Neuropsychological Development in Children with Early and Continuously Treated Phenylketonuria: Association with Biochemical Markers

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A thesis submitted in accordance with the requirements for admission to the degree of Doctor of Philosophy

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and

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June 2011
Keywords

Phenylketonuria, phenylalanine, tyrosine, dopamine, executive function, working memory, phenylalanine:tyrosine ratio, depression, practice survey
Preface

Ethical approval for the studies described was obtained from the Queensland University of Technology Human Research Ethics Committee (0800000055) and the Royal Children’s Hospital, Brisbane, Queensland Ethics Committee (2005/085).

The format of the research presented is in accordance with guidelines prescribed by the American Psychological Association (5th ed.), with the exception of large tables that were spaced to improve layout where necessary. All statistical analyses were conducted using PASW version 18.0.
Abstract

PKU is a genetically inherited inborn error of metabolism caused by a deficiency of the enzyme phenylalanine hydroxylase. The failure of this enzyme causes incomplete metabolism of protein ingested in the diet, specifically the conversion of one amino acid, phenylalanine, to tyrosine, which is a precursor to the neurotransmitter dopamine. Rising levels of phenylalanine is toxic to the developing brain, disrupting the formation of white matter tracts. The impact of tyrosine deficiency is not as well understood, but is hypothesized to lead to a low dopamine environment for the developing brain.

Detection in the newborn period and continuous treatment (a low protein phenylalanine-restricted diet supplemented with phenylalanine-free protein formulas) has resulted in children with early and continuously treated PKU now developing normal I.Q. However, deficits in executive function (EF) are common, leading to a rate of Attention Deficit Hyperactivity Disorder (ADHD) up to five times the norm. EF worsens with exposure to higher phenylalanine levels, however recent research has demonstrated that a high phenylalanine to tyrosine ratio (phenylalanine:tyrosine ratio), which is hypothesized to lead to poorer dopamine function, has a more negative impact on EF than phenylalanine levels alone. Research and treatment of PKU is currently phenylalanine-focused, with little investigation of the impact of tyrosine on neuropsychological development. There is no current consensus as to the veracity of tyrosine monitoring or treatment in this population. Further, the research agenda in this population has demonstrated a primary focus on EF impairment alone, even though there may be additional neuropsychological skills compromised (e.g., mood, visuospatial deficits).
The aim of this PhD research was to identify residual neuropsychological deficits in a cohort of children with early and continuously treated phenylketonuria, at two time points in development (early childhood and early adolescence), separated by eight years. In addition, this research sought to determine which biochemical markers were associated with neuropsychological impairments. A clinical practice survey was also undertaken to ascertain the current level of monitoring/treatment of tyrosine in this population.

Thirteen children with early and continuously treated PKU were tested at mean age 5.9 years and again at mean age 13.95 years on several neuropsychological measures. Four children with hyperphenylalaninemia (a milder version of PKU) were also tested at both time points and provide a comparison group in analyses. Associations between neuropsychological function and biochemical markers were analysed. A between groups analysis in adolescence was also conducted (children with PKU compared to their siblings) on parent report measures of EF and mood.

Minor EF impairments were evident in the PKU group by age 6 years and these persisted into adolescence. Life-long exposure to high phenylalanine:tyrosine ratio and/or low tyrosine independent of phenylalanine were significantly associated with EF impairments at both time points. Over half the children with PKU showed severe impairment on a visuospatial task, and this was associated only with concurrent levels of tyrosine in adolescence. Children with PKU also showed a statistically significant decline in a language comprehension task from 6 years to adolescence (going from normal to subnormal), this deficit was
associated with lifetime levels of phenylalanine. In comparison, the four children with hyperphenylalaninemia demonstrated normal function at both time points, across all measures.

No statistically significant differences were detected between children with PKU and their siblings on the parent report of EF and mood. However, depressive symptoms were significantly correlated with: EF; long term high phe:tyr exposure; and low tyrosine levels independent of phenylalanine.

The practice survey of metabolic clinics from 12 countries indicated a high level of variability in terms of monitoring/treatment of tyrosine in this population. Whilst over 80% of clinics surveyed routinely monitored tyrosine levels in their child patients, 25% reported treatment strategies to increase tyrosine (and thereby lower the phenylalanine:tyrosine ratio) under a variety of patient presentation conditions.

Overall, these studies have shown that EF impairments associated with PKU provide support for the dopamine-deficiency model. A language comprehension task showed a different trajectory, serving a timely reminder that non-EF functions also remain vulnerable in this population; and that normal function in childhood does not guarantee normal function by adolescence. Mood impairments were associated with EF impairments as well as long term measures of phenylalanine:tyrosine and/or tyrosine. The implications of this research for enhanced clinical guidelines are discussed given varied current practice.
Publications Relating to Specific Thesis Chapters

Chapter 7


Presentations Relating to Specific Thesis Chapters

Peer-reviewed Oral Presentations


Peer-reviewed Poster Presentations

Other PKU Publications Completed During Candidature


# Table of Contents

## Chapter 1  1-18

Phenylketonuria: the disorder and its current treatment.  1-7

Residual deficits in early and continuously treated phenylketonuria.  7-8

Biochemistry of PKU and effect on brain development.  9-16

Rationale for the PhD research.  16-18

## Chapter 2  19-33

Findings and limitations of previous PKU research: A critical analysis.

## Chapter 3  34-68

Study 1: Longitudinal analysis of executive function: Associations with biochemical markers

## Chapter 4  69-76

Study 2: Between groups executive function assessment: Associations of EF impairment with biochemical markers.

## Chapter 5  77-87

Study 3: Early and continuously treated PKU and mood: Between groups analysis of depression symptoms in children with PKU and their siblings. Associations of mood with biochemical markers
Chapter 6

Study 4: Hyperphenylalaninemia. Neuropsychological development over an eight year period in comparison to children with PKU; and associations with biochemical markers.

Chapter 7

Study 5: Tyrosine monitoring and treatment: Current clinical practice.

Chapter 8

The final word: Children with PKU and hyperphenylalaninemia describe their views on their disorder and how it affects their lives.

Chapter 9

Summary and Conclusions.

References

Appendix A – Screening questionnaire for children with PKU
Appendix B – International practice survey questionnaire
Appendix C – Information and Consent forms
Appendix D – example Rey Complex Figure drawing from an 11 year old male with phenylketonuria
List of Tables

Table 1. Disability Outcomes From Untreated Phenylketonuria

Table 2. Recommended Phenylalanine Guidelines by Country

Table 3. Design and Conclusions from Previous PKU Research

Table 4. Means (SD’s) and Ranges of Biochemical Markers of 13 Children with Classical PKU

Table 5. Differences in EF T-scores Between 13 Children with Classical PKU and Eight Sibling Controls

Table 6. Significant Correlations (Pearson’s r) Between Depressive Symptoms (CDI T-score) and Biochemical Markers in Children with ECT-PKU

Table 7. Biochemical characteristics of Four Children with Hyperphenylalaninemia – Means and Standard Deviations (in brackets)

Table 8. Neuropsychological Performance of Four Adolescent Children with Hyperphenylalaninemia

List of Figures

Figure 1. Percentage of clinics reporting monthly tyrosine screening as a function of child age, across the previous 2 years compared to over 5 years ago.

Figure 2. Dangers of temptation in the PKU diet
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASIEM</td>
<td>Australian Society for Inborn Errors of Metabolism</td>
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<tr>
<td>BRIEF</td>
<td>Behavior Rating Inventory of Executive Function</td>
</tr>
<tr>
<td>CDI</td>
<td>Children’s Depression Inventory</td>
</tr>
<tr>
<td>EF</td>
<td>Executive Function</td>
</tr>
<tr>
<td>GEC</td>
<td>Global Executive Composite</td>
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<tr>
<td>IQ</td>
<td>Intelligence Quotient</td>
</tr>
<tr>
<td>NEPSY-II</td>
<td>Neuropsychological Assessment, 2nd edition</td>
</tr>
<tr>
<td>Phe:tyr</td>
<td>Phenylalanine to tyrosine ratio</td>
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<tr>
<td>RCFT</td>
<td>Rey Complex Figure Task</td>
</tr>
<tr>
<td>WISC IV</td>
<td>Weschler Intelligence Scale for Children, 4th edition</td>
</tr>
<tr>
<td>WM</td>
<td>Working Memory</td>
</tr>
<tr>
<td>WMA</td>
<td>White Matter Abnormalities</td>
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Statement of Original Authorship

I hereby certify that the work contained in this thesis is the result of original research and has not been previously submitted to meet the requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Rachael Rebecca Sharman

June, 2011
Acknowledgements

I would especially like to acknowledge the excellent level of support and training provided by my two supervisors Associate Professor Karen Sullivan and Professor Ross Young. Our collaboration has been a happy and intellectually fulfilling one, and I look forward to its continuation well into the future. Also, the support from the metabolic clinic based at the Royal Children’s Hospital, Queensland. In particular the Director of Metabolic Medicine, Dr Jim McGill and metabolic nurse Anita Inwood, who both volunteered their limited amount of free of time to support this project. Without clinical support, this kind of research is impossible to pursue. My sincere thanks also go to clinical neuropsychologist Dr Toni Jones, whose generosity in the provision of her initial research findings, made a rare longitudinal analysis in this field possible.

Funding for this project was provided by the Royal Children’s Hospital Foundation, Queensland (~ $25,000); the Institute of Health and Biomedical Innovation, Queensland University of Technology; and I was also the recipient firstly of a Queensland University of Technology scholarship, and later an Australian Postgraduate Award.

Finally I would to thank the children and adolescents who participated in this research. I’m sure that enduring continued interference and testing because of a mishap in one’s genetic code can become a rather tedious experience for the average 14 year old; however these children and their families have given cheerily of their time to benefit future generations of children born with phenylketonuria.
CHAPTER 1

Inborn Errors of Metabolism

The food we eat contains fats, carbohydrates and proteins all of which need to be broken down into components our cells can use for their daily energy requirements. This process of metabolism involves a series of specific enzymes required to metabolise each individual component of food, to break it down into a substance the body can use for maintaining healthy function. All food-based protein is made up of 20 amino acids, and each of those amino acids require a specific enzyme to break them down and into a form necessary for normal physiological activity.

Inborn errors of metabolism (or metabolic disorders) arise when an individual is born lacking one or more of the enzymes required to break down a specific component of food. Failure to metabolise a particular food component typically results in an upstream collection of that component, as well as downstream deficiencies in the usual converted product of that food component. Either the over abundance of a particular food component or the deficiency of its usual downstream product (or both) can then cause a variety of problems for physiological functions (National Institutes of Health [NIH], 2009).

