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WASHINGTON UNIVERSITY IN ST. LOUIS
Department of Psychology

Phenylalanine Control Predicts Cognition and White Matter Integrity
in Children with Phenylketonuria

by
Anna Marie Hood

A thesis presented to the
Graduate School of Arts and Sciences
of Washington University in
partial fulfillment of the
requirements for the
degree of Master of Arts

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ABSTRACT OF THE THESIS

Objective: Phenylketonuria (PKU) is a hereditary metabolic disorder in which the amino acid phenylalanine (Phe) is not properly metabolized. As a result, Phe levels in the blood are elevated, even with early and continuous treatment to control Phe levels. Individuals with PKU have lower than expected intelligence, impairments in executive abilities, and compromised brain white matter integrity. This current study was conducted to examine the relationships between indices of Phe control and IQ, executive abilities, and white matter integrity in children with PKU. The overarching goal was to determine which indices should be monitored and controlled with particular care to optimize cognitive and neural outcomes in children with PKU.

Methods: Participants were 47 children with early- and continuously-treated PKU ranging from 6 – 18 years of age. Indices of Phe control reflected average Phe, change in Phe with age, variability in Phe, and Phe exposure over the lifetime prior to cognitive and neuroimaging evaluation. IQ was estimated using the Wechsler Abbreviated Scale of Intelligence (WASI) and children completed three tasks assessing executive abilities. Microstructural white matter integrity was assessed using two complementary diffusion tensor imaging (DTI) approaches to examine mean diffusivity (MD): region-of-interest (ROI) based analysis and voxel-wise tract based spatial statistics (TBSS) analysis.

Results: Across the IQ and executive tasks administered, variability in Phe was negatively correlated with 4 of 6 cognitive variables examined; average Phe, however, was negatively correlated with only 1 of 6 cognitive variables. In addition, variability in Phe at older ages was more predictive of executive performance than was variability in Phe at younger ages. With regard to white matter integrity, ROI analyses revealed that average Phe was negatively correlated with MD in 8 of 10 ROIs; average Phe predicted MD approximately 2 standard

deviations below those of healthy control children. In addition, exposure to Phe was negatively correlated with MD in 7 ROIs, change in Phe with age was negatively correlated with MD in 6 ROIs, and variability in Phe was negatively correlated with MD in 5 ROIs. Consistent with findings from ROI analyses, voxel-wise analyses showed that average Phe and exposure to Phe were negatively correlated with MD; however, change in Phe with age and variability in Phe were not correlated with MD in voxel-wise analyses.

Conclusions: Variability in Phe was the strongest predictor of cognitive performance, whereas average Phe was the strongest predictor of white matter integrity. Exposure to Phe predicted white matter integrity but not cognition. Overall, these findings indicated that variability in Phe, average Phe, and exposure to Phe should be carefully monitored and controlled to maximize cognitive and neural outcomes in children with early- and continuously-treated PKU. Findings also indicated that Phe monitoring and control should not be liberalized as children with PKU age.

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Introduction

Phenylketonuria (PKU) is a hereditary metabolic disorder associated with a deficiency in or absence of the phenylalanine hydroxylase enzyme. As a result, the amino acid phenylalanine (Phe) is not properly metabolized, and blood Phe is elevated in individuals with PKU (De Groot, Hoeksma, Blau, Reijngoud, & Van Spronsen, 2010). In individuals without PKU, Phe levels are typically $< 60 \mu\text{mol/L}$. In individuals with PKU, the goal is to maintain Phe levels between 120 and $360 \mu\text{mol/L}$ (National Institutes of Health Consensus Development Panel, 2001) although in many cases Phe levels are often much higher. From neurophysiological perspective, elevations in Phe disrupt the neurochemical cascade by which Phe is converted to tyrosine, a precursor of dopamine, and other catecholaminergic neurotransmitters (Scriver, 2007).

Untreated PKU typically results in significant neurologic compromise and cognitive impairment (i.e., profoundly lowered IQ and intellectual disability) (Paine, 1957). These serious cognitive sequelae, however, are avoided through early detection and dietary treatment to limit Phe intake (Mitchell, Trakadis, & Scriver, 2011; Paine, 1957). That said, individuals with early- and continuously-treated PKU often have IQs that are lower than expected in comparison with peers and family members (Ris, Williams, Hunt, Berry, & Leslie, 1994), as well as impairments in executive abilities such as inhibitory control, strategic processing, and working memory (Christ, Huijbregts, de Sonnevile, & White, 2010; DeRoche & Welsh, 2008).

The specific neural mechanisms underlying cognitive impairment in individuals with PKU are not yet fully elucidated. For decades it has been hypothesized that PKU-related cognitive impairment is associated with dopamine deficiency (De Groot et al., 2010). In addition, and of particular relevance to the current study, neuroimaging studies have shown that PKU is associated with widespread compromise of the white matter of the brain (Anderson et al., 2007;

Antenor-Dorsey et al., 2013; Peng, Peck, White, & Christ, 2013; White et al., 2013). In most of the neuroimaging research conducted to date, structural magnetic resonance imaging (MRI) has been used to identify white matter abnormalities that were detectable by visual inspection of MRI images (Anderson et al., 2004; Cleary et al., 1995; Leuzzi et al., 1993; Manara et al., 2009; Thompson et al., 1993). Categorical labels were then assigned to indicate the severity of the identified abnormalities. Although useful, this qualitative approach is imprecise and has provided little information regarding the nature of the white matter pathology associated with PKU.

Recently, a specialized MRI technique, diffusion tensor imaging (DTI), has been used to examine the microstructural white matter integrity of individuals with PKU (Antenor-Dorsey et al., 2013; Peng et al., 2013; White et al., 2010; White et al., 2013). DTI provides more refined information and is sensitive to subtle changes in white matter integrity that are not detectable using visual inspection of structural MRI images (Sener, 2004). DTI studies have shown that mean diffusivity (MD) is significantly lower in individuals with PKU in comparison with healthy controls (Dezortova, Hajek, Tintěra, Hejzmanova, & Sykova, 2001; Kono et al., 2005; Vermathen et al., 2007; White et al., 2010) and lower MD values have been associated with higher Phe (Vermathen et al., 2007; White et al., 2010). Prior research has posited that the underlying MD restriction in individuals with PKU may result from the accumulation of intracellular debris produced as a byproduct of inadequate Phe metabolism (Leuzzi et al., 2007).

