Nutritional Consequences of Adhering to a Low Phenylalanine Diet for Late-Treated Adults with PKU

Low Phe Diet for Adults with PKU

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Abstract *Background:* The main treatment for phenylketonuria (PKU) is a low phenylalanine (Phe) diet, phenylalanine-free protein substitute and low-protein special foods. This study describes dietary composition and nutritional status in late-diagnosed adult patients adhering to a PKU diet.

Methods: Nineteen patients, followed at Oslo University Hospital in Norway, participated; median age was 48 years (range 26–66). Subjects were mild to severely mentally retarded. Food intake, clinical data and blood analyses relevant for nutritional status were assessed.

Results: Median energy intake was 2,091 kcal/day (range 1,537–3,277 kcal/day). Carbohydrates constituted 59% (range 53–70%) of the total energy, including 15% from added sugar; 26% was from fat. The total protein intake was 1.02 g/kg/day (range 0.32–1.36 g/kg/day), including 0.74 g/kg/day (range 0.13–1.07 g/kg/day) from protein substitutes. Median dietary Phe intake was 746 mg/day (range 370–1,370 mg/day). Median serum Phe was 542 $\mu\text{mol/L}$ (range 146–1,310 mg/day). Fortified protein substitutes supplied the main source of micronutrients. Iron intake was 39.5 mg/day (range 24.6–57 mg/day), exceeding

the upper safe intake level. Intake of folate and folic acid, calculated as dietary folate equivalents, was 1,370 $\mu\text{g/day}$ (range 347–1,744 $\mu\text{g/day}$), and resulted in high blood folate concentrations. Median intake of vitamin B₁₂ was 7.0 $\mu\text{g/day}$ (range 0.9–15.1 $\mu\text{g/day}$).

Conclusions: The diet supplied adequate protein and energy. Fortification of the protein substitutes resulted in excess intake of micronutrients. The protein substitutes may require adjustment to meet nutritional recommendations for adults with PKU.

Introduction

In Phenylketonuria (PKU; OMIM 261600), the conversion of phenylalanine (Phe) to tyrosine is restrained or blocked, due to impaired activity of the enzyme phenylalanine hydroxylase (PAH; EC 1.14.16.1) in the liver (Scriver et al. 2011). With the newborn screening programme, dietary treatment is usually started in the first days of life to avoid brain damage. The Norwegian national screening programme was instituted around 1970. Patients born prior to this date were usually diagnosed late and developed various degrees of brain damage. It has been documented that dietary treatment started on clinical indications, during childhood or in adult years, can alleviate neurological and behavioural symptoms and signs also in late diagnosed patients (Yannicelli and Ryan 1995; Baumeister and Baumeister 1998; Fitzgerald et al. 2000; Lee et al. 2009). In Norway, late diagnosed adults with PKU are offered dietary treatment on a permanent basis if they experience positive effects on neurological and behavioural symptoms during a trial period, usually lasting 3 to 6 months.

The main treatment for PKU is a low phenylalanine diet, supplemented with a Phe-free protein substitute, vitamins

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and minerals. The aim of the diet is to reduce the level of phenylalanine in the blood and brain. Small amounts of protein from natural food products provide the essential amino acid phenylalanine. The amount of Phe tolerated and protein substitute needed vary according to the rest activity in the PAH enzyme. The amount of dietary Phe is determined by regular monitoring of Phe levels in the blood (Yi and Singh 2008; Poustie and Wildgoose 2010). To meet energy requirements, specially manufactured low protein foods such as bread, pasta and biscuits, and natural foods low in phenylalanine such as fruits, vegetables, sugar and butter, are also used.

There is little documentation regarding the nutritional consequences for adults who follow a PKU diet over years. Hence the objectives of this study were to report the nutrient intake of late-treated adults with PKU and describe dietary effects on nutritional status. We wished to investigate as to whether the diet used on a daily basis conformed to nutritional recommendations and medical treatment goals.

Methods

This was an observational cross-sectional study. Food intake was registered for 4 days, serum Phe levels and several blood biochemical parameters, relevant for assessing nutritional status were evaluated. The study was organised in connection with annual outpatient follow-up.