Metabolic disorders are usually of genetic etiology, and are typically diagnosed at birth (via newborn screening programs) or in infancy/early childhood when symptoms of disordered metabolism become apparent. Symptom presentation varies considerably from largely asymptomatic (e.g., elevated levels of metabolites but no other discernable impact on physiological function) to severe disability and death (Burton, 1998). This variation in outcome is
essentially a consequence of whether or not the overabundance of the specific food component is toxic; or whether the downstream deficiencies caused by the failure of the enzyme to convert that component is harmful or deleterious.

Phenylketonuria

Phenylketonuria [PKU] is a genetically inherited metabolic disorder (autosomal recessive), requiring both parents to be carriers of the disorder, with each offspring having a one in four chance of inheriting the condition (Scriver, Kaufman, Eisensmith, & Woo, 1995). Approximately 1 in 12 000 babies in Australia will be born with PKU (Pitt et al., 1983). Incidence of PKU shows racial variations, with most Caucasian populations of European background recording incidence around 1 in 10 000 births (Eiesensmith & Woo, 1994).

PKU involves the failure of a liver-based enzyme, phenylalanine hydroxylase. Individuals with PKU are unable to metabolise one amino acid in protein, phenylalanine, and convert it to tyrosine (Scriver et al., 1995). Untreated, PKU results in rising blood levels of phenylalanine, which is toxic to developing white matter tracts in the brain, causing severe intellectual disability (Pitt & Danks, 1991). Table 1 shows the results of untreated PKU.
Table 1

*Disability Outcomes From Untreated Phenylketonuria*

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Incidence</th>
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<tbody>
<tr>
<td>IQ &lt; 35</td>
<td>45%</td>
</tr>
<tr>
<td>IQ 36 – 67</td>
<td>48%</td>
</tr>
<tr>
<td>IQ &gt; 68</td>
<td>7%</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>26%</td>
</tr>
<tr>
<td>Spasticity</td>
<td>9%</td>
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</table>

*Source:* Pitt & Danks (1999); IQ = intelligence quotient

PKU also results in downstream deficiencies in tyrosine, the effect of which is currently not fully understood (de Groot, Hoeksma, Blau, Reijngoud, & van Spronsen, 2010). As tyrosine is a precursor to the neurotransmitter dopamine, lower levels of tyrosine may create a low dopamine environment within the brain and body.

**Diagnosis and Treatment of PKU**

Newborns are typically screened for PKU via newborn screening programs using dried blood spots (NIH, 2009). Treatment consists of a medically prescribed diet to limit exposure to phenylalanine, which is found in all food-based protein. The diet is vegan and very low in natural protein, and therefore needs to be supplemented with a phenylalanine-free, protein-enriched amino acid supplement, to ensure adequate growth and to maintain tyrosine levels (Australian Society for Inborn Errors of Metabolism[ASIEM], 2005; MacDonald & Asplin, 2006).
Dietary restrictions and supplements are strongly recommended for life, although it is well recognised that the worst damage caused by high phenylalanine occurs when the brain is developing (for a review and meta-analysis see Waisbren et al., 2007). As such, dietary restrictions are somewhat relaxed from the teenage years, in the belief that high phenylalanine should not be as damaging once brain development (especially myelination) is complete. However, this is a debatable medical treatment strategy, as dietary relaxation in adulthood and the effect on cognitive and psychological outcomes is not fully understood (Feillet et al., 2010). One universally agreed upon exception is women with PKU during pregnancy (as high phenylalanine is devastating to foetal neural development), who are advised especially in the early stages of their pregnancy, to comply with a greater level of phenylalanine restriction (ASIEM, 2005; Koch, Trefz, & Waisbren, 2010).

Compliance with the PKU diet can be difficult because of the restricted variety of natural foods, the high cost of specially manufactured palatable alternatives, the perception by some that the recipes are complicated and time-consuming to prepare, or that their dietary needs place restrictions on their social life (Bilginsoy, Waiztman, Leonard, & Ernst, 2005). Attrition from diet, rebellion and apathy are typical problems in maintaining dietary adherence for some individuals with PKU, especially during adolescence (Levy & Waisbren, 1994).

Neuropsychological Outcomes of PKU

Studies have clearly indicated that intellectual impairment worsens with increasing exposure to phenylalanine (see meta-analysis by Waisbren et al., 2007); however the issue of how much phenylalanine, over what period of time
and during which stages of brain development continues to draw debate (see Cochrane review by Poustie & Wildgoose, 2010). This divergence is reflected in different International guidelines to patients (see Table 2). Table 2 demonstrates the variation in current treatment guidelines as to what constitutes a “safe” amount of phenylalanine exposure for children with PKU.

Table 2

<table>
<thead>
<tr>
<th>Country</th>
<th>&lt; 12 years of age</th>
<th>&gt; 12 years of age</th>
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<tbody>
<tr>
<td>Australia</td>
<td>up to 360 umol/L</td>
<td>&lt;500 umol/L</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>120 – 480 umol/L</td>
<td>&lt;700 umol/L*</td>
</tr>
<tr>
<td>United States of America</td>
<td>120 – 365 umol/L</td>
<td>&lt;910 umol/L</td>
</tr>
<tr>
<td>Germany</td>
<td>40 – 240 umol/L</td>
<td>&lt;900 umol/L*</td>
</tr>
</tbody>
</table>

* >10 years of age. Note: Germany allows levels up to 1200 umol/L after 15 years. Source: ASIEM, 2005; Burgard et al., 1999; National Institutes of Health, 2000; The National Society for Phenylketonuria, 2004.

Table 2 shows differences in medical advice, both in terms of amount of phenylalanine allowed, and at what age phenylalanine-restrictions can relax. This variation has largely been a result of conflicting research findings in relation to intellectual impairment as a result of phenylalanine exposure.

Whether the PKU diet needs to be continued into adulthood, after brain development is complete, is unknown (Feillet et al., 2010; Poustie & Wildgoose, 2010). Some research has shown mean differences in IQ between PKU adults off-diet and PKU adults on-diet of up to 15 points (Cabalska, Nowaczewska, Sendecka & Zorska, 1996). Other research has shown little difference (Koch et al., 2002). Partial reversibility (i.e., improvement) of intellectual impairment has been demonstrated in late-treated adults with PKU who commence the low
phenylalanine diet (Grosse, 2010). Adults with PKU who do not maintain the low-phenylalanine diet have also been reported to show personality changes, and there is evidence for an increase in the risk of developing personality and/or internalizing disorders as a consequence (Levy & Waisbren, 1994). One difficulty in interpreting the current body of literature regarding continuation of diet into adulthood, is that most of the current adult population of PKU grew up in an era that had inferior and highly variable treatment procedures. This variation in treatment practice makes it problematic to tease out the effects of possible earlier neurological damage, compounded by later dietary instability.

Given the potential for a confounding effect of variable dietary practices in adults with PKU, a cohort-based study of children with early and continuously treated PKU is a significant advantage in order to assess the intellectual outcomes, in the context of higher consistency of recent treatment. As shown by Table 2, Australia currently advises a conservative treatment approach, with more stringent phenylalanine-restrictions, and older phenylalanine-relaxation guidelines, in comparison to other countries. Australia also recommends a diet for life approach, with phenylalanine restriction and continued phenylalanine-monitoring advised throughout adulthood (ASIEM, 2005). As such, there are further advantages in conducting studies of any proposed residual neuropsychological deficits associated with early and continuously treated phenylketonuria in Australia, where the level of phenylalanine restriction advised is both conservative and life-long.

Despite disagreements internationally, detection at birth and the medically prescribed dietary treatment for PKU has resulted in a remarkable success story from the last century for the effective prevention of neurological disability.
Improvements in dietary management and formulas since the 1960’s have been associated with steadily improving outcomes in intellectual functioning, as measured by standard Intelligence Quotient (IQ) scores. Children with early and continuously treated PKU born in the 1980’s and onwards, typically develop an IQ within the normal range (Burgard, 2000), although on average their IQ is slightly but statistically significantly lower than that of their parents and unaffected siblings (Koch, Azen, Friedman, & Williamson, 1984; Weglage, Funders, Wilken, Schubert, & Ullrich, 1993).

Residual Deficits in Early and Continuously Treated PKU

Despite improvements in treatment, children with early and continuously treated phenylketonuria [ECT-PKU] also continue to be at elevated risk of developing residual neuropsychological deficits. Executive functions [EF] remain especially vulnerable. EF directs purposeful, adaptive behaviors and the regulation of mood. EF encompasses skills in integration of information across time and space, task switching, self monitoring, abstract and predictive reasoning, and working memory. EF underpins goal-directed and adaptive behaviours including organization, planning and impulse control (Welsh & Pennington, 1991). Unlike a number of other key cognitive domains which largely finalise their development during childhood or early adolescence, EF development continues well into the early 20’s (Anderson, 2002). A significant body of research points to more EF deficits in children with ECT-PKU than controls. Specific deficits are evident in processing speed or cognitive tempo (Antshel & Waisbren, 2003; Huijbregts, de Sonneville, van Spronsen, Licht & Sergeant, 2002) especially in tasks requiring an ability to manipulate and integrate
information. This has lead to closer examination of the working memory functions in this population. Vigilance and manipulation tasks within working memory are the most affected (Huijbregts, et al., 2002). Diamond, Prevor, Callendar, and Druin (1997) also found PKU children had severe difficulty with a dual working memory and impulse control task i.e., requiring them to hold information in working memory while inhibiting a prepotent response. These abnormally high levels of EF impairment, particularly in processing speed, working memory, and impulse control have resulted in an Attention Deficit Hyperactivity Disorder (ADHD) diagnosis up to five times the norm in this population (Antshel, 2010; Arnold, Vladutiu, Orlowski, Blakely, & DeLuca, 2004).

The bulk of studies have clearly indicated that EF worsens with higher exposure to phenylalanine, and in general, the PKU literature is in agreement that high levels of phenylalanine, earlier in childhood and across longer periods of time, correlate with steadily worsening EF (Koch et al., 2002; Waisbren et al., 2007). However, debate continues as to the potential for normal EF development in children with PKU, even in the context of well controlled phenylalanine levels.

