Large Neutral Amino Acid Supplementation Increases Melatonin Synthesis in Phenylketonuria: A New Biomarker

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Objective To determine whether levels of melatonin in blood and urine can serve as a peripheral biomarker to reflect brain serotonin synthesis in individuals with phenylketonuria (PKU).

Study design We measured the levels of melatonin, a serotonin metabolite in the pinealocytes, in the blood and urine of individuals with PKU in a randomized double-blind placebo controlled crossover study consisting of three 3-week phases in 10 adults with PKU: phase 1 (washout), phase 2 (supplementation of large neutral amino acid [LNAA] tablets or placebo), and phase 3 (alternate supplementation). An overnight protocol to measure blood melatonin and urine 6-sulfatoxymelatonin and dopamine in first void urine specimens was conducted after each phase for subjects with PKU and once in 10 controls.

Results Significantly lower concentrations of these neurotransmitter metabolites were observed in subjects with PKU after phase 1 compared with controls (serum melatonin $P = .008$, urine melatonin $P = .0043$, urine dopamine $P < .0001$), with significant increases after LNAA supplementation compared with the placebo phase (serum melatonin $P = .0008$, urine melatonin $P = .0008$, urine dopamine $P = .0005$). The mean tryptophan/LNAA and tyrosine/LNAA ratios were markedly lower in subjects with PKU compared with controls, and these ratios were significantly increased in the LNAA phase compared with the placebo phase ($P = .016$, $P = .0003$, respectively). Blood phenylalanine levels in subjects with PKU were not significantly different between placebo and LNAA phases ($P = .74$).

Conclusion Blood and urine melatonin levels may serve as biomarkers reflecting brain serotonin synthesis in subjects with PKU. Because this cannot be evaluated using blood phenylalanine levels, it may provide information on neurotransmitter metabolism for optimal dietary management. *(J Pediatr 2013;162:999-1003)*.

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The dietary treatment of phenylketonuria (PKU) with restriction of phenylalanine (Phe) to reduce blood Phe levels was first introduced by Horst Bickel in the early 1950s[1] and remains the mainstay of PKU management today.[2] Blood levels of other amino acids, including tyrosine (Tyr) and tryptophan (Trp), often are not monitored in individuals with PKU, despite the fact that those under poor dietary control are known to have deficiencies of neurotransmitters, including serotonin and dopamine, in the central nervous system (CNS).[3-5] Neuropsychological studies of individuals with PKU who are diagnosed early and well controlled have shown a higher prevalence of characteristic deficits such as decreased executive functioning and internalizing disorders, including anxiety and depressive disorders.[4,6] It is presumed that high blood Phe causes deficiencies of the precursor amino acids of the neurotransmitters in the brain by competitive inhibition at the transporter level. The large neutral amino acid (LNAA) transporter 1 (LAT1) is highly expressed in the brain capillaries and transports the LNAA including branched chain (valine, leucine, and isoleucine), aromatic (Phe, Tyr, and Trp), and other amino acids including histidine, threonine, and methionine.[3] Improvement in neuropsychological symptoms and electroencephalography abnormalities in individuals with PKU have been reported after supplementation of LNAA.[4,7]

Supplementation of Tyr and Trp in individuals with PKU showed increases in the previously deficient cerebrospinal fluid (CSF) concentrations of homovanillic acid and 5-hydroxyindoleacetic acid, metabolites of dopamine and serotonin, respectively.[8,9] It is, however, impractical to monitor these neurotransmitters in CSF in the clinical management of individuals with PKU.

AUC	Area under the curve
CNS	Central nervous system
CSF	Cerebrospinal fluid
LAT1	Large neutral amino acid transporter 1
LNAA	Large neutral amino acid
Phe	Phenylalanine
PKU	Phenylketonuria
TAT1	T-type amino acid transporter 1
Trp	Tryptophan
Tyr	Tyrosine
USC	University of Southern California

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Trp hydroxylase (EC 1.14.16.4) is the rate-limiting enzyme in serotonin synthesis and is unsaturated with the concentration of Trp in the brain, thus, brain Trp concentration is the most important single metabolic determinant.[10] Melatonin, a serotonin metabolite in the pineal body, is released to blood during the night time and subsequently excreted into the urine as 6-sulfatoxymelatonin.[11] We hypothesized that: (1) melatonin would serve as a peripheral biomarker, reflecting CNS serotonin synthesis; (2) individuals with PKU would have low blood and urine melatonin levels, suggesting low CNS serotonin synthesis; and (3) these levels would increase with LNAA supplementation. To test this hypothesis, a randomized, double-blind, placebo-controlled, crossover study with LNAA supplementation in subjects with PKU was conducted. Urine dopamine levels were also measured to evaluate any effects of LNAA supplementation in the dopamine metabolism.

