$ = 1123.08, SD = 334.55) [t (12) = 0.837; p= 0.419]. This study indicates that patients are getting sufficient vitamin D and calcium when compared to the RDA respectively (p=0.130 and p=0.419).

Table 2. Actual Vitamin D and Calcium Intake comparing to the subject's RDA.

Subjects	Vit D intake (mcg)	Vit D RDA (mcg)	Calcium intake (mg)	Calcium RDA (mg)
1	15	15	1110.33	1000
2	6.9	15	952	1000
3	12.75	15	1518.5	1300
4	13.75	15	1738.25	1300
5	15	15	2135.5	1300
6	0.25	15	276.75	1300
7	13	10	731	200
8	28.43	15	2193.57	1300
9	3.44	15	421.22	700
10	12.13	15	1679.63	1300
11	6.5	15	1000.25	1300
12	10.67	15	1103.83	1300
13	10.83	15	1307.5	1300

Table 3. Means and Standard Deviations for vitamin D intake, vitamin D requirement RDA, calcium intake, and calcium requirement RDA. (n=13)

	$\bar{x}$ (SD)
Vit D intake (mcg)	11.43 (6.83)
Vit D Requirement (mcg)	14.61 (1.39)
Calcium intake (mg)	1243.72 (596.36)
Cal Requirement (mg)	1123.08 (334.55)

Table 4. Dependent t test between Vitamin D verses Vitamin D requirement and between calcium and calcium requirement.

	t	df	sig (2-tailed)
Vit D vs. Vit D req	-1.635	12	.130
Cal vs. Cal req	.837	12	.419

CHAPTER V

DISCUSSION

Currently, the combination of a phe-restricted diet and medical formula has been successful in preventing devastating neurological damage in patients with PKU. However, studies have shown that patients with PKU may have specific nutrient deficiencies, such as calcium, due to difficulty adhering to dietary treatment (5).

To the best of the author's knowledge, this is the first study assessing the calcium and vitamin D intake among patients with PKU at the mid south clinic. Though some studies show the nutrient inadequacy among patients with PKU, the results from this study indicate that patients with PKU at the mid south clinic are getting the recommended amount (RDA) of calcium and vitamin D based on age. Acosta et al have been shown that the mean calcium and vitamin D met recommended amount (33), however in Acosta's study, the subjects were less than three month of age. Our subjects were wilder age range, between 0- 21 years old.

There are several factors that may explain how patients with PKU at the mid south clinic are getting enough calcium and vitamin D. First, all patients have access to the medical formula, covered by private insurance, government healthcare (Medicaid), or non-profit organization such as NORD (National Organization for Rare Disorder). Even though paying co-pay for the medical formula can add up as a significant amount of expense over time; according to the 3-day record, participants were either be able to afford the co-pay or receiving medical formula which is donated from non-profit organization. This privilege allows patients to be able to meet their vitamin and mineral's need through being compliant to their formula intake. Second, patients may be taking

vitamin and mineral supplements, despites not taking the recommended amount of medical formula. However, author rechecked the food record; no patient was taking vitamin and mineral supplements during the time of the study. Third, patients may non-compliant in lowering natural protein intake. If they are not restricting their natural protein, they may reach the calcium and vitamin D RDA despites not taking the medical formula. It is extremely dangerous to their brain development because of the high phe content in natural protein. Fortunately, eleven out of thirteen patients are taking assigned medical formula; taking more than 80% of the formula, and following the low natural protein restricted diet greater than 80% of the time. The other two patients are not as compliant as the rest of the group. One was being compliant (taking ≥ 80% of the formula) only 66% of the time; another did not take the formula almost always based on the record. Both individuals are teenagers. One of the main reasons the two teenagers choose being non-compliant most likely due to individual does not experience immediate clinical symptom after not ingesting all prescribed medical formula/food daily. The progress of the brain damage is slow and most of the time the individual is unaware of damages. Due to no immediate consequence related to non-compliance, some individuals may lose motivation in following the diet and taking adequate amount of medical formula. A study has shown that in adolescents, the non-compliant to the diet increased a risk in reducing bone mineralization (27).