Recent reviews have highlighted the general problem of minor residual impairments leading to sub-optimal outcomes in this population, and questioned whether management of phenylalanine on its own is the most effective strategy to maximise intellectual development (Brumm & Grant, 2010; Feillet et al., 2010; van Spronsen, 2010). This general notion that current phenylalanine-management protocols for individuals with PKU, whilst undeniably successful, are not as effective as they could be in maximising potential development, underpins the rationale behind this PhD.
Biochemistry of PKU and Effects on Brain Development

Phenylalanine

Phenylalanine can easily cross the blood brain barrier and high phenylalanine levels cause severe disruptions to the developing brain in particular, with hypomyelination especially in white matter tracts that myelinate post-natally (Dyer, 1999; Zhang, Zhao, Wang, & Jiao, 2010). Animal models have demonstrated that the hypomyelination caused by high phenylalanine occurs predominantly in the corpus callosum and sub cortical white matter (Reynolds, Burri, Mahal, & Herschkowitz, 1992), and this observation has since been supported by human imaging studies (Anderson et al., 2007). Other imaging studies have implicated areas such as the cerebrum and hippocampus (Pfaendner et al., 2005), and suggested atypical connectivity both within the prefrontal cortex and between the prefrontal cortex and other brain regions (Christ, Moffit, & Peck, 2010). It is important to note however, that recent research has questioned whether high phenylalanine is directly or indirectly responsible for this hypomyelination (Schoemans, 2010). Anderson and Leuzzi (2010) have suggested that white matter abnormalities (WMA) can be caused either by hypomyelination, which is typically observed in untreated or poorly treated patients; or intramyelinic edema in patients with at least some history of dietary control i.e., this would be the expected etiology of WMA in more recent cohorts with early and continuously treated PKU.
White Matter Abnormalities and EF

Despite the clear risk to children with PKU of developing WMA, consistent associations between neuroanatomical anomalies, metabolic parameters, and cognition, have been difficult to demonstrate (e.g., see Anderson et al., 2007; Pfadendner et al., 2005). There is also the potential for reversal of WMA if phenylalanine levels are again restricted throughout the lifespan (Cleary et al., 1995; McCombe et al., 1992) particularly if it is a result of intramyelinic edema (Anderson & Leuzzi, 2010).

Although both WMA and EF worsen with increased exposure to phenylalanine (Koch et al., 2002), the relationship between WMA and EF is not as direct. Of most interest is the finding that children who display no WMA nonetheless continue to display EF deficits (Anderson et al., 2007; Leuzzi et al., 2004). Studies that have attempted to find a direct relationship between WMA and IQ/EF deficits have found an absent or weak or unclear association (Cleary et al., 1994; Pietz et al., 1997; Wang, Zhou, & Yu, 2006), or suggested a relationship that appears to be further complicated by neurotransmitter dysregulation (Anderson et al., 2007).

A possible explanation for these findings is that people who display severe WMA in conjunction with poor EF tend to show poor dietary compliance (Anderson et al., 2007; McCombe et al., 1992). Given the global damage caused by very high elevations in phenylalanine i.e., those associated with no or unregulated dietary control (Anderson & Leuzzi, 2010), it is reasonable to assume that once phenylalanine has reached a level high enough to cause severe WMA, multiple intellectual functions (including EF) will be compromised. In short,
eventually EF will suffer if white matter tracts are compromised; particularly in those regions that sub serve EF (e.g., prefrontal cortex).

What the WMA hypothesis does not explain, is why children whose phenylalanine levels are under sound control and/or children with no WMA, continue to display EF deficits nonetheless (e.g., see Leuzzi et al., 2004). It is these children, with at least sound phenylalanine management who are of most interest in the current context, as they are following the current treatment protocols, yet remain at risk of impairment.

_Dopamine Production_

Secondary problems arising from the biochemistry associated with PKU are thought to primarily affect the dopaminergic systems in the brain due to the disruption of the metabolism of phenylalanine to tyrosine, a precursor to dopamine. High phenylalanine saturates the transport system in the blood brain barrier, and competes with other large neutral amino acids (e.g. tyrosine and tryptophan). This causes problems for the transport of other amino acids, along with the inhibition of other enzymes needed to synthesise dopamine and serotonin in particular (Ormazabal, Artuch, Vilaseca, Garcia-Cazorla, & Campistol, 2004), this results in a significant reduction in dopamine, adrenaline, noradrenaline and serotonin (Schulpis, Tjamouranis, Karikas, Michelakakis, & Tsakiris, 2002). Using an animal model, Diamond, Ciaramitaro, Donner, Djali, and Robinson (1994) demonstrated that high phenylalanine resulted in dopamine reductions targeting medial prefrontal cortical areas, especially those areas associated with EF. Recent improvements in technology (Positron Emission Technology) to more accurately measure dopamine activity in the human PKU brain supports this
hypothesis, in that dopamine has been found to be reduced in humans with PKU, primarily due to the competition of phenylalanine (Landvogt et al., 2008).

**Dopamine and EF.**

A large body of research exists to demonstrate that dopamine acts as a neuromodulator in the prefrontal cortex, and that dysregulation of dopamine is associated with EF impairment (Seamans & Yang, 2004). Dopamine is essential to the function of the prefrontal cortex that serves the executive functions, and even minor reductions can create profound impairments leading to ADHD-type symptoms (Arnsten & Li, 2005).

**Phenylalanine to Tyrosine Ratio**

In 1997, Diamond, Prevor, Callender, and Druin published an experimental study investigating EF development in a young sample of children with ECT-PKU, and raised the possibility that EF deficits were still evident in children whose phenylalanine levels were under sound control (i.e., 360 - 600 umol). Diamond et al. (1997) further suggested that high phenylalanine in concert with low tyrosine (a high phenylalanine:tyrosine ratio) may better explain EF deficits in children with PKU than phenylalanine levels alone. Their hypothesis was that persistently high phenylalanine:tyrosine levels led to a ‘worst case scenario’ in terms of creating a low dopamine environment for the developing brain, which in turn causes deficits in EF. As high phenylalanine concentration inhibits tyrosine transport into across the blood brain barrier, less is available for dopamine formation. Increased plasma levels of tyrosine may not improve the competition for transport if phenylalanine is also increased. A low
phenylalanine:tyrosine ratio occurs when the plasma concentration of tyrosine is high relative to phenylalanine and this should allow more tyrosine to cross into the brain (Koch et al., 2003). Furthermore, once tyrosine has crossed the blood brain barrier into the brain, research has suggested that a high phenylalanine environment will inhibit the synthesis of dopamine from tyrosine (Ormazabal et al., 2004).

Although the study by Diamond et al. (1997) is now more than 10 years old, there have been relatively few follow-up studies to confirm the findings, and it is unknown if and how these findings may have been translated into clinical practice. Experimental studies that have compared the relative impact of phenylalanine and phenylalanine:tyrosine ratio on EF development have supported Diamond’s hypothesis (Luciana, Sullivan, & Nelson, 2001; Sharman, Sullivan, Young, & McGill, 2009; 2010a). Yet the potential role of the phenylalanine:tyrosine ratio on brain development has yet to be extensively researched (Poustie & Rutherford, 1999) because the focus of most studies remains as phenylalanine only measures: concurrently; historically, or both (e.g., Arnold et al., 2004; Huijbregts et al., 2002; Leuzzi et al., 2004).

Why the research agenda has been slow to change in response to Diamond’s first suggestion of the phenylalanine:tyrosine impact on EF is not known. Tyrosine data may not have been, or may still not be, routinely collected by some centres; further, tyrosine data collection may also vary across time (Sharman, Sullivan, Young, & McGill, 2010b). This divergence in practice either between clinics or within the same clinic as a function of time, has meant tyrosine and therefore the phenylalanine:tyrosine ratio may be absent as a variable.
Clinical applications of the dopamine hypothesis.

If Diamond’s dopamine hypothesis continues to be supported through further experimental investigation, findings need to be translated into treatment recommendations. Such recommendations need to identify an acceptable target or range for the phenylalanine:tyrosine ratio during childhood, to protect EF development in the PKU population. The phenylalanine:tyrosine ratio in the non-PKU population is approximately 1:1 (Hilton, Sharpe, Hicks, & Andrews, 1986) whereas the phenylalanine:tyrosine ratio in the PKU population typically range from 2.5:1 to well above 10:1 (Chace, Sherwin, Hillman, Lorey, & Cunningham, 1998).

Sharman et al. (2010a) recently reported a clinical level of lifetime phenylalanine:tyrosine ratio that was associated with impaired EF. In this sample of children with early and continuously treated (ECT)-PKU who maintained lifetime control of phenylalanine within Australian guidelines, a phenylalanine:tyrosine ratio above 6 was significantly and strongly associated with EF impairment. Further, EF was demonstrated to be better in those children whose phenylalanine:tyrosine ratios were the lowest. It is important to note that the Sharman et al. study tested a sample of children whose phenylalanine levels were under good control (i.e. within Australian guidelines), essentially holding phenylalanine as a constant variable. Under these circumstances the impact of the phenylalanine:tyrosine ratio on EF was strong, and may have been an unintended artifact of the sample characteristics in terms of their similarity of good phenylalanine control.

The influence of phenylalanine:tyrosine ratio in children whose phenylalanine levels are less well maintained, remains unknown. It is plausible
the impact of phenylalanine:tyrosine ratio may be less influential in children with less optimal phenylalanine control, because of the well established global damage created by high phenylalanine alone. That is, as phenylalanine increases and precipitates widespread damage to underlying brain structures, the impact of the phenylalanine:tyrosine ratio on functioning may be lowered or obscured.

Given these gaps in our current understanding, there is the potential to add to the current knowledge of the relative influence of biochemical markers in subsequent neuropsychological function in this population, with a view to improving PKU treatment guidelines. Emerging evidence is suggestive of an alternative mechanism for controlling the brain biochemistry thought to produce EF deficits: that is, using phenylalanine:tyrosine ratio monitoring, in combination with the current phenylalanine guidelines already in place.

*Tyrosine and EF.*

Tyrosine levels in children with PKU are significantly lower than those observed the normal population; and compared to children with milder versions of PKU e.g., hyperphenylalaninemia (Hanley et al., 2000). Why a high phenylalanine:tyrosine ratio is strongly related to EF impairments, given that low tyrosine on its own has not shown such a strong association, is unknown. Mixed results have emerged from past ECT-PKU studies that included increased tyrosine supplementation as a variable in terms of neuropsychological function (Poustie & Rutherford, 1999). A limitation of this past ECT-PKU tyrosine research is the inclusion of participants across a relatively broad age range (e.g., from early childhood to late 20’s), which could mask effects arising from tyrosine exposure that could be linked to potential “critical periods” in brain development (e.g. see
Lykkelund et al., 1988; Smith et al., 1998). Studies have typically used dietary supplementation over a relatively short period of time (weeks), whereas in the Luciana et al. (2002) and Sharman et al. (2010a) studies, the strongest association between phenylalanine:tyrosine and EF was observed for lifetime and not concurrent or recent phenylalanine:tyrosine ratios. Therefore there is yet to be any consistent evidence presented that relatively short term or temporary fluctuations in tyrosine levels may correlate with improved EF. To the author’s knowledge, there are no published studies exclusively focusing on the effects of increasing tyrosine during childhood, and/or over a long period of time.

