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A preliminary investigation of the role of the phenylalanine:tyrosine ratio in children with early and continuously treated phenylketonuria: towards identification of "safe" levels

Abstract

Children with early and continuously treated phenylketonuria (ECT-PKU) remain at risk of developing executive function (EF) deficits. There is some evidence that a high phenylalanine to tyrosine ratio (phe:tyr) is more strongly associated with impaired EF development than high phenylalanine alone. This study examined EF in a sample of 11 adolescents against concurrent and historical levels of phenylalanine, phe:tyr, and tyrosine. Lifetime measures of phe:tyr were more strongly associated with EF than phenylalanine-only measures. Children with a lifetime phe:tyr less than 6 demonstrated normal EF, whereas children who had a lifetime phe:tyr above 6, on average, demonstrated clinically impaired EF.

Abbreviations:
PKU - Phenylketonuria
ECT-PKU – early and continuously treated phenylketonuria
Phe – phenylalanine
Tyr – tyrosine
EF – Executive function
BRIEF – Behaviour Rating Inventory of Executive Function
MI – Metacognition index
BRI – Behaviour Regulation index
GEC – Global executive composite.

Keywords: Phenylketonuria; PKU; Tyrosine; Executive Function; Phenylalanine:tyrosine ratio; dopamine
Phenylketonuria (PKU) is an inborn error of metabolism involving a deficiency in the enzyme phenylalanine hydroxylase, required to metabolize one amino acid in protein phenylalanine (phe), and convert it to tyrosine. PKU results in increased concentrations of phe, which is a known toxin to the developing brain, as well as lowered concentrations of tyrosine - a precursor to the neurotransmitter, dopamine - in the blood and cerebrospinal fluid (Scriver & Sly, 2000).

Untreated PKU causes substantial neurological damage, resulting in severe intellectual disability, motor disorders and spasticity (Pitt & Danks, 1991). Early detection and improvements in treatment for PKU (protein restricted diet and phe-free supplemental formulas) are aimed at keeping phe levels low enough to prevent this severe neurological damage. Even with stringent treatment regimes, children with early and continuously treated (ECT-) PKU will maintain higher levels of phenylalanine (phe) compared to the non-PKU population (Koch et al., 2000).

Although children with ECT- PKU typically develop an IQ within normal range, neuropsychological research shows that, compared to controls, deficits in executive functioning persist in children with ECT-PKU (Antshel & Waisbren, 2003; DeRoche & Welsh, 2008; Huijbregts et al., 2002a, 2002b; Waisbren et al., 2007). Executive functioning (EF) describes abilities in goal directed behaviour: prediction of consequences, inhibition of automatic responses, self-regulation, task-switching, abstract reasoning, integration of information across space and time and working memory. In most cases, EF worsens with exposure to higher phe (Antshel & Waisbren, 2003; Huijbregts et al., 2002a). Research suggests that earlier exposure, and longer exposure to higher phe concentrations, lead to worse outcomes (Antshel & Waisbren, 2003; Waisbren et al., 2007)
The role of tyrosine in the development of EF in children with ECT-PKU is less definitive. Diamond, Prevor, Callender and Druin (1997) proposed that a combination of high phe and low tyrosine (a high phe:tyr ratio) creates a low dopamine environment in the developing brain, which may be responsible for the EF deficits that continue to manifest in ECT-PKU. Tyrosine is a precursor to dopamine and is already lowered by the initial enzyme deficiency in PKU. High phe then “floods” the blood brain barrier, competing for transport with other amino acids (including tyrosine), along with inhibiting other enzymes needed to synthesise dopamine and serotonin in particular (Ormazabal et al., 2004). Therefore high phe acting in concert with low tyrosine (high phe:tyr ratio) is hypothesized to lead to the worst case scenario for dopamine synthesis, over and above the effects of high phe alone.

This potential role of the phe:tyr ratio on brain development in children with ECT-PKU has not been extensively researched. The focus of most studies involves phe-only measures: concurrently, historically, or both (Antshel & Waisbren, 2003; Arnold et al., 2004; Huijbregts et al., 2002b; Waisbren et al., 2007). Diamond et al. (1997), Luciana, Sullivan and Nelson (2001) and Sharman, Sullivan, Young and McGill (2009) have reported significant associations between the phe:tyr ratio and EF deficits in samples of children with ECT-PKU. Sharman et al. (2009) recently tested the relationship between EF and a range of biochemical markers, including phe-only and tyrosine-only, measured both concurrently and historically, and demonstrated that the strongest and most consistent pattern of associations were due to historical (prior to age 12 years) and lifetime measures of participants’ phe:tyr ratio. This finding, whilst consistent with previous phe:tyr research (Diamond et al., 1997; Luciana, Sullivan & Nelson, 2001) warrants further attention as the mechanism by which a high phe:tyr may negatively influence EF development in children with ECT-PKU remains a matter of some debate.
From a clinical point of view, no data have yet been presented identifying a possible target phe:tyr ratio during childhood brain development. Given these gaps in the literature, the purpose of this paper was to further examine the relationship between the phe:tyr ratio and executive function in the developing brain.