Some limitations of this study are its small sample size (n=13), the self-administered 3-day food record, and limited nutrition information from MetabolicPro. Due to the small sample size, the average nutrient intake can be skewed by extreme nutrient intake values. Some patients may not accurately record their 3-day food record,

potentially overestimating the amount of medical formula they have been taking. Patients could have changed their diet habits to be more compliant the 3 days prior to the clinic visit since they knew their diet would be analyzed. This concern can be solved if the study compare food intake to actual serum phe, calcium and vitamin D level. However, due to financial constrain and beyond the scope of practice, serum calcium and vitamin D tests were not performed. Another potential limitation is MetabolicPro does not contain all the foods that patients consumed. The dietitians had to use the best of their knowledge to estimate their nutrient intake by gathering the data online, therefore these data may not express the most accurate nutrition information. However, this should not be the main concern since most of the foods are found in the MetabolicPro System.

Additional research is needed with a larger number of subjects to assess the adequacy of nutrient intake by using separate t-tests for each age group, and possibly comparing nutrition analysis with the subjects' serum calcium and vitamin D levels.

After the Institute of Medicine's report, Dietary Reference Intake for calcium and vitamin D was released in November 2010; the recommendation for vitamin D stays controversial. Because some studies show that a vitamin D intake greater than the recommendations may be associated with better health (34). In addition, following the RDA may not raised the serum 25-hydroxyvitamin D (form of vitamin D) to the optimal level (at least 30 ng/mL). For future research, identifying the specific vitamin D recommendation for patients with PKU can be beneficial.

REFERENCES

1. Robin A Williams, Cyril DS Mamotte, John R Burnett. Phenylketonuria: An inborn error of phenylalanine metabolism. *Clin Biochem*. 2008;29(1):31-41. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2423317/.

2. Burton B.K LL. Reaching out to the lost generation of adults with early-treated phenylketonuria. *Molecular Genetics and Metabolism*. 2010:146-148.

3. Koura HM, Abdallah Ismail N, Kamel AF, Ahmed AM, Saad-Hussein A, Effat LK. A long-term study of bone mineral density in patients with phenylketonuria under diet therapy. *Arch Med Sci*. 2011;7(3):493-500.

4. Centers for Disease Control and Prevention. Using Tandem Mass Spectrometry for Metabolic Disease Screening Among Newborns. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5003a1.htm. Accessed 04/13, 2001.

5. Belanger-Quintana A, Burlina A, Harding CO, Muntau AC. Up to date knowledge on different treatment strategies for phenylketonuria. *Mol Genet Metab*. 2011;104 Suppl:S19-25.

6. Cunniff C, Frias J, Kaye C, Moeschler J, Panny S, Trotter T. Maternal phenylketonuria. *American Academy of Pediatrics*. 2001;107:427.

7. Koch R, Burton B, Hoganson G, Peterson R, Rhead W, Rouse B, Scott R, Wolff J, Stern AM, Guttler F, Nelson M, de la Cruz F, Coldwell J, Erbe R, Geraghty MT, Shear

C, Thomas J, Azen C. Phenylketonuria in adulthood: A collaborative study. *J Inherit Metab*. 2002;25:333-346.

8. Antisdel JE CJ. Comparison of eating attitudes and behaviors among adolescent and young women with type 1 diabetes mellitus and phenylketonuria. *Developmental and Behavioral Pediatrics*. 2000;21:81-87.

9. Enns GM, Koch R, Brumm V, Blakely E, Suter R, Jurecki E. Suboptimal outcomes in patients with PKU treated early with diet alone: Revisiting the evidence. *Mol Genet Metab*. 2010;101(2-3):99-109.

10. Burke W, Atkins D, Gwinn M, Guttmacher A, Haddow J, Lau J, Palomaki G, Press N, Richards C S, Wideroff L, Wiesner G. Genetic test evaluation: Information needs of clinicians, policy makers, and the public. *American Journal of Epidermiology*. 2002;156:311-318.

11. Acosta PB, Yannicelli S, Singh R, Mofidi S, Steiner R, DeVincentis E, Jurecki E, Bernstein L, Gleason S, Chetty M, Rouse B. Nutrient intakes and physical growth of children with phenylketonuria undergoing nutrition therapy. *J Am Diet Assoc*. 2003;103(9):1167-1173.