In addition to inconsistencies from the research base, some researchers have questioned both the unreliability of tyrosine sampling as well as the potential for negative side effects of supplemental tyrosine as a treatment strategy (van Spronsen, van Rijn, Bekhof, Koch, & Smit, 2001). Whilst others have prescribed supplemental tyrosine over and above that contained in PKU formula when tyrosine levels drop below normal levels in this population and/or to remediate symptoms of EF (Posner, Gorman, & Nagel, 2009; Sharman, Sullivan, Young, & McGill, 2010b).

Rationale for the PhD

Given the gaps outlined in current understanding of the biochemical mechanisms that underpin residual EF deficits observed in this population, especially those deficits that persist in children who are following current treatment protocols, there is potential to add to the current body of knowledge that is relevant to treatment. It is clear that maintaining good phenylalanine control especially during childhood is vital to protect the brain from white matter damage
and neurotransmitter dysregulation that may impair cognition. What is less well understood is the potential for phenylalanine:tyrosine and/or tyrosine control to protect the developing brain from residual impairments that may result from dopamine dysfunction.

The following programme of research aimed to further examine these relationships within a cohort of adolescents with PKU. A critical analysis of the PKU literature is followed by an assessment of EF in a sample of adolescents with early and continuously treated PKU. The assessment was both longitudinal (clinical assessment of EF development in the PKU group across two time points, separated by 8 years); and between groups (using sibling controls) from parental report of EF dysfunction during adolescence. An examination of the biochemical markers (phenylalanine; phenylalanine:tyrosine ratio and tyrosine) associated with EF impairment is presented. Other potential consequences of poor biochemical control are discussed and a between groups analysis of depressive symptoms in the PKU and sibling control group was undertaken. Associations between biochemical markers and depressive symptoms in the PKU group are presented. As will be outlined later, this sample also included four children with hyperphenylalaninemia (a milder form of PKU) and whilst they were excluded from the primary analyses, their biochemical and neuropsychological development across time is presented. To ascertain how the current research body is being translated into actual clinical practice, an international practice survey outlining current approaches in the monitoring/treatment of tyrosine levels in children with PKU is then presented. Given impairments have real and tangible consequences for individuals, the children who participated in this study were
given an opportunity to comment on their disorder and how they perceive their management of PKU may impact their lives.
CHAPTER 2

Findings and Limitations of Previous PKU Research

In 1997, Diamond et al. published a study that found that children with well controlled PKU still showed emerging EF deficits. This paper created a flurry of research into EF development in children with PKU to investigate this possibility further. Studies since this time have been mixed in their conclusions. Table 3 outlines the major post-Diamond EF research design in child populations. These studies were drawn from internet search engines including PubMed; Medline; and Psych Info, using the search terms “executive function pku”. Experimental studies (i.e., not reviews) using child participants with PKU, that tested EF as well as its association with biochemical markers were included in Table 3. The results of these studies are discussed next.
Table 3

_PKU and Executive Function: Main Research Findings Post-1997_

<table>
<thead>
<tr>
<th>Author/year</th>
<th>Design</th>
<th>IV</th>
<th>DV</th>
<th>Participants</th>
<th>EF Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al., 2007</td>
<td>BG</td>
<td>WMA ‘metabolic control’</td>
<td>IQ; EF; academic achievement</td>
<td>N = 32 ECT-PKU N = 34 controls</td>
<td>Rey complex figure; WISC III digit span; tower of London; contingency naming.</td>
</tr>
<tr>
<td>Antshel &amp; Waisbren, 2003</td>
<td>BG</td>
<td>Timing of phe exposure; concurrent phe</td>
<td>ADD/ADHD IQ; EF and non EF tasks</td>
<td>N = 46 PKU; N = 15 mPKU; N = 18 sib. controls</td>
<td>Stroop word colour; CVLT; Rey complex figure; BRIEF; ADHD rating scale.</td>
</tr>
<tr>
<td>Arnold et al., 1998</td>
<td>Correlational</td>
<td>Concurrent and lifetime phe</td>
<td>Motor skills; EF; behaviour</td>
<td>N = 18 PKU</td>
<td>AB reversal; object retrieval; visual search; finger sequencing; verbal fluency; mazes and block.</td>
</tr>
<tr>
<td>Azadi et al., 2009</td>
<td>BG</td>
<td>Concurrent phe</td>
<td>EF; IQ; ADHD Autism</td>
<td>N = 10 PKU 6 – 20 years N = 15 controls</td>
<td>Tower of London; Continuous performance test; Stroop</td>
</tr>
<tr>
<td>Study</td>
<td>Type</td>
<td>Measure</td>
<td>Sample Size</td>
<td>Age Range</td>
<td></td>
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<tr>
<td>Griffiths et al., 1997</td>
<td>Correlational</td>
<td>Concurrent and historical phe, EF; non-EF; Personality</td>
<td>N = 15 PKU</td>
<td>10 – 13 years</td>
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<tr>
<td>Griffiths et al., 1998</td>
<td>BG</td>
<td>Concurrent and historical phe, IQ; EF; non-EF</td>
<td>N = 11 PKU</td>
<td>6 – 12 years</td>
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<td></td>
<td></td>
<td></td>
<td>N = 11 control</td>
<td>6 – 13 years</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>N = 11 control</td>
<td>6 – 13 years</td>
<td></td>
</tr>
<tr>
<td>Leuzzi et al., 2004</td>
<td>BG</td>
<td>Concurrent phe, IQ; EF, WMA- all absent</td>
<td>N= 14 PKU</td>
<td>8 – 13 years</td>
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<td></td>
<td></td>
<td></td>
<td>N= 14 control</td>
<td></td>
<td></td>
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<tr>
<td>Luciana et al., 2001</td>
<td>BG</td>
<td>Concurrent phe, EF and non-EF; motor skills</td>
<td>N = 18 PKU</td>
<td>18 years</td>
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<td></td>
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<td>N = 16 control</td>
<td>16 years</td>
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<td></td>
<td>N = 17 chronically ill controls, 18 years</td>
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<tr>
<td>Malloy-Diniz et al., 2004</td>
<td>BG</td>
<td>Historical phe – split into high vs low phe, EF and global development</td>
<td>N = 21 PKU</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>N = 18 controls infants</td>
<td></td>
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<tr>
<td>Sharman et al., 2009</td>
<td>BG &amp; RM</td>
<td>Concurrent phe, EF and non-EF; motor skills</td>
<td>N = 12 PKU</td>
<td>10 – 16 years</td>
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<td></td>
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<td></td>
<td>N = 7 sib. Controls</td>
<td>8 – 18 years</td>
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</tbody>
</table>

Mazes; Stroop; letter cancellation; design fluency.
N-back tests
Wisconsin card test; Rey complex figure; Maze; sorting; tower of London; visual search; motor learning
Working memory; set shifting; tower of London.
Bayley scales of infant development
BRIEF
<table>
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<th>Study</th>
<th>Design</th>
<th>Task Type</th>
<th>Independent Variable</th>
<th>Dependent Variable</th>
<th>Sample Size</th>
<th>Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smith et al., 2000</td>
<td>BG</td>
<td>Concurrent phe</td>
<td>IQ; EF; non-EF</td>
<td>N = 19 PKU</td>
<td>6 – 14 years</td>
<td>Problem solving; verbal fluency; working memory</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>N = 19 control</td>
<td>6 – 15 years</td>
<td></td>
</tr>
<tr>
<td>Van Zutphen et al., 2007</td>
<td>BG – PKU vs test norms</td>
<td>Concurrent phe; lifetime phe</td>
<td>EF</td>
<td>N = 15 PKU</td>
<td>8 – 20 years</td>
<td>Sorting; trail making; design fluency; tower; Stroop.</td>
</tr>
</tbody>
</table>

ADHD – Attention Deficit Hyperactivity Disorder; BG – between groups; BRIEF – Behaviour Rating Inventory of Executive Function; CVLT – California Verbal Learning task; DV – dependent variable; EF – executive function; IV – independent variable; Phe – phenylalanine; RM – repeated measures; Sib. – sibling; WISC – Weschler Intelligence Scales for Children
Table 3 shows that there is variation in study design (most studies are between groups), biochemical markers selected for analysis (most studies are phenylalanine-only), exposure to those markers (almost all studies use concurrent phenylalanine, some use lifetime exposure, only three included tyrosine), test selection (highly variable) and age of participants (also highly variable). This may explain why the conclusions drawn by different studies, can at times, appear to be at odds with one another. For example Anderson et al. found stronger relationships between historical phenylalanine and cognitive performance (rather than concurrent); both Smith et al. and Malloy-Diniz et al. found no between groups differences until high phenylalanine group was split out from the PKU sample and compared separately against controls; Arnold et al. and van Zutphen et al. found relationships between concurrent phenylalanine and test performance; whereas the Griffiths studies find no between groups differences (PKU and normal controls); the Luciana et al. study finds phenylalanine:tyrosine impact on both EF and non-EF cognitive tasks. Table 3 also shows that there are common methodological limitations in PKU research, such as sample size (with N from Table 3 ranging from 11 to 46). The following section outlines some of the major issues and solutions that have been proposed to minimise these problems.

Sample Size and Study Design

A universal methodological challenge in research into rare metabolic disorders is the limited number of available participants in any geographical area. With a PKU birthrate of approximately 1 in 10 000 in Caucasian populations, recruiting the number of participants typically required to achieve a statistically
significant between-groups effect problematic. There are very small between-groups studies that have claimed to find the null effect looking at EF within PKU. The basis of this interpretation is typically on a failure to achieve statistical significance, possibly committing a Type II error in statistical calculations i.e., failure to find an effect where one really exists – an especially important consideration in small sample sizes (Tabachnick & Fidell, 2001). For example, in a between groups design, Griffiths, Campbell and Robinson (1998) looked at working memory performance via one and two N-back tests in 11 children with PKU (aged 6 -12 years) and 11 IQ matched controls (aged 6 – 13 years). The study found no between-group differences, however with only 11 participants the study would need a very large effect size (d > .5) to detect such a difference. The authors did not report effect sizes or p values, so readers are unable to judge whether this failure to find significance may be a result of a Type II error. Longitudinal studies in PKU are rare, but are important for consideration for future research design in this field, as they assist in minimizing the possibility of a Type II error, created by a lack of power, that is typically encountered by small cross sectional studies (Tabachnick & Fidell, 2001).