Subjects

The national PKU centre at Oslo University Hospital, Norway, invited 27 late-treated adult patients to participate in the study. All had adhered to diet for the last 12 months or longer. Consent was received from 21 subjects. Two patients withdrew before the study started, due to intercurrent illness and inability to record food intake. Nineteen late-treated subjects were recruited, 7 males and 12 females with a median age of 48 years (range 26–66 years). The median age at diagnosis was 3 years (range 6 months to 43 years), and median age at diet start was 27 years (range 6 months to 47 years). Subjects had bodyweight comparable to the general population in Norway; BMI showed a median 28 (range 20.2–38.5), four had BMI above 30. Before dietary treatment was instituted, participants had a median serum Phe of 1,541 $\mu\text{Mol/L}$ (range 1,131–2,468 $\mu\text{Mol/L}$). All participants were ethnic Norwegians.

Subjects and carers received written and verbal information; all communication was undertaken by the first author. Twelve subjects, six male, six female, with a median age of

49 years (range 36–66 years) suffered severe cognitive disability and could not give informed consent. These 12 subjects lived in small, staffed care homes, and professional carers acted as informants and recorded the subjects' food intake. The remaining seven participants, one male, and six female, median age 42 years (range 26–51 years), had milder cognitive disabilities and were able to give informed consent. These subjects lived by themselves or with their parents and managed the diet with no, or limited, assistance from the community. These seven plus one of the severely disabled patients visited Oslo University Hospital for annual outpatient control, information on the study and venous blood tests. The remaining 11 participants were unable to visit the hospital, due to mental and physical disability and long distance to the hospital. Instead, the first author visited the care homes, to obtain data and give information on food recording and blood tests.

The Regional Committee for Medical Research Ethics and the Commission for Privacy Protection at Oslo University Hospital approved the study. Handling of blood samples was according to provisions in the Norwegian Biobank Act.

Food Recording

The patients or their carers kept a 4 day prospective food diary. Food intake was recorded from Wednesday to Saturday, or Sunday to Wednesday. The patients were encouraged to follow their ordinary diet during food recording. Foods were weighed on digital scales with 1 g increments and drinks were measured in decilitres. The diaries, personal recipes and wrapping paper for special products were returned by mail.

The recordings were analysed using a Norwegian commercial nutrient calculation programme “Mat pa data 4a”, based on the official Norwegian table of food composition (Rimestad et al. 2001). Data for the protein substitutes and the low-protein food products were added to the database. Phenylalanine in food was calculated as 5% of the protein content or based on analysis of amino acid distribution (Weetch and MacDonald 2006). Vitamin B₁₂ content was calculated manually as it was not included in the software programme. To account for different bioavailability in folate and folic acid, intake of this vitamin was calculated as Dietary folate equivalents (DFE) (Suitor and Bailey 2000). The Nordic Nutrition Recommendations were used to compare intake with recommendations and upper intake levels (Nordic Council of Ministers 2004). All participants received individual dietary advice after the study.

Blood Sampling and Analyses

Blood tests were taken the day before or during the period of food recording and after overnight fasting. For all subjects, routine tests for serum Phe were obtained by finger pricking. These were subsequently analysed by the Neonatal Screening Laboratory at Oslo University Hospital. Phe in serum was determined fluorometrically, by a method described by M.W. McCaman (McCaman and Robins 1962). Subjects had their serum Phe levels analysed routinely, four to ten times a year.

Venous blood samples were analysed at the Department of Medical Biochemistry at Oslo University Hospital. Analyses were done routinely on arrival at the laboratory and according to standard procedures: The following equipment was used for blood analysis: Amino acid profiles in serum: Amino Acid Analyser Biochrom 30, Biochrom LTD, Cambridge, UK. Serum iron analyses: Modular P800 Roche Diagnostics, Basel, Switzerland. Ferritin analyses: Modular E170 Roche Diagnostics, Basel, Switzerland. Haemoglobin analyses: Celldyn 4000, Abbott Laboratories, CA, USA. Folate and B₁₂ analyses: Immulite 2000, Siemens Medical Solutions Diagnostics, DPC Cirrus Inc., Instrument Systems Division, Flanders, N.J., USA.

For two subjects, all analyses, apart from serum Phe, amino acid profiles, folate and vitamin B₁₂, were done at local hospital laboratories. For two other subjects, only capillary serum Phe was obtained, as venous blood drawing would have required general anaesthesia.