Method

Participants

13 children with ECT-PKU aged 10 to 17 years (Mean = 14.3 years, SD = 2.09) were tested for this study, however data from two participants were excluded from analyses involving historical or lifetime phe:tyr ratio and EF due to significant missing biochemical marker data (first 6 years of life due to movement from other centres which did not routinely measure blood tyrosine levels). This sample represents a subgroup of participants recruited for a larger between-groups study focusing on differences in EF between children with ECT-PKU and sibling controls (Sharman et al., 2009).

Measures

Executive function

EF was assessed by the parent questionnaire version of Behaviour Rating Inventory of Executive Function (BRIEF; Gioia, Isquith, Guy & Kenworthy, 2000) which is a standardized clinical measure of children’s executive function. The BRIEF measures several EF abilities however summary scales of the BRIEF (Indices) were used for this study. These indices measure the EF domains of: Behavioural Regulation Index (BRI: abilities in regulating emotions and impulse control); Metacognition Index (MI: abilities in higher-order cognitive tasks such as planning, organization and working memory) and Global Executive Composite (GEC: overall measure of executive function). T-scores were calculated for each participant to correct for
variations in age and sex. For the original study, the children were assessed over two different
time periods, separated by 3 months, to assess possible changes in EF alongside fluctuating
concurrent phe levels. Concurrent phe and tyrosine levels were collected on the day participants
completed the questionnaires. However no substantial fluctuations in concurrent phe levels or
EF across time were found. Therefore, for this study, we averaged EF scores as well as
concurrent phe levels across the two time periods for each participant.

Phe:tyr ratio

The phe:tyr ratio was calculated retrospectively, using archival hospital records. Phe and
tyrosine data were usually collected via dried blood spot analysis (e.g. Guthrie card). Phe levels
were usually recorded at least monthly for each participant, although these measurements
decreased in frequency as the child aged, especially post puberty. After 2001, both tyrosine and
phe level data were available for over 90% of the measurement points. Before 2001, tyrosine
levels were only recorded against phe levels approximately 30% of the time. This difference in
clinical records was due to a protocol change in 2001 to automatically include tyrosine in the
standard phe level screening procedure. Phe:tyr ratio was calculated using only those results
where a phe:tyr result existed. That is, lifetime phe was not averaged and then divided by
averaged lifetime tyrosine, as that figure that would have been confounded by significant missing
data. Averages of phe, tyrosine and phe:tyr ratios were also generated for each participant prior
to the age of 12 and after the age of 12 (if applicable) due to the changes in guidelines for
acceptable phe levels prior to 12 years of age (< 360 umol) and after 12 years of age (< 500
umol) (Australian Society for Inborn Errors of Metabolism; ASIEM, 2005)
The phe:tyr ratio in children with ECT-PKU

Results

The lifetime phe:tyr ratio for participants in this sample ranged from 4.2 to 9.8, with a mean of 6.75 ($SD = 1.8$). Figure 1 shows the relationship between lifetime phe:tyr and EF (GEC T-scores from the BRIEF). Using interpretative guidelines provided by the BRIEF, where T scores of 50 represent average EF, and T scores greater than 65 represent clinically impaired EF (Gioia et al., 2000) Figure 1 indicates that lower phe:tyr ratios were associated with better EF overall.

Insert Figure 1 here

In comparison, lifetime phe levels for participants indicated that this was a relatively adherent group in terms of compliance with ASIEM guidelines. Mean phe prior to age 12 years was 400 umol; mean phe after 12 years was 526 umol. Figure 2 shows participant’s GEC T scores against their lifetime phe levels. Whilst a positive trend between higher phe and deteriorating EF is evident, the relationship between lifetime phe and EF within the confines of this relatively complaint group is clearly not as direct as the relationship between phe:tyr and EF.

Insert Figure 2 here

Relationship between phe:tyr and EF

Correlations were used to test the strength of the associations between phe:tyr under different exposure conditions (concurrent; recent; lifetime and prior to age 12 years). Using the averaged T scores for each participant on the BRIEF Indices of Behavioural Regulation (BRI),
Metacognition (MI), and Global Executive Composite (GEC); Table 1 shows the results of correlations with phe:tyr ratio measures.