Outliers

A further concern arises from recent improvements in technology that has identified a small percentage of people with PKU who have high blood levels of phenylalanine which differ from phenylalanine levels detected in their brain by magnetic resonance spectrometry (MRS). Typically these patients present as “unaffected” with intact IQ and EF, despite very high levels of phenylalanine recorded in their bloodstream. These differences between individuals may be
explained by genetic variability of the transfer of phenylalanine across the blood brain barrier (Gizewska et al., 2003; Weglage et al., 2002). Associated costs with MRS mean that systematic identification of these individuals does not occur, and this may be a relevant confound for research characterized by small sample sizes.

**Operationalisation of Phenylalanine Exposure**

There are differences in the way studies operationalise “phenylalanine exposure”. The majority of studies have used phenylalanine recorded at the time of neuropsychological testing (concurrent/current phenylalanine). Studies of the effects of lifetime phenylalanine exposure, also referred to as “historical” phenylalanine, have also been reported and these studies show strong relationships with cognitive variables (for review see Waisbren et al., 2007). However, concurrent phenylalanine and historical phenylalanine measures may, or may not, be correlated casting doubt on the interpretations from those studies that only looked at one or the other variable. Further comparisons of concurrent versus recent versus lifetime levels, such as deliberate dietary manipulation to influence concurrent biochemistry (e.g., Arnold et al., 2004; Griffiths, Ward, Harvie, & Cockburn, 1998) or the inclusion of both concurrent and historical variables to ascertain their relative influences on neuropsychological functioning, would be useful (Sharman et al., 2009).

**Neuropsychological Test Selection**

The types of EF tests chosen for these assessments are important from both a theoretical and clinical point of view. Putting participants with sound phenylalanine control under relatively high cognitive load can elicit a between-
groups difference (e.g., dual EF tasks in Diamond et al., 1997). This raises the question as to whether studies utilizing less challenging tests may have missed emerging deficits due to the lack of test sensitivity. Conversely, it could be argued that the studies using complex and high cognitive load tests, whilst extremely important from a theoretical point of view to further our understanding of emerging deficits, may lead to statistically significant yet not clinically significant differences. They may not provide an indication of how the child’s functioning is being impacted outside the laboratory setting.

Coupling direct measures of EF with “ecologically valid” executive function tests (tests of learning delay; or Attention Deficit Hyperactivity Disorder [ADHD] screening tests e.g. see Antshel & Waisbren, 2003; Arnold et al., 2004; De Roche & Welsh, 2007) will give a better indication of how a child is functioning in their day to day environment. For example, the finding that children with PKU are five times more likely to be using stimulant medication to control symptoms of ADHD when phenylalanine concentration reaches a threshold (i.e., 792 umol; Arnold et al., 2004), is likely to be considered a more compelling reason for dietary restrictions than a finding that children with PKU have difficulty inhibiting a prepotent response whilst performing another EF task (at phenylalanine 360 – 600 umol; Diamond et al., 1997). The presentation of information in a functionally relevant manner, is an important advance for this population, as these children are already required to adhere to severe dietary constraints, and any changes to their treatment protocol will be better received if well supported by clear evidence of functional improvement.
**Age Ranges and EF Development**

Differences in the age range of participants is an important methodological variation in previous PKU research. EF is the last cognitive function to mature, with emerging abilities at around eight months of age, rapid development during early childhood, selective growth between the age of 7 - 9 years, maturing around 12 years, and final executive control achieved throughout adolescence into the early 20’s (Anderson, 2002). Biological insult during this period of rapid growth may affect plasticity and localization of function, with the early childhood period in particular showing volatility in EF development, which may lead to a slower emergence of deficits (Dennis, 2000). Further, deficits may be exacerbated by time, or new deficits may reveal themselves after time has elapsed (Dennis, 2000) highlighting the importance of longitudinal (rather than cross-sectional) assessments throughout this period, to better chart the EF outcomes in this population in the context of early biological insult.

Antshel and Waisbren (2003) clearly demonstrated the relative sensitivity of the developing brain in the context of phenylalanine exposure when comparing the developmental trajectory of ADHD development in children with PKU versus children born to women with PKU (maternal PKU or mPKU). In their study, children exposed in utero (i.e., prenatally) to high phenylalanine levels (via their mother with PKU) were more likely to develop ADHD hyperactivity type. Children born with PKU are not exposed to high phenylalanine levels until after birth, as the placenta removes their excess phenylalanine while in utero. The children born with PKU were more likely to develop ADHD inattentive type. Whilst both groups of children remained at elevated risk of developing ADHD after exposure to high phenylalanine, the timing of that exposure clearly impacted
the expression and development of the disorder. So the variation in age of participants may create a “timing” confound, and therefore studies may a) miss deficits because the brain’s vulnerability to damage has not yet reached its most sensitive point, b) or may fail to identify deficits because the brain requires longer exposure in order to demonstrate impairment.

Models of EF development

Unfortunately, no universally agreed-upon model of EF exists, instead a collection of “typical” cognitive processes necessary to exert control and monitor underlying brain functions are often used to describe the executive abilities needed to appropriately respond or adapt to environmental stimuli in a goal-directed manner. For example, shifting or adaptation; monitoring and integration of information; and inhibition or impulse control (Miyake, Friedman, Emerson, Witzki, & Howarter, 2000). Whether these functions are unitary or separable remains a matter of debate (Best & Miller, 2010), with most developmental models suggesting a somewhat disparate beginning, with the separate EF components finally orchestrating their functions in a more co-ordinated fashion at some stage during adolescence. For example, EF in childhood has been posited to begin with specific skills of: attentional control; cognitive flexibility; goal setting; and information processing. These four separate components are thought to undergo a transitional period at the beginning of adolescence to operate collectively in a manner that would start to resemble adult “executive control” (Anderson, 2002, p. 71). Other models posit EF beginning in early childhood with the rudimentary features of working memory, inhibition (impulse control)
and shifting (adaptation), with the addition of functions such as attentional control and integration of EF processes as children age (Garon, Bryson, & Smith, 2008).

In a recent review of EF models of development, Best and Miller (2010) suggest that the basic features of EF in early childhood (working memory, inhibition, shifting) show different developmental trajectories, with questions remaining as to the nature of developmental increments between stages, as well as if and how these functions achieve integration across time. Best and Miller (2010) further conclude that the relationships between these so-called dissociable functions (especially as children age) remains poorly understood.

In terms of the biological changes that underpin these functions, recent research has suggested that whole-brain integrity may be important for EF in childhood; compared to the more localised frontal lobe functions seen in adulthood (Jacobs, Harvey, & Anderson, 2010). Therefore, any disruptions caused by abnormal biochemistry within the whole brain may result in EF impairment during childhood; further, certain EF functions may be differentially affected across the developmental span, and certain tests of EF may prove more sensitive at different stages in development (for a fuller review of test sensitivity in this population see DeRoche & Welsh, 2007; Waisbren et al., 2010). This may further explain why the influence of certain biochemical factors may be shown to be more influential depending on age of testing. For example, phenylalanine which affects whole brain processes may show stronger impacts on EF in childhood; as opposed to the impact on EF of the phenylalanine:tyrosine ratio in adolescence/adulthood which should more selectively affect dopamine production in the pre-frontal cortex. As shown by Table 3, differences are apparent in age at testing. This could lead to quite different outcomes in terms of
neuropsychological function as, for example, some neurological functions may more susceptible to the influence of fluctuating or accumulated biochemistry dependent on both type of biochemical disruptions and age of exposure.

*Adolescent brain and executive function development.*

The key shifts in localisation of EF as the brain develops are not yet fully understood. As previously mentioned, whole brain integrity may be more important in childhood to keep EF intact, but at what point localisation of EF to the frontal cortex occurs, remains unknown. Whilst it has been suggested that the relatively late development in the frontal cortex in humans subserves the equally late development of EF, this has been hard to measure directly (Tamnes et al., 2010). A recent meta-analysis of fMRI studies (52 studies and 842 children and adolescents) to better map the localisation of EF functions confirms movement of functions between brain regions and the frontal cortex as children and adolescents age (Houde, Rossi, Lubin, & Joloit, 2010), for example a specific role of the anterior insular cortex in adolescent executive functions. In all, it can be surmised, that as adolescents age, disordered biochemistry may impact age-related changes in functions dependent on the regional specificity of that function; in ways that are not yet fully understood, and therefore not easily predicted. Further, it is unknown how selective damage to a particular component of EF (e.g. working memory) in childhood, may manifest during adolescence, when the more separable components of executive functions are hypothesised to synthesise and operate in a more co-ordinated fashion.
Measurement of Phenylalanine Exposure

Some researchers have suggested that children with PKU should be split into “high phenylalanine” and “low phenylalanine” groups, as some studies have only found differences between the “high phenylalanine” and control children. Some of the hypotheses may have obscured important findings by combining low phenylalanine and high phenylalanine participants, effectively “cancelling each other out” (Smith et al., 2000). For example, Griffiths, Campbell, and Robinson (1998) reported no significant differences in reaction time in their PKU versus control participants however, the PKU standard deviations were five times that of the controls, indicating substantially greater variability in the PKU group, possibly due to the presence of two subgroups within the PKU group (high performers and low performers) or a few participants with severely outlying scores. This null result may therefore have been obtained by combining good performers and bad performers within the PKU group. To avoid this confound, the approach of separating high phenylalanine versus low phenylalanine participants and analyzing their results separately against each other, as well as controls, is an important analytical advance. Mixing high phenylalanine and low phenylalanine groups may also be accused of exaggerating residual EF deficits i.e., deficits observed in the high phenylalanine group would be expected, and combining those children with the low phenylalanine group may inflate reported impairments across the combined PKU group. Therefore separating out high versus low phenylalanine groups will give greater veracity to those findings that claim children under sound phenylalanine control remain at risk of residual EF deficits, despite their adherence to current guidelines.
Biochemistry and Intellectual Development

Assessment of specific biochemical markers alongside particular test selection are variables that show inconsistency across studies, with some studies focussing exclusively on EF; or including non-EF tasks alongside EF assessments; or preferring global measures of intellectual function. As most studies also include an assessment of the impact of biochemical markers alongside neuropsychological assessments, it is important to select tests that have previously shown an association, or at least have some theoretical basis to suggest they would show an association. Some research assesses EF, but then tests for associations with biochemical markers that have been shown to at best weakly correlate with function (e.g., concurrent phenylalanine only, see Table 3).