Statistics

Microsoft® Excel 2002 SP3 was used for statistical analyses. Because of the small number of patients involved, only descriptive statistics such as median and range were used.

Results

Food and Nutrient Intake

Energy according to food groups are shown in Table 1. Intake of low-protein special foods together with protein substitutes constituted about half the consumed energy. Natural low-protein products like fruits, vegetables, sweets and soft drinks constituted approximately a fifth of the energy. The remaining energy stemmed from animal foods and miscellaneous food products like normal bread and cereals, edible fats and jams. The median intake of fruits and vegetables was 326 g (range 137–1,205 g/day). Median fibre intake was 16 g/day (range 8–38 g/day) or 1.7 g/MJ (range 0.9–3.4 g/MJ).

The median protein intake was 1.02 g/kg/day (range 0.32–1.36 g/kg/day). One subject reported a protein intake below the FAO/WHO recommendation of minimum 0.75 g/kg/day (Nordic Council of Ministers 2004). Natural protein with phenylalanine constituted about a quarter of the consumed protein. The median Phe intake was 746 mg/day (range 370–1,370 mg/day), or 10 mg/kg/day (range 6–21 mg/kg/day). The main source of Phe was food of animal origin.

Six subjects had a fat intake below the recommended minimum of 25% of energy, see Table 2. Main sources of dietary fat were margarine, oil, butter and mayonnaise, resulting in a median intake of 20 g/day (range 5–28 g/day) of polyunsaturated fatty acids (PUFA), according to recommendations for all but two subjects. All subjects ate some long-chain PUFA; 14 used commercial fish oil concentrates or cod liver oil daily, giving 0.5 g/day to 1.0 g/day of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) combined. Most subjects also used sandwich fillings based on mackerel or cod roe.

The amount of energy from carbohydrate was above the recommended level for eight subjects, mainly due to a high intake of added sugar. Only five subjects managed to maintain the intake of added sugar below the recommended maximum of 10% of total energy.

In order to maintain serum Phe in the therapeutic range and to meet each patient's individual protein requirement, the amounts of protein substitute taken varied. The median protein intake from protein substitute was 0.74 g/kg/day (range 0.13–1.07 g/kg/day). Five brands of protein substitute were used, see Table 3. The protein substitutes were fat free; four of them were fortified with minerals and vitamins. One subject was required to take additional supplements of vitamins, trace minerals and calcium, since the substitute used was unfortified. Use of fortified protein substitutes resulted in high intakes of most micronutrients. The highest micronutrient intakes were observed for iron, vitamin B₁₂ and folic acid, see Table 1. The mean intakes of calcium, magnesium, zinc, selenium and vitamins A, D, C and remaining B-vitamins also exceeded recommendations. However, as intakes did not exceed upper safe levels of intake (Nordic Council of Ministers 2004) and blood parameters were not adversely influenced, the results are not reported here.

The main source of folic acid was the fortified protein substitutes, with additional small amounts from some low-protein special products. About 85% of DFE originated from folic acid in the protein substitutes, ranging from 174 µg/day for the subject taking Avonil to 887 µg/day for one subject taking Lophlex. As fortification is not permitted in Norwegian food products, the additional food did not contain folic acid. Dietary sources of natural folate were vegetables, fruits, and small amounts of dairy products, providing about 10% of DFE.

Table 1 Energy and nutrient intakes according to food groups in late-treated PKU patients