Table 1 demonstrates that the lifetime phe:tyr ratio was strongly and positively correlated with the reported EF deficits in this sample. Furthermore, phe:tyr ratio prior to age 12 years showed stronger correlations with EF than lifetime phe:tyr. Recent phe:tyr (averaged across the previous year) and concurrent phe:tyr (averaged across 2 time periods separated by 3 months) showed non-significant and weak associations.

**Exploratory analysis via ANOVA**

Given that the historical measures of phe:tyr ratio were significantly associated with EF performance in children with ECT-PKU, we performed an exploratory analysis to determine the level at which EF performance might become compromised. Evaluation of Figure 1 demonstrated that all participants with a lifetime ratio of phe:tyr less than 6 had GEC T scores within the normal range (T scores of < 50 to 65), whereas five of the seven children with phe:tyr ratios above 6 scored in the clinically impaired range (GEC T score > 65).

To determine if differences in EF were due to a lifetime phe:tyr ratio above or below 6, a series of ANOVAs were conducted. For all ANOVA ratio analyses the independent variable was the phe:tyr ratio with two levels: low lifetime phe:tyr ratio (less than 6) and high lifetime phe:tyr ratio (above 6). Three separate analyses were performed using the following dependent measures of executive function: BRI; MI and GEC T scores. A conservative alpha level was
chosen for all ANOVAs (p <0.01) due the number of statistical comparisons and sample size. Table 2 presents the results.

Insert Table 2 here

The effect sizes observed demonstrated that a lifetime phe:tyr ratio above or below 6 accounted for 60% and 58% of the variance in EF T scores of the indices of MI and GEC respectively. Following Bonferroni correction (p< .01) the difference in BRI scale was not significant using ANOVA. A follow up non parametric test (Mann Whitney U) was conducted on the BRI scores because of relatively low power (.64) and moderate effect size initially identified (43%). This analysis subsequently revealed a significant result at p < .01.

Participant’s lifetime phe:tyr ratios (categorized as below or above 6) did not change for any participant after they turned 12 years of age (i.e. phe:tyr was quite consistent across the lifetime). Therefore results of this ANOVA using the phe:tyr ratio of participants below age 12 years are identical to their lifetime results.

Discussion

The results of this study highlight the potential importance of the phe:tyr in the development of EF in children with ECT-PKU. Together with previous reports of significant associations between phe:tyr ratio and EF, which were the strongest and most consistent associations of the biochemical markers analysed (Diamond et al., 1997; Luciana, Sullivan & Nelson, 2001; Sharman et al., 2009) we now propose that: (a) lifetime measures of phe:tyr ratio are strongly associated with EF development in children with ECT-PKU (b) children with a
The phe:tyr ratio in children with ECT-PKU

lifetime ratio of phe:tyr of less than 6 demonstrated normal EF, whereas children with a lifetime phe:tyr ratio above 6, on average, scored in clinically impaired range in our sample.

It is important to note that this strong association of the phe:tyr ratio and EF was confined within a sample of children whose lifetime phe levels were generally well controlled i.e. within current Australian guidelines (see Figure 2, or for a fuller outline of participant’s historical and concurrent phe levels see Sharman et al., 2009). Taken together, our results implicate the measurement and control of phe:tyr ratios as potentially important in preventing EF deficits developing in children with ECT-PKU whose phe levels are already under good control. In this sample the phe:tyr effect on EF impairment held for lifetime phe:tyr, and associations appeared stronger below age 12 years. Concurrent and recent phe:tyr measures showed no relationship with EF in this sample.

The results of this study are preliminary given the small sample size, although the effect sizes and observed power suggest that our design was adequate to detect a robust effect. Other features of this sample may limit the application of results; in particular, the average age of our sample was 14 years, so further investigations are needed to determine whether these findings generalize to samples of other age groups of children/adults with ECT-PKU.

Our results offer support for the aforementioned dopamine hypothesis (Diamond et al., 1997), in that a persistently high phe:tyr ratio may be creating a low dopamine environment for the developing brain. Recent research using positron emission tomography provides support for the notion that individuals with PKU have reduced cerebral dopamine uptake compared to the normal population (Landvogt et al., 2007). It is this dopamine deficiency that is then hypothesized to result in the development of EF impairments in children with PKU. Recent research has also implicated low dopamine in the development of EF impairment in the non-
PKU population, most notably in populations of children with Attention Deficit Hyperactivity Disorder (Prince, 2008; Stallar & Faraone, 2007).