Phe Control in Relation to Cognition and Brain White Matter

Previous studies of individuals with early- and continuously-treated PKU have focused almost exclusively on relationships between cognition and measures representing average Phe over specified periods (e.g., 1 month, 1 year, lifetime) prior to the time of cognitive evaluation. For the most part, these studies showed that lower IQ was associated with higher Phe (Azen, Koch,

Friedman, Wenz, & Fishier, 1996; Burgard, 2000; Smith, Beasley, & Ades, 1990). In fact, a meta-analysis of data from children with PKU and hyperphenylalaninemia indicated that each 100 $\mu\text{mol/L}$ increase in blood Phe predicted a reduction in IQ of 1.3 - 3.1 points (Waisbren et al., 2007). Higher Phe has also been negatively associated with performance on tasks assessing executive abilities (Albrecht, Garbade, & Burgard, 2009; Brumm et al., 2004; Christ et al., 2010; Janzen & Nguyen, 2010; Leuzzi et al., 2004).

In only a small number of studies have associations between cognition and any index other than average Phe been investigated. Vilaseca et al. (2010) and Burgard et al. (1996) found that greater variability (i.e., fluctuations) in Phe was related to lower IQ. In addition, although their results were inconclusive, Anastasoie et al. (2008) reported a statistical trend suggesting that greater variability in Phe may be associated with lower IQ in preschool and school-age children. Finally, in a small sample of preschool and early school-age children, Arnold et al. (1998) demonstrated that greater variability in Phe was related to poorer performance on tests of executive abilities. In contrast with findings from these studies, Viau et al. (2011) failed to find an association between variability in Phe and intelligence; however, in this study of children and adults, variability in Phe was examined only in relation to the verbal, processing speed, and perceptual reasoning subcomponents of IQ rather than overall IQ. Taken together, findings from the small number of studies conducted to date largely suggest that stability in Phe control is important for maximizing cognitive outcomes in individuals with PKU.

There are, however, issues that limit the interpretation of findings across these studies, such as the use of different indices of variability in Phe. Burgard et al. (1996) and Vilaseca et al. (2010) used the standard error of estimate (SEE Phe), whereas Anastasoie et al. (2008), Arnold et al. (1998), and Viau et al. (2011) used the standard deviation (SD Phe). Anastasoie et al.

(2008) also reported the number of spikes in Phe but did not conduct statistical analyses on this index of variability. In addition, sample size was quite small in the Arnold et al. (1998) study ($n = 18$), and in the study by Anastasoae et al. (2008) the relationship between SD Phe over the lifetime and IQ failed to reach statistical significance. Finally, to our knowledge, the association between variability in Phe and executive abilities has not been examined in children across the school-age range, a period during which executive abilities are rapidly developing. Thus, as pointed out in a recent review by Cleary et al. (2013), additional research is needed, using multiple methods to determine which index of Phe control is most strongly associated with cognitive outcomes.

Turning next to findings from neuroimaging, the majority of studies that have examined relationships between white matter compromise and Phe have almost exclusively focused on Phe at or near the time of neuroimaging evaluation (Anderson et al., 2007; Bick et al., 1993; Dezortova et al., 2001; Leuzzi et al., 2000; Sirrs et al., 2007; Toft et al., 1994). A few studies have examined mean Phe in the month or year preceding neuroimaging evaluation (Kono et al., 2005; Manara et al., 2009; Peng et al., 2013). Findings across these studies are somewhat inconsistent, but in general significant negative relationships have been found between white matter compromise and Phe. As these studies measured Phe in a single instance or over a short period, rather than over the lifetime, it is perhaps unsurprising that there was a lack of consensus about the relationship between white matter compromise and Phe.

Average Phe over the lifetime in relation to white matter compromise has been examined in only three studies (Anderson et al., 2004; Leuzzi et al., 1993; Pietz et al., 1996), and in all of the studies only white matter abnormalities detectable with visual inspection were used. Results from these studies are consistent, as all show that average Phe over the lifetime was negatively

related to the severity of white matter abnormalities. To our knowledge, the relationship between average Phe over the lifetime and microstructural white matter integrity has not been previously investigated utilizing DTI.

In only two studies have indices other than average Phe been examined, and in both of these studies, white matter compromise was identified via qualitative visual inspection of structural MRI images. Rupp et al. (2001) found that variability in Phe, measured as the SEE, was not related to white matter severity. In a more recent investigation, Das et al. (2013) also found no relationship between variability in Phe and white matter abnormality severity; it should be noted, however, that in this study the method of calculating variability in Phe was not reported. However, Das et al. (2013) did report a significant relationship between mean Phe at 0 - 10 years of age and the severity of white matter compromise.

Although findings from the neuroimaging studies just described are of interest, both are limited because they used qualitative categorization to describe severity of white matter compromise. In addition, both had small sample sizes, and in the Rupp et al. (2001) study, some adults were on dietary control to limit Phe intake whereas others were not. Finally, only the SEE was examined in the Rupp et al. (2001) study, whereas the measure of variability used in the Das et al. (2013) study was unreported. It is also possible that other indices of Phe control have greater power in terms of predicting compromised white matter integrity.

Rationale for the Current Study

The current study was conducted to thoroughly examine various indices of Phe control in relation to cognition and microstructural white matter integrity in school-age children with early- and continuously-treated PKU. The aspects of cognition examined were IQ and executive abilities (inhibitory control, working memory, and strategic processing). We evaluated

microstructural white matter integrity using two complementary DTI approaches: region of interest (ROI) analysis and voxel-wise tract based spatial statistics (TBSS) (Smith et al., 2004).

A variety of indices of Phe control were examined in relation to cognition and white matter integrity. Indices representing average Phe were mean Phe (the most commonly reported index) and the index of dietary control (IDC). A slope was computed to reflect change in Phe as a function of age. Three indices were computed to reflect variability in Phe: the SD Phe, the SEE Phe, and the percentage of spikes in Phe (% spikes). Two final indices represented exposure scores: mean exposure and SD exposure. Although the focus was on Phe over the lifetime, these 8 indices were computed for 3 developmental epochs (< 5, 5.0 – 9.9, and \geq 10 yrs. of age) prior to evaluation.

