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Metabolomic changes demonstrate reduced bioavailability of tyrosine and altered metabolism of tryptophan via the kynurenine pathway with ingestion of medical foods in phenylketonuria

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ABSTRACT

Background: Deficiencies of the monoamine neurotransmitters, such as dopamine synthesized from Tyr and serotonin synthesized from Trp, are of concern in PKU. Our objective was to utilize metabolomics analysis to assess monoamine metabolites in subjects with PKU consuming amino acid medical foods (AA-MF) and glycomacropeptide medical foods (GMP-MF).

Methods: Subjects with PKU consumed a low-Phe diet combined with AA-MF or GMP-MF for 3 weeks each in a randomized, controlled, crossover study. Metabolomic analysis was conducted by Metabolon, Inc. on plasma (n = 18) and urine (n = 9) samples. Catecholamines and 6-sulfatoxymelatonin were measured in 24-h urine samples.

Results: Intake of Tyr and Trp was ~50% higher with AA-MF, and AA-MF were consumed in larger quantities, less frequently during the day compared with GMP-MF. Performance on neuropsychological tests and concentrations of neurotransmitters derived from Tyr and Trp were not significantly different with AA-MF or GMP-MF. Plasma serotonin levels of gut origin were higher in subjects with variant compared with classical PKU, and with GMP-MF compared with AA-MF in subjects with variant PKU. Metabolomics analysis identified higher levels of microbiome-derived compounds synthesized from Tyr, such as phenol sulfate, and higher levels of compounds synthesized from Trp in the kynurenine pathway, such as quinolinic acid, with ingestion of AA-MF compared with GMP-MF.

Conclusions: The Tyr from AA-MF is less bioavailable due, in part, to greater degradation by intestinal microbes compared with the Tyr from prebiotic GMP-MF. Research is needed to understand how metabolism of Trp via the kynurenine pathway and changes in the intestinal microbiota affect health for individuals with PKU.

This trial is registered at www.clinicaltrials.gov as NCT01428258.

1. Introduction

Phenylketonuria (PKU; OMIM 261600) is an autosomal recessive disorder caused by deficiency of hepatic phenylalanine hydroxylase (PAH; EC 1.14.16), which catalyzes the conversion of Phe to Tyr using tetrahydrobiopterin as a cofactor [1]. In untreated PKU, consumption of a normal diet causes Phe to accumulate in blood and brain resulting in cognitive impairment. The mainstay of PKU treatment is a low-Phe diet restricted in protein from natural foods and supplemented with amino acid medical foods (AA-MF) or glycomacropeptide medical foods (GMP-MF) to provide the majority of dietary nitrogen and micronutrients [2,3]. Glycomacropeptide (GMP) is a 64-amino acid glycophosphopeptide derived from κ-casein in bovine milk that is produced during the manufacture of cheese and shows prebiotic properties [4,5]. The absence of aromatic amino acids in GMP (Phe, Tyr and Trp) enables the formulation of a variety of palatable GMP-MF for the management of PKU and tyrosinemia. Because GMP is not a complete protein, it requires supplementation with the following indispensable amino acids for PKU: Arg, His, Leu, Trp and Tyr [6,7]. Individuals who are diagnosed in infancy and maintain control of blood Phe levels within 120–360 μM/L show IQs within the normal range, although

Abbreviations: AA-MF, amino acid medical foods; CNS, central nervous system; GMP, glycomacropeptide; GMP-MF, glycomacropeptide medical foods; LNAA, large neutral amino acid; LAT1, LNAA transporter 1; PAH, phenylalanine hydroxylase; PKU, phenylketonuria.

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neurocognitive symptoms, such as decreased executive function, and neuropsychological symptoms, such as anxiety and depression, persist [8,9].

Abnormal metabolism of the monoamine neurotransmitters, particularly deficiencies of dopamine synthesized from Tyr and serotonin synthesized from Trp in the central nervous system (CNS), may relate to the occurrence of neurocognitive and neuropsychological symptoms in individuals with PKU [9,10]. High blood Phe levels are presumed to cause deficiencies of the precursor amino acids Tyr and Trp for synthesis of the monoamine neurotransmitters by competitive inhibition for the large neutral amino acid (LNAA) transporter 1 (LAT1) which transports the LNAA from blood into the brain [11]. Supplementation with LNAA, including Tyr and Trp, in individuals with PKU has been reported to increase cerebrospinal concentrations of metabolites of dopamine and serotonin [10–12]. Thus, the concentration of Tyr and Trp in medical foods for the management of PKU may impact neurotransmitter synthesis; however, the bioavailability of these amino acids for absorption from the gastrointestinal tract has not been studied. This is an important consideration, given evidence that the intestinal microbiome is altered in human PKU with ingestion of AA-MF [13] and improved with ingestion of a diet containing GMP. GMP alters microbial phyla and increases production of short chain fatty acids compared with a diet containing amino acids [4,5]. Moreover, genes for metabolism of amino acids, especially Tyr and Trp, are overrepresented in bacteria comprising the microbiome in the distal gut [14].

In our recent randomized, controlled, crossover trial, we observed a disparity between intake and plasma concentrations of Tyr and Trp [6]. Despite significantly higher intakes of Tyr and Trp in subjects consuming a low-Phe diet in combination with AA-MF compared with GMP-MF, fasting plasma concentrations of Tyr and Trp were not significantly different. This led us to the hypothesis that changes in the intestinal microbiota with ingestion of AA-MF result in degradation of Tyr and Trp, thus reducing their bioavailability for synthesis of neurotransmitters. Our objective was to utilize metabolomics analysis to assess metabolites and neurotransmitters derived from Tyr and Trp in plasma and urine samples from subjects with PKU consuming both AA-MF and GMP-MF.

