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Nutritional composition of low protein and phenylalanine-restricted dishes prepared for phenylketonuric patients

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ABSTRACT

Nutritional management is essential for Phenylketonuria (PKU) treatment, consisting in a semi-synthetic and low phenylalanine (Phe) diet, which includes strictly controlled amounts of low protein natural foods (essentially fruits and vegetables) supplemented with Phe-free protein substitutes and dietetic low-protein products. PKU diet has to be carefully planned, providing the best ingredient combinations, so that patients can achieve good metabolic control and an adequate nutritional status. Hereupon, it is mandatory to know the detailed composition of natural and/or cooked foodstuffs prepared specifically for these patients.

We intended to evaluate sixteen dishes specifically prepared for PKU patients, regarding the nutritional composition, Phe and tyrosine (Tyr) contents, fatty acids profile, and vitamins E and B_{12} amounts.

The nutritional composition of the cooked samples was 15.5–92.0 g/100 g, for moisture; 0.7–3.2 g/100 g, for protein; 0.1–25.0 g/100 g, for total fat; and 5.0–62.0 g/100 g, for total carbohydrates. Fatty acids profile and vitamin E amount reflected the type of fat used. All samples were poor in vitamin B_{12} (0.3–0.8 μg/100 g). Boiled rice presented the highest Phe content: 50.3 mg/g of protein. These data allow a more accurate calculation of the diet portions to be ingested by the patients according to their individual tolerance.

1. Introduction

Phenylketonuria (PKU; OMIM 261600) is a rare autosomal recessive inherited metabolic disorder, but it is also the most prevailing inherited defect in amino acid metabolism (van Spronsen, 2010). The deficiency of phenylalanine hydroxylase (Bélanger-Quintana, Burlina, & Muntau, 2011; Scriver & Kaufman, 2001; Smith & Lee, 2000), the liver enzyme that converts dietary phenylalanine (Phe) into tyrosine (Tyr), leads to persistent elevated Phe blood and tissue toxic concentrations. For more than 60 years, neonatal diagnosis combined with an appropriate dietary intervention, have been crucial to prevent severe mental retardation and attain a good patient prognosis (Blau, Hennermann, Langenbeck, & Lichter-Konecki, 2011; Blau, van Spronsen, & Levy, 2010; de Groot, Hoekesma, Blau, Reijngoud, & van Spronsen, 2010; MacDonald, Rocha, van Rijn, & Feillet, 2011; van Spronsen & Enns, 2010). In order to reach safe concentrations of this amino acid in blood, patients need to follow a Phe-restricted diet (Giovannini, Verduci, Salvatici, Fiori, & Riva, 2007; Rocha et al., 2012). Notwithstanding, Phe is an essential amino acid (EAA) and is required to assure normal anabolism. For PKU individuals, Tyr also becomes an EAA due to the enzymatic defect that no longer allows the liver to convert Phe into Tyr. Although restricted, the diet still has to provide an adequate intake of all nutrients, including little amounts of Phe, in order to guarantee normal development of PKU patients (MacDonald et al., 2011; van Spronsen, van Rijn, Bekhof, Koch, & Smit, 2001). The dietary management consists in a semi-synthetic low-Phe diet, including Phe-free and Tyr enriched

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amino acids mixtures and a wide variety of dietetic low-protein products necessary to accomplish the daily energy requirements of the patients (Feillet & Agostoni, 2010). Considering the nutritional recommendations and individuals' tolerance to Phe, the diet may also incorporate strictly controlled amounts of natural foods, essentially fruits, vegetables and other foodstuffs with low protein content (Bremer, Anninos & Schuldz, 1996; Feillet & Agostoni, 2010; Weetch & MacDonald, 2006), resembling a vegan like food pattern.

As a result of avoiding animal products, due to its high protein content, PKU patients are likely to present lower intakes of some micronutrients, like vitamins A, C and E, vitamins B_2, B_6 and B_{12}, and folates, as well as selenium, iron, zinc and calcium. This is often reported in PKU patients, especially if there is poor amino acid mixture compliance (Craig, Mangels, & American Dietetic Association, 2009; Feillet & Agostoni, 2010; Hvas, Nexo, & Nielsen, 2006; MacDonald et al., 2011; Walter, 2011).

Until few years ago the main goal of diet therapy in PKU was to prevent mental retardation. Nowadays, the nutritional and dietary management intends to improve diet compliance, nutritional status and patient's quality of life (Feillet & Agostoni, 2010). According to this, daily meals need to be carefully planned and Food Composition Databases (FCD) are essential tools to work within this context. Nonetheless, they offer scarce or even no information about the nutritional composition of some foods and most processed dishes, especially in what concerns to Phe or other amino acids content.

Therefore, the aim of this work was to study the nutritional composition (regarding not only macronutrients, but also Phe and Tyr contents, fatty acids profile, and vitamins E and B_{12} content) of low protein dishes, most of them selected from a list of recipes specifically intended for PKU patients (Almeida, 1995, 2001). With this, we expect to contribute to fulfill gaps mentioned by health professionals and PKU patients as well as other individuals or institutions directly or indirectly involved in PKU dietary management.

