Mediterranean Journal of Nutrition and Metabolism 7 (2014) 95–106
DOI:10.3233/MNM-140010
IOS Press

Study on the prevalence of phenylketonuria in Jordan and assessment of follow-up efforts and dietary management of patients with this disease

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Abstract. The objective of this research was to collect data on the prevalence of phenylketonuria in Jordan, to assess the nutritional status and physical growth of a sample of PKU patients in Jordan using anthropometric measurements and evaluation of the adequacy of their diets. Twenty five patients were divided, according to their age, into four groups: 4–6 months age ($n = 6$), 8–12 months age ($n = 5$), 2 years old ($n = 7$) and 4 years old ($n = 7$). Height, weight, triceps and subscapular skinfold thicknesses were measured. Dietary intake was taken using three-day food records, and blood phenylalanine (Phe) concentration was determined. The results indicated that 48% of the whole sample had poor physical growth; about (66%) of patients in group 1, (40%) of patients in group 2, (28%) of patients in group 3 and (57%) of patients in group 4 were underweight. Significant positive correlations ($p < 0.05$) were found between protein intake and each of head circumference ($r = 0.434$) weight (0.420) and height ($r = 0.502$) of patients. The protein intakes were 77% and 88% of the recommended levels for groups 1 and 2 respectively. Other low dietary intakes included energy (in groups 1 and 4), fat (in groups 1 and 3) and selenium (in group 1). All patients had controlled blood Phe levels except those in group 4 (4 years age) who had a concentration of 358 μmol/L. It is concluded that there is need for counseling the community about the relationship between PKU and consanguineous marriages and for stressing the importance of monitoring the dietary management of PKU patients.

Keywords: Phenylketonuria, PKU, Jordan, dietary management

1. Introduction

Phenylketonuria (PKU) is an inborn error of amino acid (AA) metabolism caused by deficiency or inactivity of phenylalanine hydroxylase enzyme (PAH), which is needed to convert the essential amino acid Phe to tyrosine [1]. This disease was first described as an inherited metabolic disorder characterized by severe intellectual impairment, motor problems and skin abnormalities in 1934 by the Norwegian biochemist and physician Asbjorn Folling [2]. If PKU is left untreated, toxic concentrations of Phe are rapidly accumulated in the blood, leading to severe brain damage and microcephaly [3]. The majority of PKU cases are now identified via neonatal screening program, allowing timely intervention to avoid severe consequences. Nutritional follow-up is needed to maintain Phe within safe levels to ensure adequate neurological and physical development by avoiding the lack of any essential nutrient due to a deprived diet [4].

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ISSN 1973-798X/14/$27.50

Early diagnosis and prompt intervention have allowed most individuals with PKU to avoid severe mental disability [5]. Dietary restriction of Phe remains the mainstay of treatment but PKU is an active area of research and new treatment options are emerging that might reduce the burden of the difficult and restrictive diet on patients and their families [6].

The low-Phe diet restricts the intake of high-protein foods, thus the nutritional requirements of protein and Phe must be obtained from Phe-free amino acid supplements and special or natural foods that are low in Phe [7]. To maintain a diet low in Phe, patients must eliminate from their diet foods that are high in protein, such as meat, poultry, fish, milk, nuts and eggs. Small portions of foods containing moderate amounts of protein, including fruits, vegetables, grains and potatoes are thus recommended [8].

Studies on the prevalence of PKU in Jordan are scanty. The Ministry of Health in Jordan has started screening for PKU in all of the newborns and providing care for detected cases since 2010; parents are guided to the use of the right diet and are provided by low phenylalanine flour and other low phe food products [9]. The aims of the present work were to collect data on the prevalence of phenylketonuria in Jordan and to assess the nutritional status and physical growth and dietary intake of a sample of PKU patients in Jordan.

2. Methods

This study was approved by the Research Ethics Committee at Prince Hamza Hospital to which Al-Shamel Health Center is affiliated. A written consent was obtained from the parents/guardians of the children included in the study. Data on the prevalence of Phenylketonuria were obtained from the records of the Genetic Department of the Jordanian Ministry of Health

2.1. Subjects

Twenty-five patients aged from 4 months to 4 years (15 boys and 10 girls) with classical PKU were included in the study. They were all recruited from Al-Shamel Health Center in Jabal Hussein in Amman, which is the sole center in Jordan that provides care for PKU patients. Patients were divided into the following groups: group 1 (4–6 months age) ($n = 6$), group 2 (8–12 months age) ($n = 5$), group 3 (two years age) ($n = 7$) and group 4 (four years age) ($n = 7$). The patients' parents received a questionnaire, which included questions about patient date of birth, gender, family size, education, the severity of the disease, dietary compliance, formula used, family history of PKU, consanguineous marriage, age of diagnosis, IQ, and the medical history of patients. All participants had been diagnosed by newborn screening and started dietary treatment early in life and were on a special PKU low protein diet as guided and monitored by the health care center.