Food group	Energy (kcal/day)	Energy (kJ/day)	Protein (g/day)	Fat (g/day)	Carbohydrate (g/day)	Added sugar ^a (g/day)	Iron (mg/day)	DFE ^b (μg/day)	Vitamin B ₁₂ (μg/day)
	Median (range)								
Protein substitute	406 (84–512)	1701 (351–2,142)	58.5 (12.7–75)	0.7 (0–0.9)	45.9 (0.8–57.8)	4.2 (0–5.3)	31.7 (12.3–40)	1,156 (174–1,508)	5.4 (0.9–11.8)
Dietary food ^c	678 (401–1,385)	2,738 (1,679–5,799)	3 (1–8.5)	17.0 (8.8–34.8)	129.1 (59.1–247.7)	5.2 (0–20.1)	6.9 (0–28.7)	90 (0–230)	0
Vegetables and fruits	206 (67–524)	864 (282–2,195)	5 (1.5–12.3)	0.6 (0.1–2.1)	44.6 (13.8–120.2)	0.6 (0–6.7)	1.3 (0.4–4.4)	89 (17–251)	0
Animal foods ^d	153 (83–345)	641 (346–1,445)	8 (2.9–21)	10.6 (5.2–22.6)	4.8 (1.8–24)	0 (0–0.8)	0.9 (0.1–2.1)	10 (2–36)	1.3 (0–3.3)
Sweets ^e	239 (17–620)	999 (72–2,595)	0.3 (0–1.7)	0 (0–8.8)	58.5 (4–150.9)	52.5 (4–140)	0 (0–1.3)	0 (0–24)	0
Total intake	2,091 (1,537–3,277)	8,754 (6,435–13,717)	76.9 (32.6–106.3)	65.1 (37.2–102.4)	319.9 (211.8–494.2)	73.2 (8.5–179.2)	39.5 (24.6–57)	1,370 (347–1,744)	7.0 (0.9–15.1)

^a All sugar, syrup or glucose syrup added by manufacturers or household cooking^b Folate and folic acid calculated as dietary folate equivalents (DFE)^c Dietary food manufactured with less protein (flour, baked goods, pasta, etc.)^d Food products consisting mainly of meat, fish, eggs, cows milk, yoghurt or cheese^e Candies, chocolate, popsicles, soft drinks

Table 2 Distribution of energy in the diet of late-treated PKU patients

	Proportion of total intake (%)	
	Median (range)	Nordic recommendations
Protein	14 (6–20)	10–20
Fat	26 (17–35)	25–35
Saturated fat	9 (5–17)	ca 10
Polyunsaturated fat	7 (2–11)	5–10
Carbohydrate	59 (53–70)	50–60
Added sugar	15 (2–31)	<10

The intake of vitamin B₁₂ was lower than recommended only for the subject taking Avonil. The others had intakes according to recommendations or higher. All subjects had an iron intake greater than the recommendation. Almost all dietary iron originated from fortified protein substitutes and low protein special food. The protein substitutes contributed approximately 75% of the total iron intake. Intakes exceeded the upper intake levels of 25 mg/day for 14 subjects. The use of food containing haemic iron and natural B₁₂ was minimal.

Blood Tests

Median serum Phe at the time of the study was 542 µMol/L (range 146–1,310 µMol/L). The subjects' mean serum Phe level during the 12 months preceding the study showed a median of 472 µMol/L (range 352–1,143 µMol/L). Phe levels at the time of the study and for the preceding year had a Spearman's rho correlation of 0.83 ($p < 0.01$). Other blood tests were analysed for 13 to 17 subjects. Folate, B₁₂, and iron parameters are shown in Table 4. Data for renal

function, lipids, zinc, selenium and magnesium were within normal ranges and are not reported. Neither did the serum amino acid profiles reveal discrepancies apart from elevated serum Phe.

Discussion

We wished to describe nutritional implications for late-diagnosed adults who had followed a Phe restricted diet for at least 1 year preceding the study. PKU is a rare disease, and even when all eligible patients in Norway were invited, the sample size was small. The medical ethical committee required that the burden of participation should be minimal for retarded subjects. Thus, only blood tests commonly done at annual follow-up were allowed. However, all subjects had capillary serum Phe levels analysed routinely, and the stable Phe levels indicated that the diet was well adhered to. Most subjects chose to maintain serum Phe above the Norwegian treatment goal of maximum 400 µMol/L. For these adults, the optimal serum Phe level was individually chosen and can be seen as a compromise between the effects on patients' emotional and behavioural functions and their ability to adhere to and manage the diet. The Phe levels in this study are comparable to the levels reported in other studies for late-diagnosed patients with PKU (Lee et al. 2009; Trefz et al. 2011) and to the levels reported to have positive effects on early-treated adult patients' mood and attention (Ten Hoedt et al. 2011).

The stable serum Phe levels and the relatively high energy intakes reported support our assumption that the food recordings reflected patients' habitual dietary intake. The detailed and meticulously recorded registrations supplied important data on how the patients chose to eat at home.