12. Arnold GL, Vladutiu CJ, Kirby RS, Blakely EM, Deluca JM. Protein insufficiency and linear growth restriction in phenylketonuria. *J Pediatr*. 2002;141(2):243-246.

13. Dobbelaere D, Michaud L, Debrabander A, Vanderbecken S, Gottrand F, Turck D, Farriaux JP. Evaluation of nutritional status and pathophysiology of growth retardation in patients with phenylketonuria. *J Inherit Metab Dis*. 2003;26(1):1-11.

14. Bhatia V. Dietary calcium intake - a critical reappraisal. *Indian J Med Res*. 2008;127(3):269-273.

15. Nelms M., Sucher K, Long S. Chapter 28 metabolic disorder. In: *Nutrition Therapy and Pathophysiology*. 2nd ed. Belmont, CA: Thomson; 2007:881-914.

16. Krasnopol'skaia KD, Vestinetskaia LI, Lebedev BV. The guthrie test for the determination of phenylalanine in blood. . 1971;11:687-9.

17. Downing M, Pollitt R. Newborn bloodspot screening in the UK--past, present and future. *Ann Clin Biochem*. 2008;45(Pt 1):11-17.

18. National PKU Alliance. PKU Facts. Available at: http://www.npkua.org/index.php/pku-facts2011.

19. genes-r-us. National Newborn & Genetic Resource Center. Available at: http://genes-r-us.uthscsa.edu/%3Cfront%3E2012.

20. van Calcar SC, MacLeod EL, Gleason ST, Etzel MR, Clayton MK, Wolff JA, Ney DM. Improved nutritional management of phenylketonuria by using a diet containing glycomacropeptide compared with amino acids. *Am J Clin Nutr*. 2009;89(4):1068-1077.

21. Acosta P. *Nutrition Management of Patients with Inherited Metabolic Disorder*. Sudbury MA: Jones and Barlett Publishers; 2010.

22. Duh S, Cook J. Laboratory Reference Range Values.

23. Koch R, Moseley K, Ning J, Romstad A, Guldberg P, Guttler F. Long-term beneficial effects of the phenylalanine-restricted diet in late-diagnosed individuals with phenylketonuria. *Mol Genet Metab*. 1999;67(2):148-155.

24. Academy of Nutrition and Dietetic. Nutrition Care Manual - PKU. Available at: http://nutritioncaremanual.org/content.cfm?ncm_content_id=79855. Accessed 10/12, 2011.

25. Fisch RO, Chang PN, Weisberg S, Guldberg P, Guttler F, Tsai MY. Phenylketonuric patients decades after diet. *J Inherit Metab Dis*. 1995;18(3):347-353.

26. Schulz B, Bremer HJ. Nutrient intake and food consumption of adolescents and young adults with phenylketonuria. *Acta Paediatr*. 1995;84(7):743-748.

27. Mendes AB, Martins FF, Cruz WM, da Silva LE, Abadesso CB, Boaventura GT. Bone development in children and adolescents with PKU. *J Inherit Metab Dis*. 2012;35(3):425-430.

28. Barat P, Barthe N, Redonnet-Vernhet I, Parrot F. The impact of the control of serum phenylalanine levels on osteopenia in patients with phenylketonuria. *Eur J Pediatr*. 2002;161(12):687-688.

29. Al-Qadreh A, Schulpis KH, Athanasopoulou H, Mengreli C, Skarpalezou A, Voskaki I. Bone mineral status in children with phenylketonuria under treatment. *Acta Paediatr*. 1998;87(11):1162-1166.

30. Institute of Medicine. Dietary Reference Intakes for Calcium and Vitamin D. Available at: http://www.iom.edu/~/media/Files/Report%20Files/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/Vitamin%20D%20and%20Calcium%202010%20Report%20Brief.pdf. Accessed 11, 2010.

31. Modan-Moses , Vered I, Schwartz G, Anikster Y, Abraham S, SegevR, Efrati O. Peak bone mass in patients with phenylketonuria. *J Inherit Metab Dis*. 2007;30(2):202-8.