2.2. Anthropometric measurements

Weight and height measurements were taken for PKU children. All children were weighed to the nearest 0.1 kg using adigital scale (B.A. Wang DT 150) while wearing light clothes. Recumbent length was measured in children younger than three years of age using a measuring board, whereas for children older than three years of age, height was measured using a stadiometer (Seca 220, USA). Standing height was obtained with bare feet flat on the floor of the stadiometer and heels against the backboard. Length and height were measured to the nearest 0.1 cm [10]. Then the body mass index (BMI) was calculated [11] for each patient from these data according to the formula:

$$\text{BMI} = \text{weight (kg)}/\text{height}^2\ (\text{m}^2)$$

Measurements were compared with WHO growth standards [12] for girls and boys aged from birth to 5 years old. Body composition expressed as Fat% and FFM% was also calculated according to skinfold-thickness equations for infants and children [13]:

For males: $\%\text{Fat} = 1.21\ (\text{triceps} + \text{subscapular}) - 0.008\ (\text{triceps} + \text{subscapular})^2 - 1.7$

For females: $\%\text{Fat} = 1.33\ (\text{triceps} + \text{subscapular}) - 0.013\ (\text{triceps} + \text{subscapular})^2 - 2.5$

FFM was then calculated as follows:
FFM = body weight – (%BF × body weight)/100
where BF is body fat and FFM is fat free-mass.

Skinfold thickness measurements were taken from two different sites: triceps and subscapula by using Harpenden skinfold caliper (British Indicators, St. Albans, UK). Head circumference measurement was also taken by using a flexible but non-stretchable measuring tape [14].

2.3. Blood samples collection

Blood samples were obtained from all patients by finger-prick. The blood spots were left to air dry for at least 3 hours at room temperature and away from direct sunlight. Then the blood samples were analyzed for the determination of phenylalanine concentration in blood by Elisa technique [15] at Prince Hamzah Hospital Laboratory, Amman, Jordan.

2.4. Dietary assessment

Dietary intake of PKU children was taken by mothers using a special form of a 3-day record, 2 days being in the middle of the week and 1 day during the weekend [16]. Instructions on the use of accurate weights and measures were given to mothers. Calculations of the intake of calories, protein, lipid, carbohydrate, calcium, iron, zinc and copper were done using a software program [17]. When any food was not listed in the software, the Food Composition Tables for Use in the Middle East [18], PKU Exchange List [19] and the proximate analysis of local low-Phe foods were used. Also the Phe-free formulas were entered to the software as additional food items. To assess the adequacy of nutrient intakes, the percentage of each nutrient in relation to the Dietary Reference Intakes (DRI) was calculated for each patient from the appropriate age group [20, 21] using the mean consumption of the 3-day food record.

2.5. Proximate analysis and phe content of locally-produced Low-Phe foods

The production of few local foods, special for PKU patients, has started since 2010. These food products included bread, followed by cupcakes (carrot and chocolate), *petit fours, ka'ek and ma'moul*, using low protein flour supplied by the Ministry of Health. The moisture, protein, energy, carbohydrates, ash, fat and fiber contents of those products were determined according to standard methods of AOAC [22].

2.6. Phenylalanine content

The Phenylalanine content of PKU products was analyzed by Amino Acid Analyzer using HPLC technique at Princess Haya Biotechnology Center, Irbid according to the procedure described by Fountoulakis and Lahm [23]. A methanol solution containing the PTC-amino acids was transferred to HPLC system for separation [24].

2.7. Adequacy of energy and nutrients

The adequacy of energy and nutrient intakes was calculated [10] for each patient according to the formula:
% Adequacy = [(ingested × 100)/recommended]

2.8. Statistical analysis

Statistical Analysis was performed using Statistical Program for Social Studies (SPSS) [25]. Values of the studied variables were expressed as mean ± standard error of the mean and percentile of prevalence of these variables. Pearson correlation was performed to determine any significant differences among variables. Also the analysis of variance was applied to test the differences among variables.

3. Results

3.1. Prevalence of PKU in Jordan

The prevalence of PKU in Jordan was estimated to be 1 in 5000 babies born every year, with 85% of their parents being blood relatives [9]. Among the 25 PKU patients included in this research, 80% came as a result of close relative marriage (Table 1). Eight percent had problems in the eye, 24% had learning problems and 60% consumed supplements.

In 2006, the MOH initiated the pilot National New Born Screening Project which was extended in 2007 to 7 of the 12 governorates in the Kingdom [9]. During this period new borns were screened with 28 children diagnosed to have PKU (see Table 2). By 2012, the total number of cases diagnosed to have PKU was 168; about 32% of these cases were discovered early after birth [9]. The youngest was 3 months old and the oldest was 32 years.

3.2. Anthropometric measurements

As shown in Table 3, about 48% of patients in the whole sample had a weight below the (3rd) centile, 40% where within the (‘15th–85th) centiles, and 12% were above the (85th) centile. About (66.7%) of patients in group 1 were underweight (stunted) and (33.3%) had normal weight. In group 2, (40%) of patients were underweight, (40%) had normal weight and (20%) had overweight. Whereas in group 3 (28.3%) of patients were underweight, (57.1%) had normal weight and (14.3%) had overweight and in group 4 about (57.1%) of patients were underweight, (28.3%) had normal weight and (14.3%) had overweight.