Table 3 The contents of selected nutrients in the different types of protein substitutes used by late-treated PKU patients

	Type of protein substitutes				
	XP Maxamum ^a	Lophlex powder ^a	PKU Express ^b	Avonil ^c	Prekunil ^c
Number of subjects using each substitute	13	1	3	1	1
<i>Nutrients per 100 g of substitute</i>					
Protein equivalents (g)	30	72	60	55.8	55
Iron (mg)	23.5	19.2	21.6	16	0
Vitamin B ₁₂ (µg)	3.6	6.4	9.4	1.1	0
Folic acid (µg)	500	896	400	226	0
DFE ^d (µg)	850	1,523	680	384	0

^a XP Maxamum and Lophlex powder by Nutricia

^b PKU Express by VitaFlo International Ltd

^c Avonil and Prekunil by Prekulab Ltd

^d Dietary Folate Equivalents (DFE): 1 µg folic acid as a fortificant = 1.7 µg DFE

Table 4 Nutritional biochemical parameters of the late-treated PKU patients

	Median (range)	Normal range at the laboratory used
B-Haemoglobin (<i>n</i> = 13), g/dL	13.5 (12.9–15.2)	Men: 13.4–17 Women: 11.7–17
S-iron (<i>n</i> = 15), g/L	17 (7–27)	9–34
P-Ferritin (<i>n</i> = 16), µg/L	53 (9–257)	Men: 29–383 Women: 10–167
S-Vitamin B12 (<i>n</i> = 17), pmol/L	580 (110–1,030)	160–710
ER-folate (<i>n</i> = 13), nmol/L	1,870 (605–3,165)	390–1,140
S-folate (<i>n</i> = 15), nmol/L	53.0 (22.3–>54.4)	7.1–27

N number of subjects tested

Such knowledge is of great value for dieticians and doctors when giving dietary advice and prescribing medical food.

As reported by MacDonald et al. (2003), fruits and vegetables can be used almost without restriction in the PKU diet. However, several subjects in the study consumed only small amounts of these foods, thus restricting their diet more than necessary. Intake of fruits and vegetables was comparable to the Norwegian mean intake, and might reflect what patients and carers considered normal portions. Availability and food price might also influence the use of these foods. Fruits and vegetables were the main source of fibre in the PKU diet. Only individuals with high intakes managed to reach the recommended intake of 25–35 g fibre per day. Readily available, low-cost sweets and soft drinks were used in considerable amounts.

A high sugar intake combined with low fat content in most low-protein special food used might increase the risk of deficiency of essential fatty acids, and, indeed, low intakes of PUFA in PKU diets are reported in several studies. Most studies, like the study by Rose et al., are performed on children (Rose et al. 2005). Mosely et al. reported that also adults with PKU had insufficient intakes of PUFA (Moseley et al. 2002). In contrast, our study showed a sufficient intake of essential fatty acids, when all PUFA were taken together. Omega-3 supplements and fish-based bread spreads were widely used. Such products are common in Norwegian diets, and were not viewed as special to the PKU diet by the subjects or carers. More knowledge about intake and requirements for long-chain PUFA, in particular DHA status, is needed as this might influence cognitive outcome in PKU (Yi et al. 2011). In this study, however, only the dietary amounts of total PUFA seem relevant as subjects started treatment late.

The food recordings and serum amino acid profiles indicated that the subjects had a protein intake in accordance with requirements. This indicates that a low Phe diet supplemented with protein substitute will fulfil protein requirements for adult PKU patients. However, the

amount of protein substitute necessary, coupled with the high fortification levels in these products, resulted in excessive intakes of most vitamins and trace minerals for these adults. This was reflected in high levels of folate in both erythrocytes and serum. Similar high folate levels were reported by Robinson et al. who assumed the high levels resulted from large amounts of vegetables in the PKU diet (Robinson et al. 2000). In our study, however, low vegetable consumption resulted in a low intake of natural folate. Even if the subjects had doubled their vegetable intake, the natural folate would be a minor fraction of the folic acid intake from protein substitutes. The intake and blood parameters reported indicate that the amounts of folic acid in three of the protein substitutes used are excessive for adult patients. The two subjects using Prekuniil and Avonil had intakes of folic acid and vitamin B₁₂ comparable to recommendations. They were the only subjects with erythrocyte and serum folate within normal range, see Table 4.