32. Genetic Metabolic Dietitian International. Terms of Use. Available at: https://metabolicpro.org/registration.php#2012.

33. Acosta PB, Wenz E, Williamson M. Nutrient intake of treated infants with phenylketonuria. *Am J Clin Nutr*. 1977;30(2):198-208.

34. Bischoff-Ferrari HA. Optimal serum 25-hydroxyvitamin D levels for multiple health outcomes. *Adv Exp Med Biol*. 2008;624:55-71.

APPENDIX A

METABOLICPRO NUTRITION ANALYSIS

Metabolic 10

Summary Report

Day average

Macronutients			
Nutrient	Amount	Units	%DRI
Energy	2375.4	kcal	78
Protein	96.4	g	185
% Energy as Protein	16	%	
Fat*	66.5	g	
% Energy as Fat	25	%	
Carbohydrate*	368.4	g	283
% Energy as CHO	62	%	
Dietary Fiber *	13.1	g	34
Cholesterol*	4.1	mg	

Fatty Acids			
Nutrient	Amount	Units	%DRI
Linoleic Acid*	1.99	g	12
ALPHA Linolenic Acid*	0.21	g	13
Docosahexaenoic Acid*	0.00	g	

Vitamins			
Nutrient	Amount	Units	%DRI
Vitamin A(RAE)*	795.5	mcg	88
Vitamin A*	2890.0	IU	
Vitamin C*	64	mg	85
Vitamin D*	7	mcg	47
Vitamin D*	278.2	IU	
Vitamin E(alpha-TE)*	13.6	mg	91
Vitamin K*	77	mcg	103
Thiamin*	3.16	mg	263
Riboflavin*	1.88	mg	145
Niacin*	17.1	mg	107
Vitamin B6*	1.45	mg	112
Folate *	466	mcg	117
Dietary Folate Equivalents*	65	mcg	
Vitamin B12*	5.0	mcg	208
Pantothenic Acid*	7.69	mg	154
Biotin*	90	mcg	300
Choline*	103	mg	19

Amino Acids			
Nutrient	Amount	Units	%DRI
Isoleucine	4.704	g	
Leucine	9.062	g	
Lysine	7.815	g	
Methionine	2.293	g	
Phenylalanine	0.272	g	
Threonine*	4.354	g	
Tryptophan*	1.276	g	
Valine	6.733	g	
Alanine*	7.827	g	
Arginine*	7.129	g	
Aspartic Acid*	0.582	g	
Cystine*	1.152	g	
Glycine*	5.650	g	
Glutamic acid*	1.030	g	
Histidine*	2.403	g	
Proline*	6.525	g	
Tyrosine	8.671	g	
Serine*	7.726	g	

Minerals			
Nutrient	Amount	Units	%DRI
Calcium*	1521	mg	117
Copper*	1.1	mg	124
Iron*	14.8	mg	133
Magnesium*	327	mg	80
Manganese*	0.97	mg	44
Molybdenum*	45	mcg	105
Phosphorus*	1122	mg	90
Selenium*	40.7	mcg	74
Potassium*	1690	mg	36
Zinc*	12	mg	109
Sodium*	4418	mg	295
Iodine*	171	mcg	114

* indicates some of the foods have incomplete nutrient data, but an approximate value has been noted in summary report
"-" indicates a missing or incomplete value

Food Contribution Report

Day

Day	Food Name	Units	Quantity	Energy (kcal)	Protein (g)	Phenylalanine (g)	Tyrosine (g)
4	CHEDDAR CHEESE	1.00 oz	1.00	114.25	7.06	0.372	0.341
4	MASHED POTATOES,HOME-PREPARED,WHL MILK & BUTTER ADDED	1.00 cup	1.00	237.30	3.91	0.174	0.155
4	TURKEY BREAST MEAT	1.00 serving	2.00	68.64	11.27	0.447	0.446
4	WHITE BREAD,COMMLY PREP,TOASTED	1.00 slice, large	1.00	79.11	2.43	0.120	0.070
	Total			499.3	24.67	1.113	1.012

APPENDIX B

DIETARY REFERENCE INTAKE-RECOMMENDED DIETARY ALLOWANCE (RDA)

Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Vitamins

Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Vitamin A (µg/d)[a]	Vitamin C (mg/d)	Vitamin D (µg/d)[b,c]	Vitamin E (mg/d)[d]	Vitamin K (µg/d)	Thiamin (mg/d)	Riboflavin (mg/d)	Niacin (mg/d)[e]	Vitamin B_6 (mg/d)	Folate (µg/d)[f]	Vitamin B_{12} (µg/d)	Pantothenic Acid (mg/d)	Biotin (µg/d)	Choline (mg/d)[g]
Infants														
0 to 6 mo	400*	40*	10	4*	2.0*	0.2*	0.3*	2*	0.1*	65*	0.4*	1.7*	5*	125*
6 to 12 mo	500*	50*	10	5*	2.5*	0.3*	0.4*	4*	0.3*	80*	0.5*	1.8*	6*	150*
Children														
1–3 y	300	15	15	6	30*	0.5	0.5	6	0.5	150	0.9	2*	8*	200*
4–8 y	400	25	15	7	55*	0.6	0.6	8	0.6	200	1.2	3*	12*	250*
Males														
9–13 y	600	45	15	11	60*	0.9	0.9	12	1.0	300	1.8	4*	20*	375*
14–18 y	900	75	15	15	75*	1.2	1.3	16	1.3	400	2.4	5*	25*	550*
19–30 y	900	90	15	15	120*	1.2	1.3	16	1.3	400	2.4	5*	30*	550*
31–50 y	900	90	15	15	120*	1.2	1.3	16	1.3	400	2.4	5*	30*	550*
51–70 y	900	90	15	15	120*	1.2	1.3	16	1.7	400	2.4[h]	5*	30*	550*
> 70 y	900	90	20	15	120*	1.2	1.3	16	1.7	400	2.4[h]	5*	30*	550*
Females														
9–13 y	600	45	15	11	60*	0.9	0.9	12	1.0	300	1.8	4*	20*	375*
14–18 y	700	65	15	15	75*	1.0	1.0	14	1.2	400[i]	2.4	5*	25*	400*
19–30 y	700	75	15	15	90*	1.1	1.1	14	1.3	400[i]	2.4	5*	30*	425*
31–50 y	700	75	15	15	90*	1.1	1.1	14	1.3	400[i]	2.4	5*	30*	425*
51–70 y	700	75	15	15	90*	1.1	1.1	14	1.5	400	2.4[h]	5*	30*	425*
> 70 y	700	75	20	15	90*	1.1	1.1	14	1.5	400	2.4[h]	5*	30*	425*
Pregnancy														
14–18 y	750	80	15	15	75*	1.4	1.4	18	1.9	600[i]	2.6	6*	30*	450*
19–30 y	770	85	15	15	90*	1.4	1.4	18	1.9	600[i]	2.6	6*	30*	450*
31–50 y	770	85	15	15	90*	1.4	1.4	18	1.9	600[i]	2.6	6*	30*	450*
Lactation														
14–18 y	1,200	115	15	19	75*	1.4	1.6	17	2.0	500	2.8	7*	35*	550*
19–30 y	1,300	120	15	19	90*	1.4	1.6	17	2.0	500	2.8	7*	35*	550*
31–50 y	1,300	120	15	19	90*	1.4	1.6	17	2.0	500	2.8	7*	35*	550*

NOTE: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level; sufficient to meet the nutrient requirements of nearly all (97-98 percent) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake.

[a] As retinol activity equivalents (RAEs). 1 RAE = 1 µg retinol, 12 µg β-carotene, 24 µg α-carotene, or 24 µg β-cryptoxanthin. The RAE for dietary provitamin A carotenoids is two-fold greater than retinol equivalents (RE), whereas the RAE for preformed vitamin A is the same as RE.

[b] As cholecalciferol. 1 µg cholecalciferol = 40 IU vitamin D.

[c] Under the assumption of minimal sunlight.