Table 3 also shows the distribution of patients according to height range among age groups. Fifty-two percent of the whole sample was found to be below normal height (less than 3rd centile). Among the individual groups, the

Table 1
Characteristics and clinical manifestations of the total study groups

Clinical manifestations	Number	%
Primary consanguinity	16	64
Secondary consanguinity	4	16
Family history of similar condition	12	48
History of abortion in mothers	8	32
Learning disability	6	24
Vision impairment	2	8
Taking supplements (such as iron, calcium and multivitamins)	15	60
Normal birth weight	21	84

Table 2
The prevalence of PKU in Jordan as indicated by screening in the period 2006–2012[1]

Year	No. of patients screened	No. of PKU cases
2006	2074	1
2007	4905	4
2008	13955	2
2009	31617	5
2010	47983	7
2011	93502	7
2012	30225	2
Total	224261	28

[1]Ministry of Health 2012 (Ref. 9).

Table 3
Distribution of patients according to weight and height status among age groups

Age group	Patients (n) = 25					
	Weight status			Height status		
	Underweight[1] N (%)	Normalweight[2] N (%)	Overweight[3] N (%)	Belownormal[1] N (%)	Normalheight[2] N (%)	Abovenormal[3] N (%)
4–6 months	4 (66.6%)	2 (33.3%)	–	4 (66.6%)	2 (33.4%)	–
8–12 months	2 (40%)	2 (40%)	1 (20%)	4 (80%)	1 (20%)	–
2 years	2 (28.6%)	4 (57.1%)	1 (14.3%)	1 (14.3%)	4 (57.1%)	2 (28.6%)
4 years	4 (57.1%)	2 (28.6%)	1 (14.3%)	4 (57.1%)	3 (42.9%)	–
Total	12 (48%)	10 (40%)	3 (12%)	13 (52%)	10 (40%)	2 (8%)

[1]<3rd centile for weight-for age. [2]15th–85th centile for weight-for age. [3]>85th centile for weight-for age.

Table 4
Overall correlations between anthropometric measurements and nutrients intake

Measures	*r**	*P***
Head circumference and blood Phe	0.244	0.239
Head circumference and protein intake	0.434	0.034
Weight and protein intake	0.420	0.041
Height and protein intake	0.502	0.012
Head circumference and Phe intake	0.562	0.003
Weight and Phe intake	0.703	<0.0001
Height and Phe intake	0.685	0.0002
Phe intake and protein	0.267	0.207
Phe intake and energy	0.459	0.021
Phe intake and blood Phe	0.689	0.046

*Correlation coefficient. **Significance level of *r*.

percentage of patients who were below normal height were 66.6%, 80%, 14% and 57.1% in groups 1, 2, 3 and 4 respectively. For head circumference about 36% of all patients were below the 3rd centile, 48% were within normal range, and 16% were above the 85th centile.

Table 4 shows that there were significant positive correlations ($p < 0.05$) between protein intake and each of head circumference ($r = 0.434$) weight (0.420) and height ($r = 0.502$) of patients. Also there were positive significant correlations ($p < 0.05$) between Phe intake and each of head circumference ($r = 0.562$) weight (0.703) and height ($r = 0.685$) of patients.

3.3. Concentration of phenylalanine in blood

Table 5 shows that 44% of total subjects had normal concentration of phenylalanine in blood. The patients who had high concentration of phenylalanine in blood were 24%, whereas those who had low blood phenylalanine were 32% of total patients.

3.4. Adequacy of the diet

The average protein intake for groups 3 and 4 subjects were (121.4%) and (118.6%) respectively, which is higher than the RDA. However, in groups 1 and 2 the average protein intakes were 76.9% and 87.9% respectively, which are lower than the RDA. Regarding the calorie intake in group 1 and group 4, it was lower than the RDA. The intakes of calcium, copper and zinc were higher than the RDA in all groups as shown in Figs. 1 –4.

Table 5
Distribution of PKU children according to Phe levels

Age group	Patients (n) = 25		
	Low* N (%)	Normal** N (%)	High*** N (%)
4–6 months	3 (50%)	2 (33.3%)	1 (16.7%)
8–12 months	3 (60%)	2 (40%)	–
2 years	1 (14.3%)	4 (57.1%)	2 (28.6%)
4 years	1 (14.3%)	3 (42.8%)	3 (42.8%)
Total	8 (32%)	11 (44%)	6 (24%)

*Low blood Phe concentration (<120 μmol/L). **Normal blood Phe concentration (120–360 μmol/L). ***High blood Phe concentration (>360 μmol/L).