Natural sources of vitamin B₁₂ are scarce in the PKU diet and several reports show that adults with PKU risk B₁₂ deficiency if fortified protein substitutes are not taken (Hanley et al. 1993; Robinson et al. 2000; Hvas et al. 2006). In this study, all subjects took the substitutes as prescribed, and the high intake of vitamin B₁₂ in most protein substitutes was reflected in the blood analyses, see Table 4. Vugteveen et al. report that vitamin B₁₂ concentrations in serum within reference values, do not exclude functional vitamin B₁₂ deficiency in PKU patients. In order to detect this, they recommend measuring serum methylmalonic acid or plasma homocysteine for these patients in the future (Vugteveen et al. 2011).

Despite a high iron intake, the blood analyses revealed no signs of iron overload. This indicates a low absorption of dietary iron in PKU diets. Similar lack of correlation is reported earlier in PKU children (Arnold et al. 2001; Acosta et al. 2004). Similar to reports by MacDonald, some subjects in our study complained of abdominal discomfort

after taking the protein substitutes (MacDonald 2000). The possibility that excessive amounts of intestinal iron and other micronutrients contribute to these symptoms cannot be excluded.

Fortification of protein substitutes makes the diet less complicated and makes it easier to comply to and organise the diet, as shown by MacDonald (2000). The protein substitutes used in this study were recommended for older children, adolescents and adults. Our findings raise the question of whether a single product can meet the requirements for protein and micronutrients in children as well as adults. The amounts of micronutrients in the protein substitutes might need adjustment in order to meet vitamin and mineral recommendations in adult PKU patients. Further investigations, preferably in controlled studies with larger samples of adult PKU patients are necessary. Nutrient composition in the protein substitutes should be aimed at improving nutrient status in all adults adhering to a PKU diet.

In Summary

Despite methodological limitations in the present study, the data obtained showed that late-treated adult PKU patients manage to maintain a diet with adequate protein intake and therapeutic serum Phe levels over time. Subjects had, however, problems in adhering to nutritional recommendations for fruits and vegetables, added sugar and dietary fibre. Recommended intake of essential fatty acids required use of omega-3 supplements. Intake of folic acid, vitamin B₁₂ and iron was very high, due to fortification of the protein substitutes. The food records were carefully and accurately done, showing excess intakes of micronutrients for all subjects using highly fortified protein substitutes. We presume that early and continuously treated adults with classical PKU may have similar intakes when low blood Phe levels are maintained by diet alone. Further studies are needed to determine if requirements for micronutrients and omega-3 fatty acids differ in PKU patients compared to the general population. In the meantime composition of protein substitutes intended for adults with PKU might need adjustment.

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Conflict of Interest

Ingrid Wiig has attended meetings on dietetic management for metabolic disorders arranged and paid by Nutricia.

Kristina Motzfeldt has received compensation from Merck Serono as a member of the European Nutritionist Expert panel in PKU, and she has received fees for consulting or lecturing from Nutricia and Vitaflo Scandinavia.