Methods

Participants and Phe Levels

Children with PKU ($n = 47$; 22 male, 25 female) were recruited through metabolic clinics at Washington University in St Louis ($n = 17$), Oregon Health & Science University ($n = 25$), the University of Missouri ($n = 3$), New York Medical College ($n = 1$), and the University of Nebraska ($n = 1$). Although 62 children were initially considered for inclusion in the study, 15 were excluded due to gaps in available Phe levels of greater than 2 years at some point prior to cognitive and neuroimaging evaluation. All children were diagnosed with PKU soon after birth and received early and continuous treatment through dietary management to limit Phe intake. Across the sample of 47 children, age ranged from 6 – 18 years ($M = 11.9$, $SD = 3.6$), and education ranged from 0 – 13 years ($M = 6.1$, $SD = 3.3$). No child had a reported history of major medical, psychiatric, or learning disorder unrelated to PKU, and no child was being treated

with sapropterin dihydrochloride (a pharmaceutical agent prescribed to reduce Phe levels) at the time of evaluation.

The number of blood Phe levels available across the lifetime of the 47 children comprising the sample ranged from 85 – 489 ($M = 221.3$, $SD = 104.7$), with 10,399 total Phe levels. Data from these 47 children were also available to examine indices of Phe control during the < 5 and 5.0 – 9.9 yrs. epochs, because all were at least 9.9 years of age at the time of evaluation. During the ≥ 10 yrs. epoch, data were available for only 29 children, because 18 children in the sample of 47 had not yet reached 10 years of age at the time of evaluation. The number of blood Phe levels available for the < 5, 5.0 – 9.9, and ≥ 10 yrs. developmental epochs ranged from 18 – 288 ($M = 133.5$, $SD = 61.8$), 14 – 154 ($M = 62.4$, $SD = 35.9$), and 2 – 214 ($M = 41.6$, $SD = 54.9$), respectively, with considerably more Phe levels obtained during the younger than older epochs.

Procedures

Approval to conduct this study was obtained from institutional review boards for the protection of human subjects at Washington University in St. Louis, Oregon Health & Science University, and the University of Missouri, the sites at which cognitive and neuroimaging evaluation was completed. All participants and/or their guardians provided written informed consent prior to engagement in study procedures. Measures of IQ and executive abilities were administered in a quiet room as components of a larger study that included additional measures of cognition that were not analyzed for this report. Administration of all cognitive and neuroimaging procedures occurred during a single session lasting approximately 4 hours. The metabolic clinics from which children were referred provided blood Phe levels over the lifetime based on available medical records.

Indices of Phe control

Eight indices of blood Phe control were computed over the lifetime prior to cognitive and neuroimaging evaluation; for secondary analyses, these indices were computed for 3 developmental epochs (< 5, 5.0 – 9.9, and \geq 10 yrs. of age). Two indices reflected average Phe: mean Phe and the IDC. Mean Phe was simply the mean of all available Phe levels for each child prior to cognitive and neuroimaging evaluation. To compute the IDC, median Phe for each year of age was calculated for each child; the mean of all median Phe levels was then calculated to determine the IDC for each child. The IDC was included because a greater number of Phe levels are typically obtained during earlier than later childhood, which results in a heavier weighting of earlier Phe levels when computing mean Phe. The IDC circumvents this issue because Phe levels from each year of age are given equal weight. We also obtained the slope from a regression function to represent change in Phe as a function of age.

To assess variability, 3 indices of Phe control were computed: SD Phe, SEE Phe, and % spikes in Phe. SD Phe indicated the degree of dispersion in Phe around the mean. SEE Phe indicated residual variation in Phe around a regression line, which reflected fluctuation in Phe that was not influenced by the mean or slope. Finally, similar to Anastasoiaie et al. (2008), spikes were counted as the number of Phe levels that were at least 600 $\mu\text{mol/L}$ greater than either the preceding or succeeding Phe level. Unlike Anastasoiaie et al. (2008), however, we examined % spikes (rather than number of spikes), which represented the number of spikes in relation to the total number of Phe levels available. This approach was used because children with more available Phe levels had more opportunities for spikes to be detected, although as a percentage their spikes may have been equivalent to that of children with fewer available Phe levels.

Finally, two Phe exposure indices (mean exposure and SD exposure) were computed,

which accounted for the duration of exposure (i.e., years) to elevated Phe or variability in Phe. As the first step in calculating each exposure score, we obtained the mean and standard deviation for mean Phe, SD Phe, and age across the entire sample of children. Z scores were then computed for each child based on the mean and standard deviation of the sample. Mean exposure for each child was then calculated by summing the z scores of mean Phe and the z scores of age. SD exposure for each child was calculated by summing the z scores of SD Phe and the z scores of age. The rationale for examining these two indices was that older children with PKU have experienced more prolonged exposure to elevations in Phe and variability in Phe than younger children (Perantie et al., 2007).

Cognition

General Intellectual Abilities.

The Wechsler Abbreviated Scale of Intelligence (WASI; Psychological Corporation, 1999) was used to estimate general intellectual abilities (IQ) based on a composite of Vocabulary and Matrix Reasoning subtest scores. The Vocabulary subtest comprises 31 items and is designed to measure word knowledge and verbal concept formation. Children defined either pictures or words. Scores for each item are 0, 1, or 2, which were summed to obtain the subtest score. (The Matrix Reasoning subtest is described in the Executive Abilities section below).

Administration of WASI subtests was in accordance with test manual instructions, and age-referenced normative data from the test manual were used to generate an estimated IQ. In addition to IQ, standard scores from Vocabulary and Matrix Reasoning subtests were examined separately to determine their unique relationships with indices of Phe control. Matrix Reasoning was of particular interest, as this subtest assesses strategic processing, which is a component of executive abilities.

Executive Abilities.

Three tests of executive abilities were administered to assess strategic processing, working memory, and inhibitory control. Matrix Reasoning is a 30-item subtest of the WASI designed to measure perceptual reasoning and requires strategic processing in order to perform effectively. During this subtest, children viewed an incomplete matrix or series and selected one of five responses that completed the matrix or series. The subtest score was obtained by counting the number of correct responses.