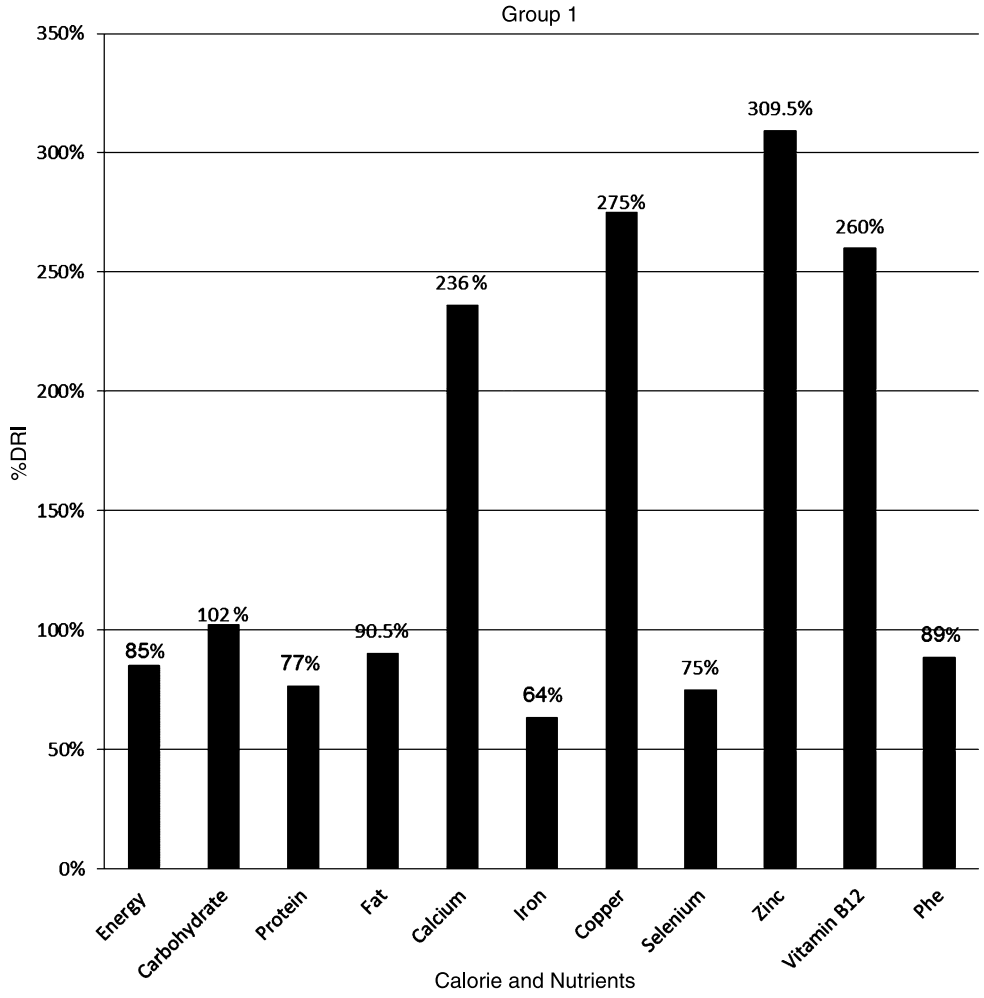

Fig. 1. Mean daily intake expressed as a percentage of dietary reference intakes (DRI) of calories and nutrients in group 1.

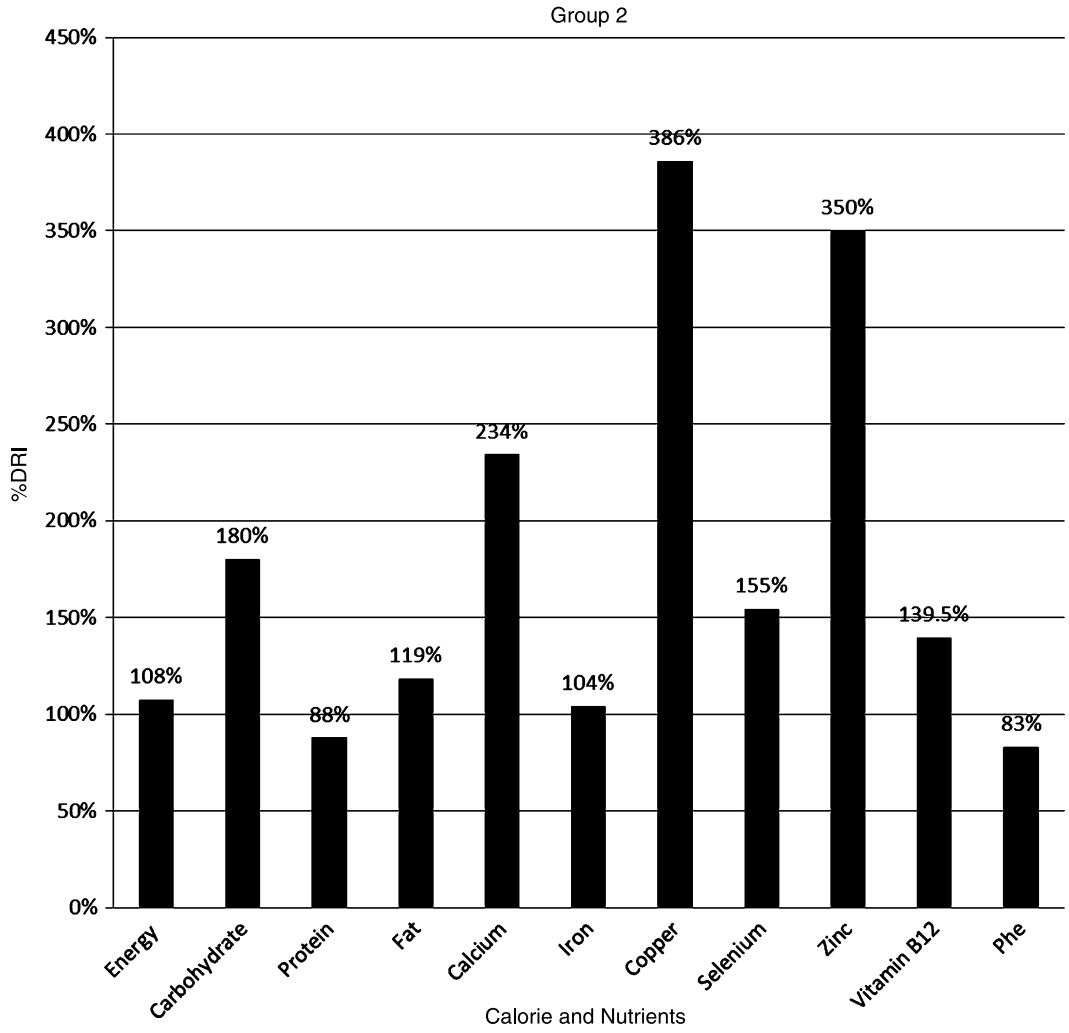

Fig. 2. Mean daily intake expressed as a percentage of dietary reference intakes (DRI) of calories and nutrients in group 2.