In a recent review, Waisbren et al. (2010) have attempted to identify key neuropsychological tests with a high sensitivity to the most “at risk” functions in PKU. Standardising test selection in this population will also move research toward a more consistent assessment of brain development in children with PKU, to allow for future meta-analyses and reviews, that will help overcome the small sample size issue in this population. Studies in this population also require a more informed focus on brain-based vulnerabilities with appropriate test selection to more accurately chart developmental impairments incurred across time. Biochemical markers selected for analysis alongside impairments should include as many known or reasonably hypothesised influential markers as practical (i.e., both concurrent and lifetime levels of phenylalanine, phenylalanine: tyrosine ratio, and tyrosine). Finally, future research should favour longitudinal designs to overcome the challenges of small sample sizes and developmental changes.
Consideration has been given to these advances in methodological understanding in the design of this PhD research.
CHAPTER 3

Study 1: Longitudinal Assessment of Executive Function

As outlined in the previous two chapters, executive functions remain the primary deficit now observed in children with early and continuously treated PKU and this PhD research was particularly interested in examining the issue of why children with PKU who are maintaining sound phenylalanine control, remain at risk of developing EF deficits. Working memory, processing speed, planning, and impulse control/inhibition remain the EF deficits typically observed in this group (Antshel & Waisbren, 2003; DeRoche & Welsh, 2008; Huijbregts et al., 2002; Sharman et al., 2009).

Of all the EF functions usually tested within this population, working memory is the EF that shows the most consistent impairment in children with PKU, including among children whose phenylalanine levels have been maintained within current guidelines. Working memory is the cognitive process that allows information to be stored temporarily, and maintained or manipulated to underpin thought processes (Baddeley, 2003). The model described by Baddeley (2003) posits that working memory is comprised of phonological and visuospatial storage systems, with a central executive postulated to control what stimuli will be attended to by the phonological and visual slave systems. Working memory is considered to be one of the early, rudimentary functions of EF (Garon et al., 2008). The maintenance and manipulation of information within working memory is necessary to sub serve other important components of EF such as planning and goal setting.
As children age, they tend to shift strategies from a passive maintenance of a memory trace (their ability recall largely dependent on duration of maintenance) to strategies that actively refresh or recode the information to keep it available in working memory from around the age of 7 years (Camos & Barrouillet, 2011). Working memory has been demonstrated to be particularly susceptible to impairments as a result of the biochemical changes caused by PKU (Huijbregts et al., 2002; Sharman et al., 2009). Closer examination of the working memory functions in this population, has demonstrated that vigilance, manipulation tasks within working memory, and impulse control are the worst affected (Diamond et al., 1997; Huijbregts, et al., 2002). Working memory deficits lead to obvious functional impairments (such as being unable to remember what to bring to school or a series of instructions given by a teacher). Working memory impairment is also the cognitive function most strongly impaired affected in children diagnosed with ADHD (Gioa, Isquith, Guy, & Kenworthy, 2000), and it is probably this impairment that is primarily responsible for the high prevalence of ADHD diagnosis in this population (Arnold et al., 2004). Dual EF tasks have also been shown to demonstrate strong differences between children with PKU and controls (e.g. Diamond et al., 1997). The dopamine-deficiency hypothesis would infer that as EF tasks are added together and performed concurrently (e.g. both working memory and impulse control); performance of these more complex dopamine-mediated functions should show a stronger level of impairment. Although it may be argued these types of tasks show poorer ecological validity or functional relevance, they remain important to further our understanding of the biochemical basis of residual impairments. Consideration was given to selecting both ecologically valid / functional EF
assessments to better inform relevant clinical guidelines, as well EF assessments that will further test the (dopamine) model proposed to account for residual EF impairments in children with early and continuously treated phenylketonuria.

How EF impairments in children with early and continuously treated PKU manifest across time remains poorly understood in this population, due to a lack of longitudinal research. As demonstrated by the Antshel and Waisbren study (2003), it may be that certain domains of EF are more vulnerable during certain sensitive periods, and may show differential vulnerability to certain biochemical markers (phenylalanine or tyrosine) across time. Given the continued debate regarding an appropriate level of phenylalanine restriction, and age that phenylalanine restrictions may relax (see Table 2), there is potential for longitudinal research to better map EF development over time, to ascertain the relative influence of biochemistry during sensitive periods of development, and to provide a clearer evidence-based rationale for phenylalanine or tyrosine control across the lifespan.

The following series of EF assessments measured EF impairment in a sample of children with PKU, and reassessed those children eight years later in adolescence. EF trajectory was investigated to determine any change in EF function across time, and the relative influence of phenylalanine and tyrosine on EF development is presented.

Hypotheses

In general, children with PKU were expected to demonstrate EF impairments (in comparison to age-expected normative data) at both time points. Whether EF impairments would persist, worsen or improve over time was not
known. Consistent with the dopamine-deficiency hypothesis and previous experimental research, EF impairments were expected to be more strongly associated with long term exposure to a high phenylalanine:tyrosine ratio than phenylalanine-only measures. Further, EF performance across all instruments chosen for re-analysis was expected to be strongly correlated.

**Method**

*Participants*

18 children with ECT-PKU (Mean age at testing 5.8 years; *SD* 1.65 years; range 3.4 years to 7.9 years) were originally assessed by then PhD candidate Toni Jones in 2001/2 (Jones, 2003). The children were assessed via a battery of neuropsychological tests as part of Dr Jones’ PhD project. All children were recruited from the Queensland children’s metabolic clinic, located at the Royal Children’s Hospital, Brisbane.

In 2009 the current PhD candidate re-contacted each family to request their participation in the current PhD project. Out of the 18 original child participants, one child was excluded from re-recruitment as the metabolic physician advised the child’s phenylalanine levels were so mild as to always be considered borderline. His last known biochemical level of phenylalanine (100 umol), was unlikely to meet current criteria for diagnosis of even mild hyperphenylalaninemia (Arnold, 2009). The remaining 17 children with PKU were retested on a select number of measures (justification for the each measure chosen is outlined in *Materials*).
Characteristics of the PKU sample.

17 children with ECT-PKU (nine male; eight female) agreed to participate in the study as part of the current PhD project, representing a 100% response rate of those children recontacted. Mean age at re-testing was 13.6 years; \( SD = 1.77 \) years; range 10.96 – 16.26 years. 13 children were assessed by the consultant metabolic physician as having “classical” phenylketonuria, the most common form of PKU. Four children were assessed by the consultant metabolic physician as having hyperphenylalaninemia, a variant form of PKU that results in milder expression of the disease and lower lifetime levels of phenylalanine (Arnold, 2009). Although differential diagnostic criteria for these two conditions are not universally agreed upon, in general phenylalanine levels either at the newborn screening period, or whilst on unrestricted diet are used to determine whether a child fits the criteria for hyperphenylalaninemia compared to PKU. Arnold (2009) suggests that hyperphenylalaninemia is indicated when phenylalanine levels exceed the upper limits of normal (120 umol) but remain persistently lower than the cut off recommended for a diagnosis for classical PKU (1200 umol). All child participants with classical PKU exhibited either a newborn screening result of > 1200 umol or an excess of that phenylalanine level cut off during their lifetime (even whilst on a phenylalanine-restricted diet); whereas the children with hyperphenylalaninemia exhibited levels well below 1200 umol at either newborn screening or during their lifetime (highest peak level of any of these four children was < 500 umol).

For reasons previously outlined in the critical review (Chapter 2), the following analyses only included the data from the 13 children with classical PKU; as combining the children with a variant form of PKU with substantially
lower lifetime expression of phenylalanine would have confounded the results.

Characteristics of the 13 children with classical PKU were: seven male, six female; mean age 13.95 years; SD 1.8 years; range 10.26 years to 16.26 years.

Demographic, neuropsychological and biochemical characteristics of the four children with hyperphenylalaninemia are presented in Chapter 6.

A substantial body of health data existed on these children, both from the Royal Children’s Hospital metabolic clinic in Queensland and Dr Jones’ original study. This data base was examined for any significant co-morbidities, accidents, head injury, childhood diseases (especially those that may impact EF), and none were observed for the children with either classical PKU or hyperphenylalaninemia. Minor illnesses such as flu, fever, measles (n=2) were reported, but were not reported to have had any ongoing or substantial impact on development. Likewise indicators of developmental delay (e.g., age at walking, talking) were available for examination, and no remarkable deviations from expected developmental trajectory was noted for any child.

*Biochemical characteristics of the PKU sample.*

Table 4 presents means, standard deviation (in brackets) and ranges of phenylalanine; tyrosine and phenylalanine:tyrosine ratio across the children’s lifetime, prior to age 12 years and concurrent with test administration.
Table 4

*Means (SD's) and Ranges of Biochemical Markers of 13 Children with Classical PKU*

<table>
<thead>
<tr>
<th></th>
<th>Phenylalanine mean (SD)</th>
<th>Tyrosine mean (SD)</th>
<th>Phen:tyr ratio mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td>Lifetime</td>
<td>438 (149)</td>
<td>97 (17)</td>
<td>7.4 (4.2)</td>
</tr>
<tr>
<td></td>
<td>226 – 735</td>
<td>67 - 121</td>
<td>3.6 – 19.5</td>
</tr>
<tr>
<td>&lt; 12 years</td>
<td>415 (146)</td>
<td>95 (15)</td>
<td>7.1 (4.1)</td>
</tr>
<tr>
<td></td>
<td>226 - 706</td>
<td>67 – 114</td>
<td>3.6 – 18.9</td>
</tr>
<tr>
<td>Concurrent</td>
<td>713 (273)</td>
<td>105 (40)</td>
<td>7.9 (4.4)</td>
</tr>
<tr>
<td></td>
<td>200 – 1200</td>
<td>45 – 170</td>
<td>1.3 – 16</td>
</tr>
</tbody>
</table>

Note: explanation of how these figures were calculated is provided under the heading *Biochemical markers* on p. 49.

Table 4 shows that on average this group displayed slightly higher phenylalanine levels prior to age 12 years than recommended by the ASIEM (< 360 umol), although these levels would be considered under sound control by countries outside of Australia (see Table 2). ASIEM guidelines suggests an acceptable relaxation of phenylalanine post age 12 years (to < 500 umol); given the mean age of participants (13.95 years), concurrent phenylalanine levels were also higher than recommended, although again, within limits set by other countries.