An n-back task was administered to assess working memory. Two conditions were presented: letter and location. In both conditions, children observed one of eight letters (C, F, H, J, N, P, Q, and S) positioned at one of eight locations along an imaginary circle that was eccentric to central fixation (+) on a computer monitor. In the location condition, children were asked to press a target button when any letter appeared in the same location as two trials ago (regardless of letter identity). In the letter condition, children were asked to press a target button when a letter appeared that was identical to the letter presented two trials ago (regardless of location). In both conditions, stimuli remained on the screen for 2,500 ms, with an inter-trial interval of 1,000 ms. Children heard a “beep” following correct responses and a “bloop” following incorrect responses. Children completed a practice block of 24 trials followed by 96 experimental trials. The condition presented first was counterbalanced across children. The number of correct responses was averaged across location and letter conditions for analyses, as was the number of correct nonresponses, both of which were used in analyses.

A Go/no-go task was used to assess inhibitory control. On each trial of this task, one of four shapes (square, triangle, diamond, or circle) appeared at the center of a computer monitor, with an inter-trial interval of 2,000 ms. At the beginning of the task, one shape was designated as

the non-target, with the designated shape counterbalanced across children. Children were instructed to press a centrally-positioned response key as quickly as possible when any shape other than the non-target shape appeared (go trials) and to withhold their response when the non-target shape appeared (no-go trials; inhibitory control condition). Non-targets were randomly presented on 25% of the trials. If children responded to a non-target (commission error), a brief tone and the message “No response needed” were presented. Children completed 20 practice trials followed by 200 experimental trials. The number of errors made during no-go trials (i.e., commission errors) was used in analyses.

White Matter Integrity

Diffusion Tensor Imaging (DTI) data were obtained for a subset of 36 children using a Siemens Tim Trio 3.0 T imaging system (Erlangen, Germany). DTI data were acquired using an echo planar imaging sequence along 6 ($n = 24$) and 25 ($n = 12$) non-collinear diffusion gradients. Parametric maps were generated for MD. Fractional anisotropy was not examined because previous findings from our laboratory indicated no significant difference between PKU and healthy control groups on this variable (Antenor-Dorsey et al., 2013; White et al., 2010).

MD was examined using two analytic approaches: ROI-based analysis and voxel-wise TBSS analysis (Peng, Tseng, Chien, Hwu, & Liu, 2004). We focused on 10 ROIs across a variety of brain regions: prefrontal cortex, centrum semiovale, posterior parietal–occipital cortex, optic radiation, putamen, corpus callosum (CC; genu, body, and splenium), thalamus, and hippocampus. MD values across right and left homologous regions were averaged.

Voxel-wise TBSS analysis was used to permit identification of compromised white matter integrity without regard to the strict anatomical boundaries imposed by ROI analysis. TBSS is sensitive to combining DTI images collected using different non-collinear diffusion

gradients, thus we limited our TBSS analysis to the 24 participants with data collected along 6 diffusion gradients.

Data Analyses

The range, mean, and SD for each of the 8 indices of Phe control over the lifetime were computed first. Associations among these lifetime indices of Phe control were examined using Pearson correlations. Next, the range, mean, and SD for each of the 8 indices of Phe control across the 3 developmental epochs (< 5, 5.0 – 9.9, and \geq 10 yrs.) were computed. Paired samples t-tests were used to identify possible differences in indices of Phe control across epochs, with the exception of mean exposure and SD exposure because these were z scores for which the means summed to zero.

Given that a number of statistical analyses were performed for this study, statistical rigor was increased by considering findings significant only if $p < .05$ and effect sizes were either medium or large. Following Cohen's conventions, for Pearson correlations (Cohen, 1988), $r = .1$, $r = .3$, and $r = .5$ represented small, medium, and large effect sizes, respectively. Following Cohen's convention, for paired-samples t-tests (Cohen, 1988), $d = .2$, $.5$, and $.8$ represented small, medium, and large effect sizes, respectively.

For cognitive analyses, standard scores ($M = 100$; $SD = 15$) based on normative data reflected IQ, Vocabulary, and Matrix Reasoning performance. Normative data were not available for the experimental working memory and inhibitory control tasks administered; however, to provide a similar context for the interpretation of results, standard scores ($M = 100$; $SD = 15$) were computed for the PKU group based on data previously collected in our laboratory from a group of 80 typically-developing healthy children with a comparable age range. Associations between indices of Phe control and standard cognitive scores were examined using Pearson

correlations. Hierarchical regression analyses were then conducted to determine the degree to which indices of Phe control predicted losses in standard score points in general intellectual and executive abilities over the lifetime. Specifically, unstandardized regression coefficients (B) were used to determine the number of standard score points lost for every 100 $\mu\text{mol/L}$ increase in indices of Phe control.

For DTI ROI analyses, z scores ($M = 0$; $SD = 1$) were generated for the PKU group based on data previously collected in our laboratory from a group of 62 typically-developing healthy children with a comparable age range. All ROI analyses were conducted using these MD z scores. For 5 ROIs (i.e., centrum semiovale, posterior parietal-occipital cortex, putamen, body of the CC, and thalamus), there was a relationship with age in the healthy control children; as such, age was statistically controlled when computing z scores for these ROIs. We examined associations between indices of Phe control and MD z scores using Pearson correlations. Hierarchical regression analyses were then conducted to determine the degree to which indices of Phe control predicted MD z scores over the lifetime. These analyses were conducted using only those ROIs that were significantly correlated with indices of Phe control in earlier analyses.

Finally, for voxel-wise data, regression analyses were performed to examine relationships between indices of Phe control and raw MD values. These analyses were conducted using Threshold-Free Cluster Enhancement and permutation analyses implemented in FSL (“randomize”) (Nichols & Holmes, 2002). All statistically significant results were reported for $p < .05$ and were individually corrected using family-wise error.

Results

Indices of Phe Control over the Lifetime

For illustrative purposes, in Figure 1 we provide three exemplar profiles of Phe control across the

lifetime of three children in our sample. Panel (a) illustrates a profile in which average Phe (represented in the figure by mean Phe) over the lifetime was low, the slope representing change in Phe with age was flat (indicating little change in Phe as a function of age), and variability in Phe (represented in the figure by SD Phe) was relatively low. Phe control in this child was quite good in comparison with that of many children who participated in our study. Panel (b) demonstrates a similar profile in terms of a low average Phe and a flat slope, but variability in Phe was high. Based on only the average and slope, one might assume that Phe control in this child was quite good, but variability in Phe points to substantial instability. In sharp contrast, panel (c) shows a profile in which average Phe was high, the slope increased steeply (indicating poorer Phe control with increasing age), and variability in Phe was high. As such, Phe control in this child was poor in all regards.