3.5. Proximate analysis of locally produced low-phenylalanine foods

The proximate analysis was done to know the nutritive value of few locally produced low-Phe foods. The proximate composition of the PKU products and phenylalanine content are presented in Table 6. This data was the average of three determinations and the result obtained showed that PKU products had low protein and phenylalanine contents

4. Discussion

This relatively high rate of PKU found amongst Jordanian babies may be attributed to the continuing custom of marriage between close relatives, particularly first cousins, within the Kingdom; as mentioned previously, the prevalence of PKU in Jordan was estimated to be 1 in 5000 babies born annually, with 85% of their parents being blood relatives [9]. This figure is close to the finding in the present research that 80% of the study sample came from marriages of close relatives.

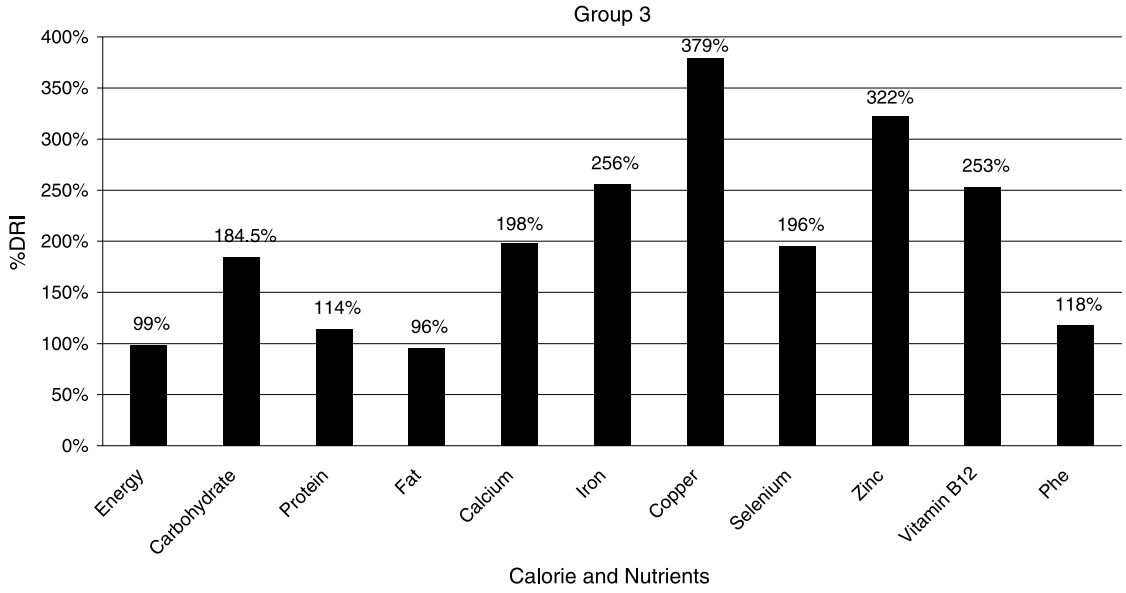

Fig. 3. Mean daily intake expressed as a percentage of dietary reference intakes (DRI) of calories and nutrients in group 3.