[d] As α-tocopherol. α-Tocopherol includes *RRR*-α-tocopherol, the only form of α-tocopherol that occurs naturally in foods, and the *2R*-stereoisomeric forms of α-tocopherol (*RRR*-, *RSR*-, *RRS*-, and *RSS*-α-tocopherol) that occur in fortified foods and supplements. It does not include the *2S*-stereoisomeric forms of α-tocopherol (*SRR*-, *SSR*-, *SRS*-, and *SSS*-α-tocopherol), also found in fortified foods and supplements.

[e] As niacin equivalents (*NE*). 1 mg of niacin = 60 mg of tryptophan; 0–6 months = preformed niacin (not NE).

[f] As dietary folate equivalents (DFE). 1 DFE = 1 µg food folate = 0.6 µg of folic acid from fortified food or as a supplement consumed with food = 0.5 µg of a supplement taken on an empty stomach.

[g] Although AIs have been set for choline, there are few data to assess whether a dietary supply of choline is needed at all stages of the life cycle, and it may be that the choline requirement can be met by endogenous synthesis at some of these stages.

[h] Because 10 to 30 percent of older people may malabsorb food-bound B_{12}, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with B_{12} or a supplement containing B_{12}.

[i] In view of evidence linking folate intake with neural tube defects in the fetus, it is recommended that all women capable of becoming pregnant consume 400 µg from supplements or fortified foods in addition to intake of food folate from a varied diet.

Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements
Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Calcium (mg/d)	Chromium (µg/d)	Copper (µg/d)	Fluoride (mg/d)	Iodine (µg/d)	Iron (mg/d)	Magnesium (mg/d)	Manganese (mg/d)	Molybdenum (µg/d)	Phosphorus (mg/d)	Selenium (µg/d)	Zinc (mg/d)	Potassium (g/d)	Sodium (g/d)	Chloride (g/d)
Infants															
0 to 6 mo	200*	0.2*	200*	0.01*	110*	0.27*	30*	0.003*	2*	100*	15*	2*	0.4*	0.12*	0.18*
6 to 12 mo	260*	5.5*	220*	0.5*	130*	11	75*	0.6*	3*	275*	20*	3	0.7*	0.37*	0.57*
Children															
1–3 y	700	11*	340	0.7*	90	7	80	1.2*	17	460	20	3	3.0*	1.0*	1.5*
4–8 y	1,000	15*	440	1*	90	10	130	1.5*	22	500	30	5	3.8*	1.2*	1.9*
Males															
9–13 y	1,300	25*	700	2*	120	8	240	1.9*	34	1,250	40	8	4.5*	1.5*	2.3*
14–18 y	1,300	35*	890	3*	150	11	410	2.2*	43	1,250	55	11	4.7*	1.5*	2.3*
19–30 y	1,000	35*	900	4*	150	8	400	2.3*	45	700	55	11	4.7*	1.5*	2.3*
31–50 y	1,000	35*	900	4*	150	8	420	2.3*	45	700	55	11	4.7*	1.5*	2.3*
51–70 y	1,000	30*	900	4*	150	8	420	2.3*	45	700	55	11	4.7*	1.3*	2.0*
> 70 y	1,200	30*	900	4*	150	8	420	2.3*	45	700	55	11	4.7*	1.2*	1.8*
Females															
9–13 y	1,300	21*	700	2*	120	8	240	1.6*	34	1,250	40	8	4.5*	1.5*	2.3*
14–18 y	1,300	24*	890	3*	150	15	360	1.6*	43	1,250	55	9	4.7*	1.5*	2.3*
19–30 y	1,000	25*	900	3*	150	18	310	1.8*	45	700	55	8	4.7*	1.5*	2.3*
31–50 y	1,000	25*	900	3*	150	18	320	1.8*	45	700	55	8	4.7*	1.5*	2.3*
51–70 y	1,200	20*	900	3*	150	8	320	1.8*	45	700	55	8	4.7*	1.3*	2.0*
> 70 y	1,200	20*	900	3*	150	8	320	1.8*	45	700	55	8	4.7*	1.2*	1.8*
Pregnancy															
14–18 y	1,300	29*	1,000	3*	220	27	400	2.0*	50	1,250	60	12	4.7*	1.5*	2.3*
19–30 y	1,000	30*	1,000	3*	220	27	350	2.0*	50	700	60	11	4.7*	1.5*	2.3*
31–50 y	1,000	30*	1,000	3*	220	27	360	2.0*	50	700	60	11	4.7*	1.5*	2.3*
Lactation															
14–18 y	1,300	44*	1,300	3*	290	10	360	2.6*	50	1,250	70	13	5.1*	1.5*	2.3*
19–30 y	1,000	45*	1,300	3*	290	9	310	2.6*	50	700	70	12	5.1*	1.5*	2.3*
31–50 y	1,000	45*	1,300	3*	290	9	320	2.6*	50	700	70	12	5.1*	1.5*	2.3*