Turning to our PKU group as a whole, the range of blood Phe over the lifetime was 0 – 2,742 $\mu\text{mol/L}$. Indices of Phe control over the lifetime are reported in Table 1 (as are indices for each developmental epoch, which will be discussed later). Inspection of these data revealed that average Phe (i.e., mean Phe, IDC) exceeded the recommended range of 120 – 360 $\mu\text{mol/L}$ (National Institutes of Health Consensus Development Panel, 2001). The positive slope indicated that Phe increased as children with PKU aged. Indices of variability (i.e., SD Phe, SEE Phe, % spikes) indicated considerable instability in Phe over the lifetime (in absolute terms, lifetime number of spikes ranged from 0 – 28; $M = 6.1$, $SD = 7.4$).

As shown in Table 2, a significant correlation was found between mean Phe and the IDC. In addition, significant correlations were found between mean Phe, the IDC, the slope, SD Phe, SEE Phe, and % spikes; in other words, higher Phe levels on average were associated with greater variability in Phe over the lifetime and a greater increase in Phe with age. Indices of

variability in Phe were significantly correlated with one another. The slope was not significantly correlated with any index of variability. With regard to exposure scores, there were significant correlations between both mean exposure and SD exposure and all average Phe and variability in Phe indices, with the exception of the relationship between mean exposure and % spikes.

Indices of Phe Control during Developmental Epochs

The range of blood Phe for the < 5, 5.0 – 9.9, and ≥ 10 yrs. epochs was 0 – 2742, 0 – 1580, and 30 – 1689 μmol/L, respectively. Indices of Phe control for each developmental epoch are reported in Table 1. Inspection of these data revealed that, with the exception of the youngest epoch, mean Phe and the IDC exceeded the recommended range of 120 – 360 μmol/L (National Institutes of Health Consensus Development Panel, 2001). Phe exposure scores were not included in the table because the means were zero. In terms of the range and SD for exposure scores, mean exposure during the < 5, 5.0 – 9.9, and ≥ 10 yrs. developmental epochs was -2.83 – 4.51 (*SD* = 1.67), -2.43 – 5.07 (*SD* = 1.69), and -2.41 – 3.81 (*SD* = 1.62), respectively. SD exposure during the < 5, 5.0 – 9.9, and ≥ 10 yrs. developmental epochs was -2.03 – 3.33 (*SD* = 1.44), -2.41 – 4.50 (*SD* = 1.48), and -2.12 – 3.40 (*SD* = 1.51), respectively. The pattern of exposure scores was similar across epochs, as all had large ranges and comparable SDs.

Results of paired samples t-tests showed that mean Phe was significantly higher during the ≥ 10 yrs. epoch than during either the < 5 [$t(28) = 5.76, p < .001, d = .96$] or 5.0 – 9.9 [$t(28) = 5.13, p < .001, d = .51$] yrs. epochs. The pattern was identical for the IDC, which was significantly higher during the ≥ 10 yrs. epoch than during either the < 5 [$t(28) = 6.16, p < .001, d = .96$] or 5.0 – 9.9 [$t(28) = 4.48, p < .001, d = .51$] yrs. epochs. With regard to slope, the increase in Phe with age was significantly greater during the 5.0 – 9.9 than < 5 yrs. epoch [$t(46) = 3.62, p < .001, d = .75$].

In terms of variability in Phe, there were no significant differences across developmental epochs for SD Phe, although SEE Phe was significantly greater during the < 5 than ≥ 10 yrs. epoch [$t(28) = 3.69, p < .001, d = .57$]. In absolute terms, spikes in Phe decreased across developmental epochs. Specifically, the number of spikes for the < 5, 5.0 – 9.9, and ≥ 10 yrs. epochs ranged from 0 – 17 ($M = 3.9, SD = 4.9$), 0 – 11 ($M = 1.6, SD = 2.4$), and 0 – 8 ($M = 1.0, SD = 2.1$), respectively. Although these absolute values are of interest, as noted earlier, it is more informative to examine % spikes because a larger number of Phe levels was obtained during younger than older epochs. No significant differences were found in % spikes across any of the developmental epochs.

Overall, these findings point to a general pattern in which average Phe and change in Phe with age were greater during older than younger epochs. In contrast, with a single exception, there were no significant differences in indices of variability in Phe across developmental epochs.

Cognition

IQ for the children with PKU in our sample ranged from 75 – 122 ($M = 101.3, SD = 10.6$). Scores from the Vocabulary and Matrix Reasoning subtests ranged from 72 – 126 ($M = 102.2, SD = 13.3$) and 75 – 118 ($M = 99.6, SD = 11.0$), respectively. Although the descriptive range of scores across individual children was considerable (borderline to superior), at the group level IQ and subtest scores were average.

For the working memory n-back task, the number of correct responses and nonresponses ranged from 18 – 32 ($M = 26.3, SD = 3.3$) and 27 – 64 ($M = 52.7, SD = 9.7$), respectively; standard scores ranged from 53 – 116 ($M = 92.5, SD = 14.8$) and 17 – 116 ($M = 86.8, SD = 26.1$), respectively. For the inhibitory control Go/no-go task, number of commission errors for

children with PKU ranged from 0 – 15 ($M = 5.1, SD = 3.8$), with standard scores ranging from 34 – 122 ($M = 92.2, SD = 22.4$). Overall, the range of scores across individual children was quite broad on the working memory and inhibitory control tasks; at the group level; however, performance was low average to average in relation to that of our typically-developing sample.

Indices of Phe Control as Predictors of Cognition

Correlations between cognitive variables and indices of Phe control are reported in Table 3.

Mean exposure and SD exposure are not included in the table, as the only significant correlation was between SD exposure and Matrix Reasoning ($r = .51, p < .05$) during the > 10 yrs. epoch.

Correlations between IQ and indices of Phe control were significant only for SD Phe and SEE Phe over the lifetime, as well as SD Phe during the ≥ 10 yrs. epoch. Performance on the Vocabulary subtest was not significantly correlated with any index of Phe control.