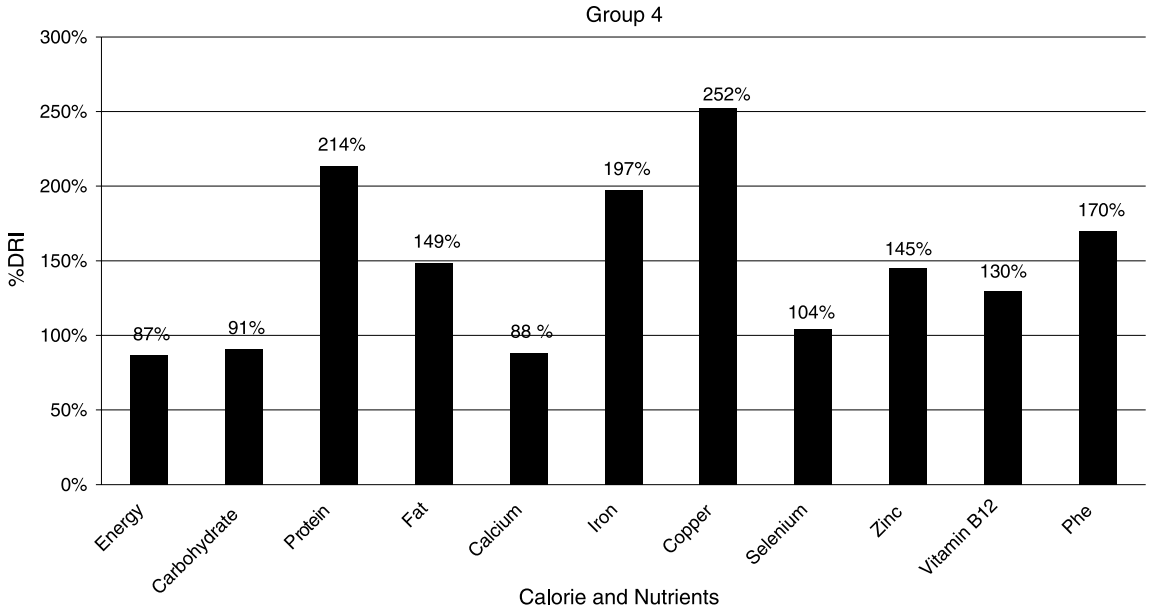

Fig. 4. Mean daily intake expressed as a percentage of dietary reference intakes (DRI) of calories and nutrients in group 4.

Neonatal screening for PKU has been recently applied in Jordan. The use of a special feeding formula and the continuous follow up of the infant directly after birth are essential for preventing the serious consequences of the disease including growth and mental abnormalities.

Previous studies on the physical growth of PKU patients showed that they suffer growth retardation, especially during the first years of life, even if they were on a diet that meets the age-specific dietary reference intake (DRI) for protein [26]. Our results are in agreement with other studies that reported protein intake above the DRI in PKU

Table 6
The results of the proximate analysis and phenylalanine content in local low-phenylalanine bakery products*

Nutrients	Flour		Low-Phenylalanine Products				
	Wheat flour	PKU flour	Bread	Cupcakes	*Petit fours*	*Ka'ek* (plain)	*Ma'moul* with date
Moisture (%)	10.3	6.6	35.4	16.9%	6.1	7.8	11.8
Protein (%)	13.7	0.4	1.9	2.5%	0.5	1.9	1.8
Phe (mg/100 g)	717.7	6.3	14.5	84.9 mg	13.7	27.1	91.1
Fat (%)	1.9	0.4	2.1	19.3%	26.8	13.2	20.4
Crude fiber (%)	12.2	2.9	1.9	2.9%	0.7	1.2	1.9
Ash (%)	1.6	1.1	4.2	2.6%	1.6	1.9	1.8
Carbohydrate (%)	72.6	88.6	54.5	55.8%	64.3	74	62.3
Food energy (Kcal)	362.3 kcal	359.6 kcal	244.5 kcal	406.9 kcal	500.4 kcal	422.4 kcal	440 kcal

*Results are average of triplicate sample with coefficient of variation (C.V.) <5%.

patients. We found that older children in groups 3 (2 years) and 4 (4 years) had higher protein intakes than DRI (121%, 214%) respectively (Figs. 3 and 4). This finding suggests that the quantity of protein intake may not be the only factor for determining growth; therefore, and as stated by Acosta et al. [27], energy and trace element intakes, phe concentrations in the blood and Phe intake by PKU patients should also be considered. The optimal supply of protein and essential amino acids is very important not only for normal growth, but also for the development of the central nervous system, which can be negatively influenced by unbalanced amino acid intake [28]. Experimental data suggest that amino acids delivered as dietary protein (casein) may support whole-body protein utilization better than ingestion of crystalline amino acids or casein hydrolysates provided by the PKU formulas [29]. Sibinga et al. [30], while studying 60 children with PKU receiving treatment, found a substantial growth deficit (weight and height), regardless of the age at which the diet was initiated. Comparing their results with those obtained from other centers, they concluded that PKU, even with treatment, is associated with poor growth. Our results show a relationship ($r = 0.502$; $p < 0.05$) between protein intake and height of patients, as well as between Phe intake and height of patients ($r = 0.685$) ($p < 0.05$). However, and as mentioned before, it is not only protein adequacy that is necessary for growth, but also dietary balance as a whole. In a prospective controlled trial, the ingestion of protein rather than an amino acids mixture resulted in lower nitrogen excretion for the same energy and amino acid intake [31].

In infancy and early childhood, Phe-free formula (or Phe-restricted formula) provides most of the protein, energy and nutrient needs for growth. Dietary Phe requirements are met by small amounts of breast milk or infant formula. As the child grows up and starts having solid food additives, Phe requirements are met through small amounts of rice cereal and puréed fruits and vegetables [33]. Thus in the present study, because the food choices are limited for PKU infants of groups 1 and 2, we found serum Phe to be controlled; only 17.1% of patients in group 1 (4–6 months) and none of patients in group 2 (8–12 months) had high blood Phe concentrations. Compliance with the PKU diet becomes more difficult as children grow older and start school, because they make their own food choices and develop taste preferences which are often similar to those without PKU [34]. Thus, children in group 3 and 4 are seen to have less compliance to the PKU diet; in group 4 (4 years old) 42.8% had high (uncontrolled) Phe levels. Frequent consumption of the formula throughout the day results in stable plasma Phe concentrations and higher Phe tolerance as protein retention and synthesis improve [35]. One problem in consuming an amino acid formula is the challenging aspect of compliance as formula looks different and has a distinctive poor odor and taste, in spite of efforts to improve formulations and packaging [36]. Most children refuse to drink the formula at school, thus are without a good source of protein for 8 h resulting in catabolism of body protein and release of the phe into blood.

