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Protein intake and physical activity are associated with body composition in individuals with phenylalanine hydroxylase deficiency☆

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ABSTRACT

Objective: Determine whether body composition as it relates to dietary protein in patients with phenylalanine hydroxylase (PAH) deficiency is associated with genotype, dietary factors, and lifestyle choices.
Methods: We examined associations between protein intake (intact and medical foods: MF) and body composition in PAH-deficient patients along with, physical activity, and genotype. Protein intakes (total, intact, and MF) were analysed from three-day food records with Nutrition Data System for Research (NDSR) in 59 children and 27 adults (N = 86, median age = 16.0 years). The severity of PAH deficiency was classified using the genotype assigned value method (AV sum). Physical activity was assessed using a study-developed question (light vs. intense activity). Body composition was measured by DXA, including android:gynoid ratio (A:G), fat-free mass index (FFMI), fat mass index (FMI), and FMI:FFMI ratio.
Results: High intact protein intake was associated with high FFMI ($r_s = 0.75$, $p = 0.008$) and low FMI:FFMI ($r_s = -0.59$, $p = 0.04$) in adults. Only in children, MF protein ($r_s = 0.38$, $p = 0.04$) was directly proportional to FFMI. Median intact protein intakes of adults (25.1 vs. 9.9 g/d, $p < 0.001$) and children (11 vs. 6 g/d, $p < 0.001$) were higher than prescribed. Only in adults, the actual median MF protein intake was lower than prescribed (53 vs. 60 g/d, $p = 0.03$). In adults and children, light activity was associated with higher fat mass indices compared to intense activity (adults: FMI:FFMI: $\beta = 1.1$, $p = 0.001$, children: FMI:FFMI: $\beta = 1.1$, $p = 0.007$; FMI $\beta = 2.1$, $p = 0.01$; A:G $\beta = 1.1$, $p = 0.04$). All associations remained significant after covariate adjustment. Genotype was not associated with body composition.
Conclusions: Although fat-free mass in adults was positively associated with intact protein intake, it should be consumed as prescribed per individual tolerance to maintain plasma Phe concentrations within treatment range. In children, total protein maximized with MF should be encouraged to promote lean mass. Nutrition counselling could be complemented with physical activity recommendations for optimal clinical outcomes.

1. Introduction

Phenylalanine hydroxylase (PAH) deficiency, an autosomal recessive inborn error of metabolism, occurs in 1:10,000 live births [1]. Mandatory newborn screening for PAH allows early, asymptomatic detection and early treatment intervention [2]. However, overweight/obesity has been reported in adults and children with PAH deficiency, with prevalence estimates ranging from 10%–55% among various international studies [2–7]. The study of body composition changes in health and disease have led to a greater understanding of growth, aging, malnutrition, and obesity as well as the impact of medical interventions and assessment methods in human body composition [8]. This is especially pertinent to patients with PAH deficiency given this patient population is reliant on synthetic amino acid formula for most of their protein requirements and many nutrients, which may have an impact on lean body mass compared to a regular diet containing intact protein.

With respect to diet, medical nutrition therapy is the cornerstone for managing PAH deficiency. Goals are to maintain serum phenylalanine (Phe) concentrations between 2 and 6 mg% (120–360 μmol/L) by limiting intact or dietary protein intake, and to incorporate the intake of synthetic amino acid mixtures devoid of phenylalanine or medical food (MF). Based on PAH severity, clinicians recommend a ratio of intact protein:MF ranging from 20:80 to 10:90 [9]. The latest phenylketonuria (PKU) guidelines recommend individuals consume 120–140% more total protein than the recommended protein intake for the general

Abbreviations: PAH, phenylalanine hydroxylase; MF, medical foods; A:G, android:gynoid ratio; FFMI, fat-free mass index; FMI, fat mass index; Phe, phenylalanine; DXA, dual-energy X-ray absorptiometry; METs, metabolic equivalents; MHP, mild/mild hyperphenylalaninemia; PA, physical activity; RDA, recommended dietary allowances.

☆ Clinical Trial Registry: KUVAN study, NCT00688844; CAMP study, NCT01659749.

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population, largely met through MF [9,10]. A synthetic amino acid mixture (e.g., MF devoid of phenylalanine) is absorbed more rapidly and used less efficiently than intact protein [11,12], triggering more rapid ghrelin-induced appetite stimulation [13]. Restriction of dietary phenylalanine often results in meeting energy requirements through energy-dense, nutrient-poor "Phe-free foods," high in fat and simple sugars [14]. As a result, the rapid absorption of synthetic amino acid mixtures, and secondary appetite stimulation coupled with energy-dense, nutrient-poor diet, could promote unfavourable body composition, particularly low lean mass and high fat mass. To study the effect of protein consumption on body composition, energy intake should be controlled. At present, only an Austrian study in PAH-deficient subjects 2–15 years of age (N = 22) showed a direct association between intact protein and FFM [15], although no association was reported with MF protein.

Among several methods for studying in vivo human body composition, dual-energy X-ray absorptiometry (DXA) has emerged as one of the most commonly used clinical standards [16,17]. To our knowledge, only one published study has utilized DXA to measure body composition. This study reported that pediatric PAH deficient patients in Greece had similar lean mass and fat mass compared to healthy controls, however within the patient group a direct association was found between plasma Phe control and fat mass, becoming more apparent during puberty [18]. All other studies have utilized indirect methods such as bioelectrical impedance, skin fold measurements, and Bod Pod (or air displacement plethysmography). In addition, no other studies have investigated the potential role of physical activity and PAH genotype in body composition outcomes.