2. Material and methods

2.1. Standards and reagents

For the macronutrients analysis all analytical grade reagents were purchased from Panreac (Barcelona, Spain) and Merck (Darmstadt, Germany). L-phenylalanine, L-tyrosine, L-norleucine and dansyl chloride were obtained from Sigma (St. Louis, MO, USA). The fatty acid methyl ester standard mixture was acquired from Supelco (Bellefonte, PA, USA). Tocopherols (α, β, γ and δ) and tocotrienols (α, β, γ and δ) were purchased from Calbiochem (*La Jolla*, California, USA) and tocol was obtained from Matreya Inc. (Pennsylvania, USA). Butylated hydroxytoluene (BHT) used was from Aldrich (Madrid, Spain).

HPLC-grade acetonitrile and 1,4-dioxane were from Fluka (Madrid, Spain). HPLC grade n-hexane was from Merck (Darmstad, Germany). Purified water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Samples and sample preparation

The low-protein dishes, including soups ($n = 2$), main courses ($n = 7$), desserts ($n = 3$) and other daily basic foods ($n = 4$) analyzed in this study were selected from recipe books specifically planned for PKU patients (Almeida, 1995, 2001). Regular ingredients were purchased in local supermarkets. Dietetic low protein and low-Phe products were kindly supplied by the Medical Center of Genetics *Doutor Jacinto de Magalhães* (INSA, IP, Porto, Portugal).

The dishes were prepared and thermally processed, in triplicate, according to the instructions of the respective recipes, using domestic scale utensils and equipments. Samples were homogenized (Classical A320R1, Moulinex, France) and immediately used to quantify moisture, protein, fat and ash contents. For the other chemical parameters (amino acids, fatty acids, and vitamins E and B_{12}) homogenized samples were kept at −20 °C until analyses.

2.3. Macronutrients analysis

Moisture content was instrumentally determined using an infrared moisture analyzer (Model SMO 01, Scaltec Instruments, Germany). The ash content was determined by incinerating the sample in a muffle furnace at 500–550 °C, according to 923.03 method (AOAC, 2000a). Protein content was determined using the Kjeldahl procedure (Egli, 2008) and calculated using the conversion factor 6.25 (Tontisirin, MacLean, Warwick, & Food and Agriculture Organization of the United Nations, 2003). Total fat was determined by Soxhlet (method 920.39, AOAC, 2000b). Total carbohydrate content was determined by difference (Tontisirin et al., 2003). The energetic values were calculated in accordance to the Atwater factors. Analyses were performed in triplicate and results are expressed as g/100 g.

2.4. Phe and Tyr determination

Phe and Tyr were analyzed by reversed-phase HPLC with fluorescence detection after submitting samples to an acidic hydrolysis (HCl 6 mol/l, 110 °C, 24 h) (Fountoulakis & Lahm, 1998; Paramás, Bárez, Marcos, García-Villanova, & Sánchez, 2006), and derivatization with dansyl chloride (Navarro, Aristoy, & Izquierdo, 1984).

The chromatographic analysis was carried out in an HPLC integrated system equipped with an AS-950 automated injector, a PU-980 pump, a CO-2060 Plus oven, and an FP-920 fluorescence detector (Jasco, Japan) programmed for excitation at 335 nm and emission at 514 nm. The compounds separation was achieved in a Tracer Excel ODS-A column (5 μm; 250 × 4 mm, Teknokroma, Spain) according to Pimentel (2011) Chromatographic data were analyzed using a Borwin-PDA Controller Software (JMBS, France). The amino acids were identified by retention time comparison with authentic standards and by standards addition. Quantification was carried out on the basis of the internal standard method. Results are expressed in mg/g of protein. Analyses were performed in triplicate.

2.5. Total vitamin E determination

Lipid fraction for vitamin E quantification was obtained by Soxhlet extraction with petroleum ether (2.5 h). The chromatographic analysis was carried out in an HPLC integrated system equipped with an AS-950 automated injector, a PU-980 pump, a MD-910 multiwavelength diode array detector (DAD) and a FP-920 fluorescence detector (Jasco, Japan), programmed for excitation at 290 and emission at 330 nm. The chromatographic separation of the compounds was achieved on a normal phase Supelcosil™ LC-SI (3 μm; 75 × 3.0 mm; Supelco, Bellefonte, PA, USA) according to Alves, Casal, and Oliveira (2009). Chromatographic data were analyzed using a Borwin-PDA Controller Software (JMBS, France).

2.6. Fatty acids (FA) profile

Fatty acid methyl esters (FAME) were prepared, in triplicate, by transmethylation using boron trifluoride (Sigma Aldrich St. Louis, MO, USA) according to Shantha and Ackman (1990) and analyzed in a Shimadzu GC-2010 gas chromatograph with a flame ionization