Given the strongest associations between phe:tyr and EF were found using phe:tyr measures taken prior to the age of 12 years, coupled with the lack of any relationship between concurrent or recent phe:tyr and EF, these results may indicate the existence of a potential critical period of brain development where the phe:tyr ratio exerts its strongest effect, similar to established phe critical periods (Waisbren et al., 2007). Further research focusing on differences in biochemical markers (phe; tyr and phe:tyr) from birth to adulthood, and their effects on EF development should help specify any sensitive periods of the brain to a high phe:tyr ratio, as well at teasing out the relative contributions of phe and phe:tyr.

From a clinical perspective, the best EF outcomes in this sample were observed in those children who maintained relatively lower phe:tyr ratios alongside recommended lifetime phe levels in the first instance. Although we have nominated a phe:tyr ratio of 6 as a cut-off, Figure 1 clearly shows that as the phe:tyr ratio lowers (to below 6), EF appears to improve, therefore further investigations may demonstrate that “healthy” phe:tyr levels belong within a range rather than a definitive cut-off. This range requires further investigation within a larger sample.

Overall, our results suggest that maintaining a low (below 6) lifetime phe:tyr ratio together with well controlled phe levels requires further evaluation as a potential clinical goal to improve EF outcomes. This may be particularly evident in children below the age of 12 years who are already maintaining good phe control.
References


The phe:tyr ratio in children with ECT-PKU


Figure 1. Lifetime phe:tyr ratios of 11 children with ECT-PKU and overall EF in adolescence as measured by BRIEF Global Executive Composite T scores.
Figure 2. Lifetime phe levels of 11 children with ECT-PKU and overall EF in adolescence as measured by BRIEF Global Executive Composite T scores.
Table 1. Correlations between phe:tyr measures from 11 adolescent children with ECT-PKU, using T scores from Behavioural Regulation Index (BRI), Metacognition Index (MI), and Global Executive Composite (GEC) from the BRIEF.

<table>
<thead>
<tr>
<th></th>
<th>BRI</th>
<th>MI</th>
<th>GEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe:tyr &lt; 12 years</td>
<td>.817**</td>
<td>.741**</td>
<td>.832**</td>
</tr>
<tr>
<td>Lifetime phe:tyr</td>
<td>.696*</td>
<td>.603*</td>
<td>.699*</td>
</tr>
<tr>
<td># Recent phe:tyr</td>
<td>.276</td>
<td>.253</td>
<td>.286</td>
</tr>
<tr>
<td>+ Concurrent phe:tyr</td>
<td>.161</td>
<td>.143</td>
<td>.161</td>
</tr>
</tbody>
</table>

Note: 2 tailed correlations; * = sig. at p<.05; ** = sig. at p<.01 # Recent phe:tyr = phe:tyr for each participant averaged over preceding year (2005) + Concurrent phe:tyr = average concurrent phe:tyr at 2 time periods (separated by 3 months).
Table 2. Lifetime phe:tyr ratio of above 6 versus below 6, in a sample of 11 adolescent children with ECT-PKU, and effects on Behavioural Regulation Index; Metacognition Index and Global Executive Composite T-scores from the BRIEF. Means (M), standard deviations (SD), F statistics (with degrees of freedom in brackets) are shown.

<table>
<thead>
<tr>
<th></th>
<th>BRI</th>
<th>MI</th>
<th>GEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe:tyr 6+</td>
<td>M(SD)</td>
<td>63.8 (14.8)</td>
<td>67.2 (7.1)</td>
</tr>
<tr>
<td>n = 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe:tyr 6-</td>
<td>M(SD)</td>
<td>44.0 (1.4)</td>
<td>52.3 (4.8)</td>
</tr>
<tr>
<td>n = 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F (9)</td>
<td>6.81</td>
<td>13.7</td>
<td>12.62</td>
</tr>
<tr>
<td>Sig.</td>
<td>$p = .028$</td>
<td>$p = .005$</td>
<td>$p = .006$</td>
</tr>
<tr>
<td>Partial eta²</td>
<td>0.43</td>
<td>0.60</td>
<td>0.58</td>
</tr>
<tr>
<td>Power</td>
<td>0.64</td>
<td>0.91</td>
<td>0.88</td>
</tr>
</tbody>
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Note: Given power for the BRI analysis was inadequate, we performed a non-parametric Mann-Whitney test for the BRI scale which indicated a statistically significant result ($z = -2.65$, exact significance $p = .006$).