Matrix Reasoning performance was of particular interest due to its relevance to executive strategic processing. For this subtest, significant correlations were found between performance and SD Phe and SEE Phe over the lifetime and during all developmental epochs; particularly robust correlations with large effect sizes were found during the ≥ 10 yrs. epoch. In addition, Matrix Reasoning performance was significantly correlated with % spikes over the lifetime and during the ≥ 10 yrs. epoch. With regard to average Phe, the only significant relationships for Matrix Reasoning performance were with mean Phe and the IDC during the ≥ 10 yrs. epoch.

Standard scores for the working memory and inhibitory control tasks were also examined in relation to indices of Phe control. As shown in Table 3, the pattern of relationships between correct nonresponses on the n-back task and SD Phe and SEE Phe was particularly striking, with significant correlations over the lifetime and across all development epochs with one exception (SEE Phe during the < 5 yrs. epoch). Nonresponses were also significantly correlated with our

final index of variability in Phe, % spikes, over the lifetime and during the 5.0 – 9.9 yrs. epoch. Correct responses on the n-back task were significantly correlated with SD Phe and SEE Phe over the lifetime, as well as with SD Phe during the < 5 yrs. In terms of average Phe, number of correct nonresponses was significantly correlated with mean Phe during the 5.0 – 9.9 and ≥ 10 yrs. epochs, as well as with the IDC during the ≥ 10 yrs. epoch. Number of correct responses was significantly correlated with mean Phe and the IDC over the lifetime and during the < 5 yrs. epoch, as well as with the IDC during the 5.0 – 9.9 yrs. epoch.

Turning to inhibitory control, as shown in Table 3, number of commission errors on the Go/no-go task was not significantly correlated with any index of Phe control over the lifetime. Commission errors were, however, significantly correlated with slope during the < 5 yrs. epoch and with mean Phe and the IDC during the 5.0 – 9.9 and ≥ 10 yrs. epochs.

To determine the degree to which indices of Phe control predicted losses in standard score points in general intellectual and executive abilities over the lifetime, we conducted hierarchical regression analyses. For the sake of parsimony and because mean Phe and SD Phe are the most easily and commonly computed indices, only SD Phe and mean Phe were included in these analyses. Further, indices of variability in Phe (SD Phe, SEE Phe, % spikes) were highly correlated, indices of average Phe (mean Phe, IDC) were highly correlated, and there were few significant findings related to slope.

Analyses in which only SD Phe was used as a predictor of general intellectual and executive abilities were conducted first; these analyses were conducted using only those cognitive variables that were significantly correlated with SD Phe in earlier analyses. As shown in Table 4, the decrease in standard score points for every 100 $\mu\text{mol/L}$ increase in SD Phe over the lifetime ranged from 3.8 – 6.4. Extrapolating from the lifetime SD Phe of 217 $\mu\text{mol/L}$ in our

sample, standard scores for IQ, Matrix Reasoning, and number of n-back nonresponses and responses decreased by 8.2, 10.2, 13.9, and 11.5 points in relation to lifetime variability in Phe. Across developmental epochs, there were a number of significant results. Findings from Matrix Reasoning and n-back nonresponses revealed that more standard score points were lost in relation to SD Phe during the older than youngest epochs.

We then used hierarchical regression to examine both mean Phe and SD Phe as predictors of executive abilities; these analyses were conducted using only those cognitive variables that were significantly correlated with mean Phe in earlier analyses. Mean Phe was entered as an independent variable in the first step of the analyses, whereas SD Phe was entered in the second step. This approach allowed determination of the degree to which mean Phe alone predicted performance, as well as the degree to which SD Phe predicted performance beyond that attributable to mean Phe.

As shown in Table 5, the only cognitive variable with which mean Phe was significantly correlated over the lifetime was number of correct n-back responses, with a decrease of 3.2 standard score points for every 100 $\mu\text{mol/L}$ increase in mean Phe. Extrapolating from the lifetime mean Phe of 369 $\mu\text{mol/L}$ in our sample, the standard score for n-back responses decreased by 11.8 points in relation to mean Phe over the lifetime. SD Phe was not a significant predictor of n-back responses after accounting for mean Phe in the regression model.

Turning to developmental epochs, mean Phe was a significant predictor of Matrix Reasoning performance during the ≥ 10 yrs. epoch. There was a loss of 2.0 points for every 100 $\mu\text{mol/L}$ increase in mean Phe; after accounting for the contribution of mean Phe, SD Phe remained a significant predictor, with 6.2 points lost for every 100 $\mu\text{mol/L}$ increase in SD Phe. A similar pattern was observed for number of n-back nonresponses during the 5.0 – 9.9 yrs. epoch.

In this instance, there was a loss of 2.4 points for every 100 $\mu\text{mol/L}$ increase in mean Phe; after accounting for the contribution of mean Phe, SD Phe remained a significant predictor, with 8.4 points lost for every 100 $\mu\text{mol/L}$ increase in SD Phe. Additional significant results for mean Phe were found in relation to Go/no-go errors during the 5.0 – 9.9 and ≥ 10 yrs. epochs, n-back responses during the < 5 yrs. epoch, and n-back nonresponses during the ≥ 10 yrs. epoch, with no additional contribution of SD Phe after accounting for mean Phe.

White Matter Integrity

For descriptive purposes, the mean and SD for MD values in each ROI are reported in Table 6. In addition, mean MD z scores in relation to the healthy control sample are included. It is notable that, in all instances, MD z scores are lower (negative values) than those of healthy control children, with some regions as much as 1 SD lower (i.e., centrum semiovale and genu of the CC).

Indices of Phe Control as Predictors of White Matter Integrity

Correlations between MD z scores and indices of Phe control are reported in Table 7. For the sake of parsimony, Table 7 does not include the IDC, as the pattern of results was identical to mean Phe for both the lifetime and < 5 yrs. The pattern for the IDC only differed from that of mean Phe during the 5.0 – 9.9 yrs. epoch (with a significant correlation between the IDC and MD for the genu of the CC; $r = .35$) and during the ≥ 10 yrs. epoch (with a non-significant correlation between the IDC and MD for the putamen; $r = .40$). In addition, neither the SEE Phe nor % spikes are included in the table because no significant correlations were found over the lifetime and only 1 significant correlation was found during developmental epochs (with a significant correlation between the SEE Phe and MD for the parietal-occipital cortex during the ≥ 10 yrs. epoch; $r = -.49$). Neither the body of the CC nor the thalamus is included in the table

because no significant correlations were found over the lifetime. Further, only 2 significant correlations were found during developmental epochs (with significant correlations between the slope and MD for the thalamus during the < 5 yrs. epoch, $r = -.34$, and between the IDC and MD for the thalamus during the 5.0 – 9.9 yrs. epoch, $r = -.34$).