References

- Acosta PB, Yannicelli S, Singh RH, Elsas LJ, Mofidi S, Steiner RD (2004) Iron status of children with phenylketonuria undergoing nutrition therapy assessed by transferrin receptors. *Genet Med* 6:96–101
- Arnold GL, Kirby R, Preston C, Blakely E (2001) Iron and protein sufficiency and red cell indices in phenylketonuria. *J Am Coll Nutr* 20:65–70
- Baumeister AA, Baumeister AA (1998) Dietary treatment of destructive behavior associated with hyperphenylalaninemia. *Clin Neuropharmacol* 21:18–27
- Fitzgerald B, Morgan J, Keene N, Rollinson R, Hodgson A, and Rymple-Smith J (2000) An investigation into diet treatment for adults with previously untreated phenylketonuria and severe intellectual disability. *J Intellect Disabil Res* 44: t-9
- Hanley WB, Feigenbaum A, Clarke JT, Schoonheydt W, Austin V (1993) Vitamin B12 deficiency in adolescents and young adults with phenylketonuria. *Lancet* 342:997
- Hvas AM, Nexø E, Nielsen JB (2006) Vitamin B12 and vitamin B6 supplementation is needed among adults with phenylketonuria (PKU). *J Inherit Metab Dis* 29:47–53
- Lee PJ, Amos A, Robertson L, Fitzgerald B, Hoskin R, Lilburn M, Weetch E, Murphy G (2009) Adults with late diagnosed PKU and severe challenging behaviour: a randomised placebo-controlled trial of a phenylalanine-restricted diet 1. *J Neurol Neurosurg Psychiatry* 80:631–635
- MacDonald A (2000) Diet and compliance in phenylketonuria. *Eur J Pediatr* 159(Suppl 2):136–141
- MacDonald A, Rylance G, Davies P, Asplin D, Hall SK, Booth IW (2003) Free use of fruits and vegetables in phenylketonuria. *J Inherit Metab Dis* 26:327–338
- McCaman MW, Robins E (1962) Fluorimetric method for the determination of phenylalanine in serum. *J Lab Clin Med* 59:885–890
- Moseley K, Koch R, Moser AB (2002) Lipid status and long-chain polyunsaturated fatty acid concentrations in adults and adolescents with phenylketonuria on phenylalanine-restricted diet. *J Inherit Metab Dis* 25:56–64
- Nordic Council of Ministers (2004) Nordic nutrition recommendations 2004. Nordic Council of Ministers, Aarhus
- Poustie VJ, Wildgoose J (2010) Dietary interventions for phenylketonuria. *Cochrane Database Syst Rev* CD001304
- Rimestad AH, Borgejordet Å, Vesterhus KN, Sygnetveit K, Løken EB, Trygg K, Pollestad ML, Lund-Larsen K, Omholdt-Jensen G, Nordbotten A (2001) Den store matvaretabellen (The official Norwegian table of food composition), 2nd edn. Gyldendal Norsk Forlag, Oslo
- Robinson M, White FJ, Cleary MA, Wraith E, Lam WK, Walter JH (2000) Increased risk of vitamin B12 deficiency in patients with phenylketonuria on an unrestricted or relaxed diet. *J Pediatr* 136:545–547
- Rose HJ, White F, MacDonald A, Rutherford PJ, Favre E (2005) Fat intakes of children with PKU on low phenylalanine diets. *J Human Nutr Dietetics* 18:395–400
- Scriver CR, Levy H, Donlon D (2011) Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. In: Valle D, Beaudet A, Vogelstein B, Kinzler KW, Antonarakis SE, Ballabio A (eds) *The*

- online metabolic & molecular bases of inherited disease. The McGraw-Hill Companies
- Sutor CW, Bailey LB (2000) Dietary folate equivalents: interpretation and application. *J Am Diet Assoc* 100:88–94
- Ten Hoedt AE, de Sonnevile LM, Francois B, Ter Horst NM, Janssen MC, Rubio-Gozalbo ME, Wijburg FA, Hollak CE, Bosch AM (2011) High phenylalanine levels directly affect mood and sustained attention in adults with phenylketonuria: a randomised, double-blind, placebo-controlled, crossover trial. *J Inherit Metab Dis* 34:165–171
- Trefz F, Maillot F, Motzfeldt K, Schwarz M (2011) Adult phenylketonuria outcome and management. *Mol Genet Metab* 104(Suppl):S26–S30
- Vugteveen I, Hoeksma M, Monsen AL, Fokkema MR, Reijngoud DJ, van RM, van Spronsen FJ (2011) Serum vitamin B12 concentrations within reference values do not exclude functional vitamin B12 deficiency in PKU patients of various ages. *Mol Genet Metab* 102:13–17
- Weetch E, MacDonald A (2006) The determination of phenylalanine content of foods suitable for phenylketonuria. *J Hum Nutr Diet* 19:229–236
- Yannicelli S, Ryan A (1995) Improvements in behaviour and physical manifestations in previously untreated adults with phenylketonuria using a phenylalanine-restricted diet: a national survey. *J Inherit Metab Dis* 18:131–134
- Yi SH, Kable JA, Evatt ML, Singh RH (2011) A cross-sectional study of docosahexaenoic acid status and cognitive outcomes in females of reproductive age with phenylketonuria. *J Inherit Metab Dis* 34:455–463
- Yi SH, Singh RH (2008) Protein substitute for children and adults with phenylketonuria. *Cochrane Database Syst Rev* CD004731