Methods

Ten adult patients with classic PKU and 10 adult control subjects without PKU were enrolled after obtaining informed consent. All study subjects were recruited from our outpatient population, and healthy controls were recruited from our research team and medical students. Subjects with PKU ranged in age from 20-49 years (mean ± SD: 29.1 ± 9 years), and the controls' ages ranged from 25-67 years (mean ± SD: 44.3 ± 15.7 years). The study protocol was approved by the University of Southern California (USC) Health Science Institutional Review Board.

The subjects with PKU completed three 3-week phases. After an initial washout phase (phase 1) without use of any medical food products, subjects with PKU were randomly assigned in a double-blind manner to take either LNAA or placebo tablets during the next 3 weeks (phase 2), then crossover to the alternate supplementation for the final 3 weeks (phase 3). They consumed a regular diet but avoided high protein foods and no dietary changes were made throughout the study. LNAA or placebo tablets (PheBloc; Applied Nutrition, Cedar Knolls, New Jersey) were given following the manufacturer's recommendation. The total number of study tablets for daily intake was determined based on the subject's weight: body weight × 0.5 (maximum total daily intake was 45 tablets per day). The LNAA tablets provided the following amount of LNAAs (mg/kg/d): Tyr 98.4, Trp 30.6, histidine 15.6, isoleucine 15.7, leucine 15.4, methionine 24.8, threonine 16.4, valine 16, and Phe 0.

The study subjects stayed overnight at the Clinical Trials Unit at USC University Hospital at the end of each phase. The 10 control subjects received no supplementation and were studied during 1 overnight stay. All subjects were given the same protein-controlled meal during the overnight evaluation. Serum melatonin was measured every 2 hours from 7 p.m.-7 a.m., and first void urine specimens were collected at 7 a.m. to measure dopamine and 6-sulfatoxymelatonin, to which 80%-90% of melatonin is metabolized and is excreted into urine.[10] Plasma amino acids were obtained before dinner at 7 p.m. Serum and urine specimens were processed and kept in a freezer (−20°C) until analyzed. Serum melatonin and urine 6-sulfatoxymelatonin were measured as described elsewhere,[12] and urine dopamine and plasma amino acids were measured by a commercial laboratory. Blood specimens for serum melatonin were obtained and processed under dim light after 11 p.m. to avoid subjects' eyes from being exposed to bright light, which potentially inhibits melatonin synthesis.

Statistical Analyses

The area under the curve (AUC) of melatonin during the overnight stay was calculated using the trapezoidal method. The ratios of Trp and Tyr to the sum of all 9 measured LNAAs, including leucine, valine, isoleucine, histidine, methionine, threonine, Phe, Tyr, and Trp, were also calculated for each subject. Comparisons between controls and subjects with PKU at the end of phase 1 and after LNAA supplementation on outcomes of interest were made with the *t* test or Wilcoxon rank sum test. Within-subject differences in the subjects with PKU over the 3 phases were examined using repeated measures ANOVA with post hoc contrast to compare the LNAA supplemented phase with the washout and placebo phases. Prior to analyses, a log transformation was applied to variables, as needed, to normalize distributions. Regression lines and correlation coefficient were calculated between urine 6-sulfatoxymelatonin and Trp/LNAA ratio and between urine dopamine and Tyr/LNAA ratio in controls and subjects with PKU after LNAA supplementation. Statistical tests were 2-sided with $P < .05$ considered statistically significant. Statistical analyses were performed using SAS v. 9.2 (SAS Institute, Cary, North Carolina).

Results

One of the control subjects (because of an accidental exposure to bright light during the overnight stay) and 1 subject with PKU (because of poor compliance) failed to complete the study. Nine subjects with PKU completed the study without experiencing any untoward effects. Dopamine and 6-sulfatoxymelatonin levels could not be measured in 1 of the 9 subjects with PKU because of an accidental loss of the urine specimens. The 9 subjects with PKU who completed the study were significantly younger than controls ($P = .026$) but had comparable sex distribution (PKU: 67% vs control: 44% male, $P = .64$).

Repeated measures ANOVA showed no differences within the PKU group between placebo and washout phases on any outcome measures. Therefore, all within group comparison reported below are for LNAA supplementation versus placebo phases.

Serum Melatonin

After the 3-week washout phase, subjects with PKU showed significantly lower nocturnal serum melatonin AUC than controls ($P = .008$). The subjects with PKU on LNAA supplementation showed significantly higher nocturnal melatonin AUC than after the placebo phase ($P = .0008$), although it did not reach the AUC of control group ($P = .0169$) (**Figure 1**).