Starting at the broadest level, in relation to results reported in Table 7, lifetime mean Phe was significantly related to MD z scores in 8 ROIs with medium to large effect sizes (i.e., $r \geq .3$). The slope over the lifetime was significantly related to MD in 6 ROIs with medium to large effect sizes (i.e., $r \geq .3$) with two exceptions (i.e., prefrontal cortex and the genu of the CC). Lifetime SD Phe was significantly related to MD in 5 ROIs (prefrontal cortex, centrum semiovale, posterior parietal occipital cortex, optic radiation and putamen) with medium effect sizes (i.e., $r \geq .3$). Finally, lifetime mean and SD exposure was significantly related to MD in 7 ROIs with medium to large effect sizes (i.e., $r \geq .3$) with only one exception (i.e., putamen).

Turning to developmental epochs, mean Phe was significantly related to MD for most ROIs with the exception of the centrum semiovale and splenium of the CC during the > 5 yrs. epoch, the genu of the CC during the 5.0 – 9.9 yrs. epoch, and the prefrontal cortex, genu and splenium of the CC during the ≥ 10 yrs. epoch. The slope was significantly related to MD in most ROIs during the > 5 yrs. epoch, with the exception of the prefrontal cortex and the genu of the CC. The slope was not significantly related to MD of any ROI during the 5.0 – 9.9 yrs. or during the ≥ 10 yrs. epoch. SD Phe was only significantly related to MD of the prefrontal cortex during the > 5 yrs. epoch and the posterior parietal–occipital cortex during the ≥ 10 yrs. epoch.

In terms of exposure, mean exposure was also significantly related to MD of most ROIs with the exception of centrum semiovale of the CC and putamen during the > 5 yrs. epoch, the putamen during the 5.0 – 9.9 yrs. epoch, and the putamen, genu and splenium of the CC during

the ≥ 10 yrs. epoch. The SD exposure was significantly related to MD of ROIs during the > 5 yrs. epoch with the exception of the centrum semiovale and the putamen. In addition, the SD exposure was significantly related to MD of ROIs during the 5.0 – 9.9 yrs. epoch with the exception of the centrum semiovale, putamen, and the genu of the CC. SD exposure was significantly related to only the posterior parietal–occipital cortex during the ≥ 10 yrs. epoch.

Hierarchical regression analyses were conducted to determine the degree to which indices of Phe control predicted lower MD z scores. Analyses were conducted using MD for only those ROIs that were significantly correlated with mean Phe, slope, and SD Phe in earlier analyses. The IDC was not included as the pattern of correlations was identical to lifetime mean Phe. In addition, SEE and % spikes were not included as neither was significantly related to any MD for any ROI over the lifetime. Mean exposure and SD exposure were not included in analyses due to high multicollinearity with mean Phe and SD Phe.

Mean Phe was entered as an independent variable in the first step of the analyses, slope in the second step and SD Phe in the third step. This order was chosen as mean Phe had the strongest correlations with MD, followed by the slope, and then SD Phe. These analyses allowed us to determine the degree to which mean Phe alone predicted performance, as well as the degree to which the slope or SD Phe predicted performance beyond that attributable to mean Phe.

Regression analyses showed that neither the slope nor SD Phe significantly predicted MD z scores after accounting for the contribution of mean Phe. As such, findings for only mean Phe are reported in Table 8. Mean Phe was a significant predictor of MD for all 8 ROIs submitted in regression analyses, accounting for 21 to 39% of the variance in MD. Given that MD z scores in our PKU sample ranged from $-.3$ to $-.7$, for every 100 $\mu\text{mol/L}$ increase in mean Phe, children with PKU had MD z scores that were between approximately $.3$ and $.7$ lower than those of

healthy children. Extrapolating from the lifetime mean Phe of 369 $\mu\text{mol/L}$ in our sample, MD z scores for children with PKU were on average, 2 standard deviations lower than those of healthy control children.

Specifically, the MD z scores for the hippocampus were 2.6 lower than that of healthy control children, whereas MD z scores for the prefrontal cortex, optic radiation, and genu and splenium of the CC were 2.2 lower than those of healthy control children. The z score for the posterior parietal-occipital cortex was 1.8 lower, whereas z scores for the centrum semiovale and putamen were 1.1 lower than those of controls. These results indicate that higher average Phe levels have a significant negative influence on the microstructural white matter integrity of diffuse brain regions in children with PKU.

Voxel-wise Analyses

Figure 2 shows that for voxel-wise analyses, raw MD values of multiple white matter tracts of children were negatively correlated with mean Phe and IDC in a similar pattern over the lifetime. As the pattern of results on the ROI-based DTI analysis was identical between mean Phe and the IDC over the lifetime, it is reassuring to confirm a similar result using a separate analytical approach. However, the white matter tracts showing significant correlations were not as widespread as seen in the mean Phe and the IDC over the lifetime in ROI-based analyses. The voxel-wise analyses also showed that similar to earlier ROI-based DTI analyses, both lifetime mean exposure and SD exposure were negatively correlated with MD values; in contrast with mean Phe and the IDC, mean exposure and SD exposure showed more widespread correlations in diffuse white matter tracts. Finally, the SEE and slope (change in Phe) were not statistically correlated with MD values in our TBSS analysis.

Discussion

A number of studies have shown that compromises in cognition (Brumm & Grant, 2010) and brain white matter (Anderson et al., 2004; Thompson et al., 1993) are associated with higher average Phe in individuals with PKU (Azen et al., 1996; Burgard, 2000; Smith et al., 1990; Anderson et al., 2004; Leuzzi et al., 1993). In only a small number of studies have associations between indices of Phe control other than average Phe been examined in relation to cognition (Anastasoiaie et al., 2008; Arnold et al., 1998; Burgard et al., 1996; Viau et al., 2011; Vilaseca et al., 2010) and white matter abnormalities (Das et al., 2013; Rupp et al., 2001). Interpretation of findings across these studies has left questions unanswered due to a number of issues, such as inconsistency in the measures used to index Phe control and small sample size. In addition, cognition has primarily been examined within only the broadest context through the assessment of IQ, whereas white matter pathology has primarily been examined through qualitative assessment of observable white matter abnormalities.