2. Materials and methods

2.1. Experimental design

We conducted metabolomics analysis of a subset of plasma (n = 18) and 24-h urine (n = 9) samples obtained from PKU subjects who completed our randomized, controlled, crossover trial where 30 early-treated PKU subjects consumed, for 3-wk. each, their usual low-Phe diet combined with AA-MF or GMP-MF [6]. The University of Wisconsin-Madison Health Sciences Institutional Review Board approved the study protocol. All subjects provided written informed consent. The trial was registered at www.clinicaltrials.gov as NCT01428258.

Participants consumed their preferred AA-MF resulting in the use of 8 different AA-MF for the urine study and 14 different AA-MF for the plasma study. The GMP-MF were donated by Cambrooke Therapeutics and contained Glytactin™, a proprietary formulation of ~70% GMP (cGMP-20, Arla Foods Ingredients) and ~30% supplemental AAs (Arg, His, Leu, Trp, Tyr). For the urine study, participants recorded all nutritional intake for 48–72 h, starting 24–48 h prior to and during the final days of each diet treatment. For the plasma study, participants recorded all nutritional intake on a consecutive 3-d food record during the final days of each treatment. Intake of macronutrients and amino acids was estimated from the food records using Food Processor SQL (Version 10.12.0, ESHA) for medical foods and natural foods; natural foods are defined as all food and beverages consumed that are not PKU medical foods.

Metabolomics analysis was performed on fasting plasma samples obtained at the final study visits after each subject consumed a low-Phe diet with AA-MF or GMP-MF for 3-wk. each at home. There was a 3-wk. washout period between treatments during which subjects consumed their usual AA-MF [6]. The 18 plasma samples were chosen to include the 9 subjects who consented to provide 24-h urine samples for both AA-MF and GMP-MF treatments and to represent the median plasma Phe response from the total sample of 30 subjects [6]. Urine samples were collected after subjects followed their low-Phe diet combined with AA-MF and GMP-MF for 3-wk. (n = 4) or 1-wk. (n = 5); seven of the nine subjects completed the urine collection with the AA-MF treatment first. Plasma and urine samples were processed and stored frozen (−70 °C) until analyzed.

2.2. Amino acids and neurotransmitter metabolites in blood and urine

The fasting plasma amino acid profiles were determined using a Hitachi L-8900 amino acid analyzer [15]. Concentrations of dopamine, norepinephrine and epinephrine were determined in 24-h urine samples using standardized techniques in a commercial clinical laboratory (LabCorp; Dublin, OH, USA). The concentration of the serotonin metabolite 6-sulfatoxymelatonin, used as a biomarker to reflect serotonin synthesis in the CNS [16], was analyzed in 24-h urine samples using a commercial ELISA (EK-M6S, Buhlmann Laboratories AG, Switzerland) [17]. Concentrations of neurotransmitter metabolites in urine were assessed per mg creatinine and per 24-h which yielded similar statistical significance.

2.3. Metabolomics

The non-targeted metabolomics analysis on plasma and urine samples from PKU subjects was carried out by Metabolon, Inc. (Durham, NC, USA). Compounds were identified by comparison to Metabolon's

Table 1
Characteristics of PKU subjects.

Subject	Age	Mutation	Phe[b] (μmol/L)	Tyr[b] (μmol/L)	Trp[b] (μmol/L)
Classical[a]					
Female					
1	34	L242F; R408W	829–1009	23–29	15–19
2	28	F55 > Lfs; R408W	583–1160	30–55	18–28
3	23	R408W; IVS12	459–649	17–40	36–45
4	26	R408W; IVS7 + 3G > C	535–624	34–42	22–29
5	28	E280K; IVS12 + 1G > T	626–852	20–39	37–39
Male					
6	32	IVS1 + 5G > T; IVS12 + 1G > A	418–1086	38–42	32–41
7	35	R408W; R261Q	378–754	43–47	17–20
8	18	R408W; Y356X	417–851	38–57	5–31
9	29	F55 > Lfs; IVS5 + 1G > A	921–1152	39–46	24–57
10	18	R408W; IVS12 + 1G > T	284–600	35–36	19–24
11	44	R261Q; IVS10-11G > A	1059–1240	27–33	21–25
Variant					
Female					
12	34	E280K; E390G	541–583	20–28	21–25
13	15	R157N; L348 V	597–652	31–38	19–25
14	15	R158Q; R408W	640–823	26–34	18–22
15	49	L48S; 195_K96delinsK	180–297	47–53	32–35
16	24	165 T; IVS10-11G > A	451–532	34–59	30–32
Male					
17	34	R408W; IVS12 + 1G > A	178–821	28–37	19–26
18	17	R68S; IVS12 + 1G > T	206–263	28–39	30–39

Urine samples were obtained from subjects 1, 2, 6, 8, 12, 13, 14, 17, and 18. Subjects 13 and 14 maintained stable doses of sapropterin dihydrochloride during the study.

[a] Subjects classified as having a variant form of PKU displayed a phenylalanine hydroxylase genotype and/or response to sapropterin dihydrochloride that was consistent with a milder or variant form of PKU. Mutation names are defined at http://www.pahdb.mcgill.ca and http://www.biopku.org.

[b] The range of fasting plasma concentrations of Phe, Tyr and Trp reflect three determinations over a period of 8 weeks while subjects were following their usual diet with amino acid medical food.