Intercorrelations between the blood markers were as follows:

Lifetime phenylalanine: < 12 years and concurrent; $r = .985$ and $r = .791$ (p < .01) indicating a high level of consistency across childhood and adolescence, but a lower level of consistency with concurrent phenylalanine. Lifetime tyrosine: <
12 years and concurrent; $r = .987 \text{ (p}< .01\text{)}$ and $r = .158 \text{ (NS)}$ indicating a similar level of consistency across childhood and adolescence as phenylalanine; but no significant relationship between concurrent tyrosine and that recorded across the lifespan. This pattern was repeated with the phenylalanine:tyrosine ratio.

Lifetime phenylalanine:tyrosine: < 12 years and concurrent; $r = .996 \text{ (p}< .01\text{)}$ and $r = .170 \text{ (NS)}$. The lifetime phenylalanine:tyrosine ratio showed a stronger association with lifetime phenylalanine than it did tyrosine $r = .813 \text{ (p}< .01\text{)}$ and $r = -.625 \text{ (p}< .05\text{)}$ but both were significant.

**Materials and Procedure**

The series of assessments that form the longitudinal study of EF in this sample follows earlier research investigating this sample of children with PKU conducted in 2001 and 2002 by then PhD candidate Toni Jones (Jones, 2003). Therefore, tests selected for the longitudinal analyses were partly constrained by the tests selected at baseline. Dr Jones selected tests to inform a cognitive processing model (relational processing) and these included instruments sensitive to both PKU biochemistry and EF impairment in this population (DeRoche & Welsh, 2008).

The general rationale behind tests selected for longitudinal analyses were that they a) were considered to be (at the very least in part) a test of EF; b) previously been found to be sensitive to differences in function in children with PKU compared to controls or; c) sensitive to biochemical variations in children with PKU. Further, tests were chosen to tap domains of EF via as many different senses/domains as practical i.e., visual, language, impulse control. As can be seen in Table 3, the type of neuropsychological instruments chosen for longitudinal
analyses in the current study represent archetypal forms of assessment of EF in children with PKU e.g., Stroop tasks, Rey Complex figure task, working memory task.

Careful consideration was given to selecting tests that were practical in terms of testing time and environment to maximize participation. Given the sample size was still quite small (even for a longitudinal analysis), the PhD candidate was especially concerned with ensuring the best possible follow up participation rate. In order to achieve this, the decision was made to test children just prior to their normal consult time with the metabolic physician, and to select a number of tests that would not be onerous or so time-consuming as to be a barrier to participation.

All tests were administered onsite at the Royal Children’s Hospital immediately prior to children (and their parents or carers) seeing the metabolic physician and dietician for their regular clinic appointment. Parents or carers filled out the questionnaires whilst children were tested in a separate room. Parents were offered the option of sitting in the room with the child (typically younger children preferred this), parents were asked not interrupt or interfere with the test administration and all parents complied with this request.

Children were seated at a desk opposite the test administrator and provided with writing implements and paper as necessary for the drawing task (Rey Complex Figure). The test administrator conducted all experimental tests blind (prior to) accessing the children’s current and historical biochemical levels, as well as baseline results.

No issues arose during test administration, and in general the children displayed a high level of enthusiasm for the process. Both children and their
families typically expressed a level of interest and novelty in being able to “do something” rather than sitting in the hospital waiting room prior to their regular appointment.

In terms of order of administration, tests were administered as such: 1) a brief screening test developed by the PhD candidate to assess children’s own perceptions of the impact of their condition (if any) on schoolwork, thinking tasks (see Appendix A). Questions included “what are your best subjects at school?” and “do you notice any changes in your thinking if your phenylalanine levels are high?” 2) Rey Complex Figure task, copy trial only (Meyers & Meyers, 1996); 3) Weschler Intelligence Scale for Children, 4th edition [WISC IV] digit span, forwards then backwards (Weschler, 2003); 4) Neuropsychological Assessment, 2nd edition [NEPSY-II] Comprehension of Instructions; 5) NEPSY-II Inhibition (Stroop) tasks (Korkman, Kirk, & Kemp, 2007). Parents filled out their questionnaire instruments (described in Chapters 4 and 5) concurrent with child test administration: the Behaviour Rating Inventory of Executive Function (BRIEF; Gioia, Isquith, Guy, & Kenworthy, 2000) and Children’s Depression Inventory [CDI] (Kovacs, 1992). Total test administration rarely exceeded 30 minutes in duration.

Rey Complex Figure Task.

The Rey-Osterreith Complex Figure Test (RCFT; Meyers & Meyers, 1996), is commonly used as a measure of EF in children with ECT-PKU (Anderson et al., 2007; DeRoche & Welsh, 2008). Along with the well known EF deficits in this population, visuospatial deficits in children with PKU have been
reported (for a review see Janzen & Nguyen, 2010), although their cause is unknown.

The RCFT is a drawing task used as a measure of complex visual planning. The RCFT in children has been shown to measure visuoperceptual and visuoconstructional abilities, as well as EF domains, especially planning and organization (Watanabe et al., 2005) as well as the core components of contemporary models of EF of working memory and inhibitory control (Miller & Hinshaw, 2010). Therefore it was chosen for retesting given it is also reported to encompass EF abilities. Performance on the RCFT has previously shown strong differences in children with PKU compared to controls (for review see DeRoche & Welsh, 2008).

This study used the copy trial only, where children are shown a complex geometric figure on the top half of an A4 piece of paper and asked to copy the figure in the space available on the bottom half of the paper. The copy trial of the RCFT in particular, measures visuospatial processing and problem solving abilities in children (Smith & Zahka, 2006). Variables derived include an accuracy score using the traditional scoring system with a possible maximum of 36 points. Time taken to complete the task is also measured.

Although the RCFT consistently shows strong differences between children with PKU and controls (DeRoche & Welsh, 2008), the reason for these differences are not clear. As the RCFT is thought to encompass EF abilities, it could be that differences between children with PKU and controls are due to the well known residual EF deficit in this population. However, the RCFT is also a test of visuospatial abilities, and these abilities independent of EF have also been found to be compromised in children with PKU (Janzen & Nguyen, 2010).
Performance on the RCFT therefore, could be an additive effect of two different impairments, which may explain why it consistently shows such strong differences in children with PKU compared to controls. The study was to ascertain 1) changes over time in performance on the RCFT in children with PKU 2) biochemical markers associated with performance on the RCFT and 3) correlations between performance on the RCFT and measures of EF to determine whether performance on the RCFT was associated with EF in this sample.

Children were given pencils and an eraser, instructions were given as per the RCFT test manual (Meyers & Meyers, 1996), which is to copy the figure as best they could and to stop when they felt they had done a good job. Children were timed using a stopwatch. One male participant was excluded from the Rey Complex Figure analyses as he had a visual deficit (corrected squint and hypermetropic refractive error) and was awaiting eye surgery.

_WISC IV Digit Span._

As previously mentioned, working memory remains the most vulnerable EF in this population, and highly sensitive to biochemical variations. Previous research has further suggested that the manipulation component of working memory is especially compromised in this population of children (Huijbregts et al., 2002). The Weschler Intelligence Scale for Children, 4th edition ([WISC IV] Weschler, 2003), digit span test (forwards and backwards) provides an assessment of working memory, where children are required to recall increasingly longer strings of numbers. The forwards test requires that children simply repeat the numbers read out to them; the backwards component requires a manipulation task in working memory where children must repeat the numbers in backwards order.
Instructions and test administration were as per the Weschler Intelligence Scale for Children test manual (Weschler, 2003). Children were asked to repeat numbers read to them aloud by the test administrator. The forwards digit span starts with a practice task, and then the strings of numbers read to the participant, and repeated back to the test administrator, increase from two, to three, to four … up to nine. The backwards digit span starts with a practice task, and children were asked to repeat numbers read aloud by the test administrator in a backwards order. Strings of numbers increase one at a time, to eight in total.

Comprehension of Instructions.

The Comprehension of Instructions subtest from the NEPSY-II (Korkman, Kirk, & Kemp, 2007) is a language comprehension task. The nature of the test may also relate to working memory – especially the auditory processing capacity of the phonological loop (Baddeley, 2003). Instructions such as “point to the blue circle above the yellow square” become increasingly complex, with children needing to recall several components of an instruction to select the correct answer. Assessment of language development in children with PKU has produced mixed results, with some suggesting impairment and others arguing for an absence of pathology (for a review see Janzen & Nguyen, 2010). Griffiths, Demellweek, Fay, Robinson, and Davidson (2000) explored possible biochemical relationships with language ability in this population, and found that primary school children whose phenylalanine levels had been kept below 360 umol demonstrated a higher mean verbal IQ (by 10 points on average) than children
with higher levels. Their study using the Weschler Intelligence Scales for children further indicated that language functions in particular seemed to normalize if phenylalanine levels remained below 360 umol, suggesting that language function may be particularly susceptible to phenylalanine variations.

In this study, the NEPSY-II Comprehension of Instructions task was chosen for reassessment in because the nature of the task may partly incorporate a measure of EF. Further, as previously outlined this PhD was particularly concerned with tapping EF domains via as many measures available from the baseline measures.

Instructions and test administration were as per the NEPSY-II test manual (Korkman et al., 2007). A series of instructions were read aloud to children, and children were expected to respond by pointing to the shape that corresponded to the instruction, such as “point to the blue circle in the third row”. Instructions become increasingly complex both in terms of comprehension (e.g., knowing the meaning of being asked to point to “every other” shape in the top row) and length, with the last few instructions typically involving two or more directives to be performed in a certain order.

**Inhibition and impulse control.**

The children's Stroop Day-Night task was developed by Diamond and colleagues (Diamond, Kirkham, & Amso, 2002; Gerstadt, Hong, & Diamond, 1994) and was used in Dr Jones’ original study to assess verbal inhibition in the context of prepotent responses. Children are instructed to say "day" when they are shown a picture of a moon and stars on a black card and to say "night" when shown a picture of a yellow sun on a white card. This task has been described as
requiring inhibitory control of a prepotent response and the ability to hold two rules in mind (Diamond et al., 2002; Gerstadt et al., 1994).

The current PhD project used a similar Stroop-type task contained in the NEPSY- II where children were asked to say “black” when shown a white square; further this older cohort were asked to say the opposite colour and shape of a stimulus figure when presented, again requiring inhibitory control and the ability to hold more than one rule in mind. The children’s ability to inhibit a prepotent response (measured by time taken to complete the set of responses) as well impulse control (measured by their total error rate) were used in analyses.

This measure was chosen for retesting as it has proven to be an extremely sensitive test of subtle EF deficit in this population, detecting biochemical associations with impairments among children whose phenylalanine is under sound or even good control (Diamond et al., 1997). This loss of ability to perform a dual EF task of manipulating information in working memory, whilst inhibiting a prepotent response, has proven in past examinations of this population to be somewhat of a “coalminer’s canary” for children who show even the mildest of elevations in phenylalanine levels. It was also the task that proved most sensitive to phenylalanine:tyrosine ratio effects in Diamond et al’s original (1997) experimental study.