The current research was conducted to thoroughly evaluate the contribution of Phe control to cognitive outcomes and white matter integrity in school-age children with early- and continuously-treated PKU. To do so, we examined IQ, executive abilities, and white matter integrity in relation to 8 indices of Phe control reflecting average Phe (mean Phe and IDC), change in Phe with age (slope), variability in Phe (SD Phe, SEE Phe, % spikes), and exposure to Phe (mean exposure and SD exposure). All indices were calculated over the lifetime and during 3 developmental epochs (< 5, 5.0 – 9.9, and \geq 10 yrs. of age).

Across the 8 indices of Phe control examined, single indices of average Phe (i.e., mean Phe) and variability in Phe (i.e., SD Phe) over the lifetime were most strongly associated with cognition. Similarly, mean Phe and SD Phe over the lifetime were strongly associated with white

matter integrity. In addition, both exposure indices (i.e., mean exposure and SD exposure) were strongly associated with white matter integrity but not cognition. As such, this discussion will focus on mean Phe and SD Phe over the lifetime in relation to cognition and on mean Phe, SD Phe, mean exposure, and SD exposure over the lifetime in relation to white matter integrity.

Turning first to cognition, our findings indicated that performance on IQ and executive abilities tasks was more often correlated with SD Phe than mean Phe. Across the IQ and executive tasks administered, SD Phe was significantly correlated with 4 of 6 cognitive variables examined. Mean Phe over the lifetime, however, was significantly correlated with only 1 of 6 cognitive variables examined. In addition, SD Phe more negatively affected performance on IQ and executive abilities tasks than mean Phe. Standard scores on the IQ, Matrix Reasoning, and working memory n-back tasks decreased by an average of 5 points for every 100 $\mu\text{mol/L}$ increase in SD Phe over the lifetime. Extrapolating from the lifetime SD Phe of 217 $\mu\text{mol/L}$ of our sample shows that standard scores decreased by an average of 11 points in relation to SD Phe.

There was a single instance in which mean Phe over the lifetime predicted a significant loss in standard score points; for n-back responses, 3.2 points were lost for every 100 $\mu\text{mol/L}$ increase in mean Phe. In this instance, SD Phe did not make a significant contribution after accounting for the points lost due to mean Phe. However, it is interesting to keep in mind that 5.3 points were lost for every 100 $\mu\text{mol/L}$ increase in SD Phe when this index was used as the sole predictor of n-back responses, whereas only 3.2 points were lost for every 100 $\mu\text{mol/L}$ increase in mean Phe when it was used as the sole predictor.

Briefly turning to developmental epochs, in general, variability in Phe during the older epochs was more robustly correlated with executive performance than during the youngest

epoch. In turn, a greater loss of standard score points was associated with increases in SD Phe during the older epochs than during the youngest epoch. The pattern of performance on the strategic Matrix Reasoning task was particularly striking in this regard, with every 100 $\mu\text{mol/L}$ increase in SD Phe during the youngest and oldest epochs associated with losses of 3.8 and 6.8 points, respectively. These findings suggest that liberalization of Phe control as children with PKU age is unwise, as variability in Phe in older children may be particularly detrimental to executive abilities. These findings also suggest that Matrix Reasoning may be useful as a screening tool to evaluate the effects of variability in Phe on cognition.

In contrast with our findings related to cognition, white matter integrity was more often correlated with mean Phe than SD Phe. Mean Phe over the lifetime was significantly correlated with MD in 8 of 10 ROIs, whereas SD Phe was correlated with MD in only 5 of 10 ROIs. In addition, mean Phe more negatively affected white matter integrity than SD Phe. On average, every 100 $\mu\text{mol/L}$ increase in mean Phe over the lifetime predicted half a standard deviation decrease in MD z scores. Extrapolating from the lifetime mean Phe of 369 $\mu\text{mol/L}$ of our sample, MD z scores decreased by an average of nearly 2 standard deviations in relation to mean Phe.

In terms of mean exposure and SD exposure, the pattern of results for both indices in relation to white matter integrity was identical, as both were correlated with MD z scores for the same 7 ROIs. The pattern of findings for mean exposure was quite similar to the pattern for mean Phe. SD exposure, however, was significantly correlated with MD z scores in 7 ROIs, whereas SD Phe was significantly correlated with MD z scores in only 5 ROIs. SD exposure was also more strongly associated with MD than was SD Phe. These findings indicate that lengthier exposure to higher mean Phe or SD Phe results in greater compromise to white matter integrity. Findings from voxel-wise analyses generally supported findings from ROI analyses.

Overall, our findings clearly indicate that maintaining lower Phe on average and avoiding variability in Phe are crucial for optimizing cognitive and neural outcomes in children with early- and continuously-treated PKU. Variability in Phe appears to be particularly detrimental to cognition, whereas higher average Phe appears to be particularly detrimental to white matter integrity. In addition, exposure to higher average Phe and variability in Phe were particularly detrimental to white matter integrity but not cognition. The reasons for the discrepancy in cognitive and white matter findings in relation to indices of Phe remain unclear, and further research is clearly needed to understand the mechanisms that link cognitive and white matter findings.

Finally, there were a number of possible limitations to our study. Although we had a relatively large number of children with PKU compared with previous studies, it is possible that additional relationships between cognition, white matter integrity, and indices of Phe control would be identified in a larger sample. It should also be noted that it is quite possible that the level of statistical significance required in our study prevented us from identifying additional relationships. Nonetheless, we believe it is unlikely that our general pattern of findings would change with a larger sample or less statistical rigor.

Conclusion

The current study included multiple indices of Phe control, a relatively large sample of children with PKU, a large number of blood Phe levels, measures of both general intellectual and executive abilities, and DTI analyses of white matter integrity. Findings strongly indicated that both variability in Phe and average Phe should be carefully monitored and controlled to maximize cognitive and neural outcomes in children with PKU. Findings also indicated that Phe monitoring and control should not be liberalized as children with PKU age because executive

performance was better predicted by variability in Phe at older than younger ages and because lengthier exposure to higher average Phe and variability in Phe were particularly detrimental to white matter integrity.

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