Previous studies showed that for children with PKU, it was more difficult to obtain their recommended calories than obtaining the recommended protein; since the special formula supplied a small amount of calories [10]. PKU formulas normally use fats and sugars to increase the total calorie intake of these PKU children. Nevertheless, children do not always accept the greater amounts of oil in their diet as they get older. In the present study, some of the PKU children mothers stated that they do not always follow the recommendations in feeding their children who need low protein and adequate calories in their diets.

The average calcium intake in groups 1, 2 and 3 was higher than the DRI (Figs. 1–3); this is because the formula given to patients was fortified with an adequate amount of calcium, as shown in the ingredients of the formula used and because these patients consumed calcium supplements. About 32% of the study patients were given calcium supplements. Also as age increases, a greater amount of Phe-free formula was taken. Consequently, the amount of calcium ingested increased when expressed as a percentage of RDA [36]. The iron intake in all groups was higher than the DRI except group 1, as derived from information provided on the formula. It was found by other authors [37] that the iron intake by children with PKU (4 to 7 years old) comprised 199% of RDA and increased to 296% of RDA for older children (7 to 11 years). Regarding selenium intake this study shows that it was high for groups 2, 3 and 4 but low (75% of RDA) for group 1; this is probably attributed to the low level of selenium (15 mcg/100 g) in the formula given to groups 1 and 2 (from birth to 12 months of age). However, in group 2 the selenium intake was higher than the RDA (155%) probably because this group received higher amounts of formula compared to group 1. In older children (group 3 and 4), the selenium intake was high; this can be explained by the fact that the formula used by these groups has higher content of selenium (37 mcg/100 g). In our investigation, all PKU patients had relatively high vitamin B12 intake in comparison with the DRI. The vitamin B12 intake by group 1 infants (4–6 months) was 260% and for group 2 (8–12 months) it was 140% (Fig. 2). For group 3 (2 years old) vitamin B12 intake was 253% of DRI (Fig. 3) and for group 4 children (4 years old) it was 130% (Fig. 4). These vitamin B12 values and values of other minerals (zinc, copper) were relatively high depending on their content in the formulas used. It is worth mentioning that most of the trace element intakes were mainly obtained from the feeding formulas, and that two types of formulas were given: Comida A (for children aged 1–12 months) and Comida B (for children older than 1 year), with different micronutrient contents. Regarding phenylalanine intake, it was adequate in older patients in groups 3 and 4, but lower than that recommended for groups 1 and 2. Probably this is connected to lack of compliance with the formula in older children as previously mentioned under protein intake.

It is very helpful for health workers involved in dietary planning of PKU patients to find available locally produced and cheap foods with low phenylalanine content. Results showed that some of such foods especially prepared for PKU patients in Jordan was low in this amino acid facilitating their use for such patients (see Table 6).

It may be concluded from the present study that the studied PKU children had poor physical growth that could be related to inadequate energy and protein intakes and nutritional imbalances, particularly at younger age of patients in whom growth in height was clearly affected. The diet of PKU patients in this study was adequate or high in calcium, copper, zinc, and iron, this indicates that the Phe-free formula is sufficiently supplemented with these trace elements. Also it may be concluded that in Jordan consanguineous marriage seems to be a main contributing factor in the etiology of PKU; and this confirms the need for more efforts in counseling the community about this disease.

Conflict of interest

The authors declare that there is no conflict of interest among them in this manuscript.

Acknowledgments

The authors would like to thank the Deanship of Academic Research at the University of Jordan for their financial support (No. 82/2010/2011) and the Ministry of Health, particularly the Head of the Department of the Genetic Diseases Dr. Safwan Dababneh for facilitating the data collection. Also the authors thank Al-Sufara Bakeries for providing the PKU food products.

References

[1] Donlon J, Levy H, Scriver C. Metabolic and molecular basis of inherited disease, (8 Edition). New York: McGraw-Hill, 2007.

[2] Christ SE. Asbjorn folling and the discovery of phenylketonuria. J Hist Neurosci. 2003;12:44-54.

[3] Koch R, Cruz F. Historical aspects and overview of research on phenylketonuria. Ment Retard Dev Disabil Res Rev. 1999;5:101-3.

[4] Lage S, Bueno M, Andrade F, Prieto JA, Delgado C, Legarda M, Sanjurjo P, Aldámiz-Echevarría LJ. Fatty acid in patients with phenylketonuria and its relationship with bone mineral density. J Inherit Metab Dis. 2010;30:38-430.

[5] Blau N, van Spronsen FJ, Levy H. Phenylketonuria. Lancet. 2010;376:1417-27.

[6] Francjan J, Spronsen V. Phenylketonuria management from an European perspective: A commentary. Mol Genet Metab. 2010;100(2):107-10.

[7] Ahring K, Bélanger-Quintana A, Dokoupil K, Ozel H, Lammardo A, MacDonald A, Motzfeldt K, Nowacka M, Robert M, Rijn M. Dietary management practices in phenylketonuria across European centres. Clin Nutr. 2009;28(3):231-6.

[8] Singh R, Quirk M, Douglas T, Brauchla M. BH4 therapy impacts the nutrition status and intake in children with phenylketonuria: 2-year follow-up. J Inherit Metab Dis. 2010;33:689-95.