NOTE: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97-98 percent) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); and *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate* (2005); and *Dietary Reference Intakes for Calcium and Vitamin D* (2011). These reports may be accessed via www.nap.edu.

APPENDIX C

INSTITUTIONAL REVIEW BOARD APPROVAL

THE UNIVERSITY OF MEMPHIS

Institutional Review Board

To:	Chien-Yung Kuo
From:	Chair or Designee, Institutional Review Board For the Protection of Human Subjects irb@memphis.edu
Subject:	ADEQUACY OF CALCIUM AND VITAMIN D INTAKE IN PATIENTS WITH PHENYLKETONURIA (#2217)
Approval Date:	June 6,2012

This is to notify you that the Institutional Review Board has designated the above referenced protocol as exempt from the full federal regulations under category 4. This project was reviewed in accordance with all applicable statuses and regulations as well as ethical principles.

When the project is finished or terminated, please submit a Human Subjects Research Completion Form (COMP) to the Board via e-mail at irbforms@memphis.edu. This form can be obtained on our website at http://www.memphis.edu/irb/forms.php.

Approval for this protocol does not expire. However, any change to the protocol must be reviewed and approved by the board prior to implementing the change.

Chair or Designee, Institutional Review Board
The University of Memphis

Cc: Dr. Margaret Williams

THE UNIVERSITY OF TENNESSEE
Health Science Center

Institutional Review Board
910 Madison Avenue, Suite 600
Memphis, TN 38163
Tel: (901) 448-4824

May 01, 2012

CHELSEA KUO
UTHSC - COM - Boling Center for Developmental Disabilities

Re: 12-01854-XM
Study Title: ADEQUACY OF CALCIUM AND VITAMIN D INTAKE IN PATIENTS WITH PHENYLKETONURIA

Dear Ms. Kuo,

The Administrative Section of the UTHSC Institutional Review Board (IRB) has received your written acceptance of and/or response dated May 1, 2012 to the provisos outlined in our correspondence of April 16, 2012 concerning the application for the above referenced project. The IRB determined that your application is eligible for **exempt** review under 45CFR46.101(b)(4) in that it involves the study of existing data or other materials that are publicly available or the information will be recorded in a way that subjects cannot be individually identified. Informed consent is waived in accord with 45CFR46.116 (d). Your application has been determined to comply with proper consideration for the rights and welfare of human subjects and the regulatory requirements for the protection of human subjects. Therefore, this letter constitutes full approval of your application (version 1.2) for the above referenced study.

This study may not be initiated until you receive approval from the institution(s) where the research is being conducted.

In addition, the request for waiver of HIPAA authorization for the conduct of the study itself is approved. The waiver applies to the medical records of patients with PKU who received care at the University of Tennessee Boling Center from June 30, 2012 and February 29, 2012.

In the event that volunteers are to be recruited using solicitation materials, such as brochures, posters, web-based advertisements, etc., these materials must receive prior approval of the IRB.

Any alterations (**revisions**) in the protocol must be promptly submitted to and approved by the UTHSC Institutional Review Board prior to implementation of these revisions. You have individual responsibility for reporting to the Board in the event of unanticipated or serious adverse events and subject deaths.

Sincerely,

Signature applied by Donna L Stallings on 05/01/2012 10:58:21 AM CDT

Signature applied by Terrence F Ackerman on 05/01/2012 11:20:33 AM CDT

Donna Stallings, CIM
IRB Administrator
UTHSC IRB

Terrence F. Ackerman, Ph.D.
Chairman
UTHSC IRB