Thus, the purpose of this study was to identify the relationship between dietary protein (as measured accurately with 3-day diet record), physical activity, and PAH genotype on lean mass and other body composition parameters in a large subject sample with PAH deficiency by using the DXA gold standard method.

2. Methods

Data from 2 investigator-initiated studies were included: the Kuvan study (N = 58) and the Camp study (N = 28). Participants were males and females diagnosed with PAH deficiency, 4–50 years of age. The Kuvan study was a one-year longitudinal study conducted at Emory University examining the impact of BH4, i.e., sapropterin dihydrochloride (Kuvan®, BioMarin Pharmaceutical Inc., Novato, CA, USA), on health outcomes in BH4-responsive individuals. Camp is a 1-week education and research-based event hosted annually at Emory University since 1994. The camp provides socialization and lifestyle coaching to adolescent and adult females with PAH deficiency and other amino acid disorders. Baseline data (year 2008) only from Kuvan and Camp (years: 2013–2015) were included in this cross-sectional analysis.

All participants provided written informed consent (≥18 years) or verbal assent with the legal guardian's written informed consent (<18 years). Both study protocols and informed consent/assent procedures were approved by the Emory University Institutional Review Board (Kuvan: IRB00007828; Camp: IRB00002447). Details of the Kuvan study are published elsewhere [19,20].

2.1. Inclusion/exclusion criteria

Inclusion criteria for the Kuvan study were: a medical diagnosis of PAH deficiency, minimum age of 4 years, not consuming sapropterin within 8 weeks prior to study involvement, and not having self-reported co-morbidities (e.g., diabetes, hypertension). Camp inclusion criteria were similar, except generally healthy females with PAH deficiency age 11 and older were eligible. Exclusion criteria included any patient who participated in both studies, pregnant, and literacy/comprehension difficulties limiting ability to provide informed consent/assent.

2.2. Body composition and anthropometric measurements

A whole-body DXA scan was performed at Emory University Hospital on a Lunar Prodigy system (GE Healthcare, Madison, WI, USA) by trained hospital technicians. Apart from body composition parameters, namely fat mass and lean mass (kg and percent) obtained through the DXA readings, comprehensive indices were calculated: android:gynoid fat mass ratio (A:G), fat-free mass index (FFMI), fat mass index (FMI), and FMI:FFMI ratio. A:G ratio was calculated using the ratio of percent fat mass in the android region to percent fat mass in the gynoid region. A:G ratio > 1.0 in men and >0.8 in women indicates abdominal obesity [21]. FFMI was calculated as lean mass (kg) + bone mineral content (kg) / length $(m)^2$ [21]. FMI, an indicator of body fat mass, was calculated as fat mass (kg) / length $(m)^2$ [21]. FMI:FFMI ratio was calculated using the ratio of FMI to FFMI [21]. A higher FMI:FFMI ratio indicates less favourable body composition with higher fat mass compared to fat-free mass [21]. These indices (FFMI, FMI and FMI:FFMI) are useful in examining body composition parameters by excluding differences associated with height. Height-independent body composition indices can then offer interpretation of nutritional status, comparison of results between studies, and the development of body composition percentile tables [22].

Two trained research nurses were responsible for anthropometric measurements for both studies. Weight was measured to the nearest 0.1 kg in light clothing without shoes. Standing height was measured to the nearest cm with no hair accessories or shoes. Body mass index [BMI: weight (kg) / height (m^2)] was calculated for adults (>19.0 years) [23], and BMI z-scores were calculated for children (4.0–19.0 years) [24]. BMI (kg/m^2) was categorized according to WHO criteria: underweight (≤18.5), healthy weight (18.5–24.9), overweight (25–29.9), and obese (≥30.0 kg/m^2) [23]. In children and adolescents, BMI z-scores were classified as wasted (<−2.0), healthy weight (≥ −2.0 to <1.0), possible risk of overweight/overweight (>1.0 to ≤3.0) and obese (>3.00) [22]. In children and adolescents, globally accepted BMI z-scores suggested by the World Health Organization [24] were used rather than BMI percentiles provided by the CDC for Americans only [25] to facilitate international comparison between studies.

2.3. Plasma amino acid analysis

In the Kuvan and the Camp studies, blood was collected by a phlebotomist to evaluate plasma amino acids, which were analysed by the Emory Genetics Laboratory using the Beckman 6300 Amino Acid Analyser [26]. Plasma Phe concentrations were reported in mg% and μmol/L.

2.4. Genotype

PAH genotypes were sourced from participants' medical records. If unavailable, peripheral blood was collected from finger sticks, spotted on filter papers, and analysed using high-resolution melt profiling [27]. Mutations were classified by location (i.e., exon, intron, untranslated region) and by type (missense, mRNA processing, nonsense, or deletion). The phenotype classification of classical, moderate, mild, mild hyperphenylalaninemia, and unclassified PAH deficiency was determined for each participant using the AV sum system [28], which was adapted from the genotype classification system by Guldberg et al. [29]; example cases provided in Table 1.

2.5. Dietary assessment

Two registered dietitians were responsible for collecting and analysing dietary data for the Kuvan and Camp studies. All participants were provided uniform 3-day diet records and educated on completing records before study visits. Participants brought completed records to study visits and dietitians reviewed food records with participants or

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