[9] Dababneh S. Facts on PKU in Jordan, Department of Genetic Diseases, Ministry of Health, Amman Jordan (Personal Communication), 2012.

[10] Fisberg R, Silva-Fernandes M, Schmidt M, Fisberg M. Nutritional evaluation of children with phenylketonuria. Sao Paulo Med J. 1999;117(5):1516-3180.

[11] Belanger-Quintana A, Martínez-Pardo M. Physical development in patients with phenylketonuria on dietary treatment: A retrospective study. Mol Genet Metab 2011;104:480-4.

[12] World Health Organization. WHO child growth standards: Length/height-for-age, weight-for-length, weight for height, and body mass index for age: Methods & development. Geneva. World Health Organization, 2006.

[13] Slaughter MH, Lohman TG, Boileau RA. Skinfold equations for estimation of body fatness in children and youth. Hum Biol. 1988;60:709-23.

[14] Nellhaus G. Head circumference from birth to eighteen years. Practical Composite International and Interracial Graphs. Pediatrics. 1968;41:106-14.

[15] Dooley KC. Enzymatic method for phenylketonuria screening using phenylalanine dehydrogenase. Clin Biochem. 1992;25(4):271-5.

[16] Dwyer JT. Dietary assessment. In: Shils ME, Olson JA, Shike M. Modern nutrition in health and disease, (8th ed.). Philadelphia: Lea and Febiger, 1994;842-60.

[17] The food processor SQL, ESHA (2008). Version 10.4.0.

[18] Pellett PL, Shadarevian S. Food composition tables in the Middle East. (Second edition), Beirut: Lebanon, 1970.

[19] Schuett V (2010) Low protein food list for PKU. (Second Edition). Seattle: National PKU News.

[20] IOM, 2002/2005. Panel on Macronutrients and Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Macronutrients and healthful diets. Dietary reference intakes for energy, carbohydrates, fiber, fats, fatty acids, cholesterol, proteins, and amino acids. Washington, DC: National Academies Press, 769-879.

[21] IOM (Institute of Medicine), 2010. Dietary Reference Intakes for Calcium and Vitamin D. From: www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D.aspx

[22] Association of Official Chemists. Official Methods of Analysis of the Association of Official Analytical Chemists (18th edition). International Virginia, USA, 2006.

[23] Fountoulakis M, Lahm HW. Hydrolysis and amino acid composition of proteins. J Chromatogr A. 1998;826(2):109-34.

[24] Peace RW, Gilani GS. Chromatographic determination of amino acids in foods. J AOAC Int. 2005;88(3):877-87.

[25] SPSS. SPSS Programming and Data Management. SPSS Inc, U.S.A, 2008.

[26] Arnold GL, Vladutiu CJ. Kirby RS, Blakely EM, DeLuca JM. Protein insufficiency and linear growth restriction in phenylketonuria. J Pediatr. 2002;141:243-6.

[27] Acosta PB, Yannicelli S, Singh R. Nutrient intakes and physical growth of children with phenylketonuria undergoing nutrition therapy. J Am Diet Assoc. 2003;103:1167-73.

[28] Arnold GL, Kramer BM, Kirby RS, Plumeau PB, Blakely EM, Sanger-Cregan LS, Davidson PW. Factors affecting cognitive, motor, behavioral and executive functioning in children with phenylketonuria. Acta Paediatr. 1998;87:565-70.

[29] Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, Callier P. The digestion rate of protein is an independent regulating factor of postprandial protein retention. Am J Physiol Endocrinol Metab. 2001;280:E340e8.

[30] Sibinga MS, Friedman CJ, Steisel IM, Baker EC. The depressing effect of diet on physical growth in phenylketonuria. Dev Med Child Neurol. 1971;13:63-70.

[31] Herrmann ME, Brosicke HG, Keller M, Monch E, Helge H. Dependence of the utilization of a phenylalanine free amino acid mixture on different amounts of single dose ingested. Eur J Pediatr. 1994;153:501-3.

[32] MacLeod EL, Ney DM. Nutritional management of phenylketonuria. Ann Nestlé. 2010;68:58-69.

[33] Owada M, Aoki K, Kitagawa T. Taste preferences and feeding behaviour in children with phenylketonuria on a semisynthetic diet. Eur J Pediatr. 2000;159:846-50

[34] MacLeod EL, Clayton MK, van Calcar SC, Ney DM. Breakfast with glycomacropeptide compared with amino acids suppresses plasma ghrelin levels in individuals with phenylketonuria. Mol Genet Metab. 2010;100(4):303-8.

[35] Prince AP, McMurray MP, Buist NR. Treatment products and approaches for phenylketonuria: Improved palatability and flexibility demonstrate safety, efficacy and acceptance in US clinical trials. J Inherit Metab Dis. 1997;20:486-98.

[36] Acosta PB, Gropper SS, Clarke-Sheehan N, Wenz E, Cheng M, Anderson K, Koch R. Trace element status of PKU children ingesting an elemental diet. J Parenter Enteral Nutr. 1987;11:287-92.

[37] Gropper SS, Acosta PB, Clarke-Sheehan N, Wenz E, Cheng M, Koch R. Trace element status of children with PKU and normal children. J Am Diet Assoc. 1988;88:459-65.