As per the NEPSY-II test manual (Korkman et al., 2007), children were given the opportunity to practice the task, and actual test performance did not proceed until they successfully completed the practice task. Children were first asked to “read” a page full of shapes (circles and squares) as quickly as they could. Children were then asked to read out the opposite shape (i.e., say square when it is a circle and vice versa). Children are finally required to read the
correct/opposite shape dependent on the shape’s colour e.g., say the shape’s correct name if the shape is black; but the opposite shape’s name if the shape is white. This task is then repeated using a page full of different stimuli (direction of arrows - up versus down).

Scoring

Biochemical markers.

Lifetime measures of phenylalanine and tyrosine were available for sixteen out of the seventeen participants. Phenylalanine levels were recorded at least monthly for all participants during infancy and throughout childhood. During adolescence, screening frequency declined for some participants. Prior to the year 2000, tyrosine levels were available for approximately one third of the available phenylalanine data. Post 2000, tyrosine levels were available for over 95% of the phenylalanine measurement points. This was due to a change in protocol in 2000, where tyrosine was automatically included in the phenylalanine screening process. Calculating the phenylalanine:tyrosine ratio by averaging lifetime phenylalanine and dividing by lifetime tyrosine would have been confounded by significant missing data; therefore, the phenylalanine:tyrosine ratio was calculated by dividing the phenylalanine by the tyrosine level, for all measurement points where levels were recorded in tandem. Means for phenylalanine, tyrosine and phenylalanine:tyrosine were calculated from birth (excluding newborn screening result) to age 12 years given the suggested vulnerability of this period (ASIEM, 2005); across the lifetime; and concurrent with test administration.
One participant’s raw pre-2000 (i.e. card based) data had been lost, however, his original means of phenylalanine, tyrosine and phenylalanine:tyrosine ratio had been recorded by Dr Toni Jones in her data set. His post-2000 results were available in full. The combination of these records was considered reliable to estimate lifetime levels of phenylalanine, tyrosine and phenylalanine:tyrosine. This participant’s levels as recorded by Dr Jones and his post-2000 biochemical levels were highly consistent (his adolescent years showing a modest increase in phenylalanine and phenylalanine:tyrosine ratio as expected given the relaxation of the PKU diet).

Rey Complex Figure task.

The RCFT copy trial is scored in terms of accuracy of reproduction and correct placement of elements of the geometric figure; the scoring manual provides detailed directions as to what type of errors should attract deduction, as well as the magnitude of the deduction. Errors can include (for example) adding extra lines, drawing a square as a rectangle, failing to join lines at intersections, drawing elements as detached from the figure, omitting parts of the figure. A completely drawn figure with acceptable accuracy and correct placement of all elements is given a total possible raw score of 36.

As scoring of any drawing task may have some measure of subjectivity, five of the children’s RCFTs were double scored (blind) by a doctoral-level clinical neuropsychologist in private practice. Inter-rater reliability between the PhD candidate and the clinical neuropsychologist was very high ($r = .98$), and consistent with published inter-rater reliabilities for this instrument ($r = .93$ to .99; Meyers & Meyers, 1996). Across the five RCFT’s that were double scored, the
PhD candidate and clinical neuropsychologist were in perfect agreement for two children, the clinical neuropsychologist ranked the remaining three children slightly lower (< 1 point out of a possible 36) than the PhD candidate. As explained further in the results section, no child’s ranking of ‘impaired’ versus ‘unimpaired’ varied as a result of the very slight differences in scoring between the PhD candidate and clinical neuropsychologist, therefore, the original raw scores assessed by the PhD candidate have been used in analyses.

Supplemental RCFT norms exist for children aged 6 years to adolescence (Meyers & Meyers, 1996). The norms are separated by 6 month intervals and provide percentile rankings of raw scores for children who fall below the 16th percentile. As the RCFT can be used to identify individuals with brain damage at the severe end of the spectrum, participants who score above the 16th percentile are considered to be within acceptably normal range.

Raw scores were also converted to the number of standard deviations (SD) above, within or below each age norm to provide a standardized measure of performance given each child’s age (as per guidelines from the supplemental norms). As no scaled (standardized) scores exist for the RCFT, this was considered the best way to control for expected variations in performance as a function of age.

*Working memory – WISC IV digit span and language comprehension.*

Raw scores were converted to scaled scores to account for variations in age (and sex in the case of the language comprehension task). A scaled score of 10 represents average function, with a deviation of 3 representing the equivalent
of one standard deviation, higher scores indicate better task performance (Korkman et al., 2007; Weschler, 2003).

**Inhibition and impulse control.**

Scaled or standardized scores were not available for the day night Stroop task used in 2001/2, only raw scores were available for analysis. In the 2009 reassessment, the NEPSY-II provides scaled scores to account for variations in age and sex, a score of 10 represents average function, and 3 one standard deviation; higher scores indicate better performance. As no standardized scores existed for the 2001/2 testing, a repeated measures analysis was not possible to assess any change in the sample across time. Instead correlations are reported at both time points for performance based on raw scores (2001/2) and scaled scores (2009).

**Data Analyses**

Three paired samples t-tests were conducted to assess change in neuropsychological test performance (WISC IV Digit span; Comprehension of Instructions; Rey Complex Figure) between the two time points (separated by eight years). Given the directional nature of the hypotheses, one-tailed correlations were used to assess associations between EF performance at each of the two time points and nine biochemical variables (phenylalanine, tyrosine and phenylalanine:tyrosine ratio; concurrent, lifetime and < 12 years of age). Given the small sample size and subsequent potential for a Type II error, any correlation that was of moderate strength \((r > .4)\) but not statistically significant \((p > .05)\) was also reported in terms of its strength and \(p\) value.
Results

Rey Complex Figure Task

Out of the 12 adolescents with classical PKU, seven scored below the 5th percentile on the copy task of the RCFT; five of those seven scored well below the 1st percentile (raw scores of the classical PKU sample ranged from 17.5 to 36). Five children scored above the 16th percentile of the RCFT (i.e., within the acceptably normal range). Standard deviations for the sample ranged from one SD above their age-expected norm to down to five SD’s below the norm. One child scored one SD above the norm; four children scored within one SD of the norm; the seven children who fell below the 5th percentile scored from 1.5 to 5 SD’s below the mean for their age. Given the severe level of impairment of over half of the sample, and the fact that no child scored a milder level of impairment, children were ranked as “impaired” or “not impaired” for the purpose of statistical analyses: SD’s from age-expected norms were also used in correlational analyses. Although this is acknowledged to constrain variability, this conservative approach ensured that expected age-related variations did not confound the results.

Analysis of children’s performance in 2001/2 revealed eight valid candidates (mean age 7.2 years) for longitudinal assessment i.e. children old enough in 2001/2 to have their raw scores converted to scaled scores. In 2001/2 these eight children had a mean age of 7.2 years, and in 2009 15.2 years. Children’s raw scores were standardized according to age into impaired versus unimpaired categories; and SD’s were calculated from their age-expected mean. SD’s from the children’s age-expected mean were used to test whether any
significant change across time had occurred, as these provide a measure standardized for age.

In 2001/2 children with classical PKU scored an average of 1.25 SD’s below the mean; in 2009 these same children scored an average of 1.31 SD’s below the mean; a paired samples t test found that this difference in performance on the RCFT was not significant \( t(7) = .092, p = .929 \). Therefore, no changes in performance across time on this test were evident in this sample.

Independent samples t-tests were conducted to compare biochemical markers of the five children with unimpaired versus the seven children with impaired performance on the RCFT. One significant result was observed: children with unimpaired performance demonstrated higher concurrent tyrosine, unimpaired group mean = 135 umol (SD = 30); impaired group mean = 77 umol (SD = 24.6) \( t(10) = -3.54, p = .008 \). Correlations between children’s biochemical markers and their SD from the RCFT age norm (a measure that allows raw scores to be standardized for age) revealed the same result, with higher concurrent tyrosine (the only significant correlation) associated with better RCFT performance Pearson’s \( r = .567, p = .027 \).

Similar to testing at adolescence, during early childhood, six out of the eight children old enough to be scored, fell below the 5\(^{th}\) percentile (impaired), the remaining two scored above the 16\(^{th}\) percentile (unimpaired). This sample was too small to conduct tests of biochemical differences between children in the impaired versus unimpaired categories and no further analysis was conducted.

Associations with other EF tests revealed that the RCFT copy trial performance showed no significant relationship with children’s performance in the WISC IV digit span; NEPSY-II comprehension and inhibition tasks (nor the
parent report of executive function, BRIEF, described in Chapter 4). Therefore, children’s performance on the RCFT copy trial was not associated with EF impairment in this sample.

**Working memory – WISC IV Digit Span**

Eight children from the 2001/2 data set were old enough at time of testing to have their raw scores converted to scaled scores; the mean scaled score of the digit span of these eight children (mean age 7.2 years) was 8.25, ($SD$ 2.55); in 2009 (mean age 15.2 years) the mean scaled score of these eight participants was 8.54, ($SD$ 2.79). On both occasions children with classical PKU scored approximately half a standard deviation below their age-expected norm, and scores were stable across time. A paired samples t-test indicated no significant change over time in performance on the WISC IV digit span task, $t(7) = -.154$, $p = .882$

Correlations were used to assess phenylalanine and tyrosine levels associated with WISC IV digit span performance in the child (n = 8) and adolescent (n = 13) samples, and no statistically significant correlations were observed. However, in terms of strength of effect, moderate-strength correlations ($r > 0.4$) were: child sample lifetime phenylalanine $r = -.621$, $p = .05$; lifetime phenylalanine:tyrosine ratio $r = -.591$, $p = .062$; adolescent sample lifetime phenylalanine:tyrosine ratio $r = -.45$, $p = .061$ and phenylalanine:tyrosine below age 12 years $r = -.43$, $p = .072$. Therefore, negative correlations of moderate strength were observed between lifetime levels of phenylalanine:tyrosine ratio and performance on the working memory task were observed in both childhood and adolescence, and showed a trend towards significance.
Correlations between the WISC IV digit span and other measures of EF were strong and significant. WISC IV digit span and NEPSY-II inhibition and error were positively correlated $r = .487; .682, p < .05$ respectively, indicating better performance on the WISC IV digit span was associated with better performance on the NEPSY-II inhibition (Stroop) tasks. The WISC IV digit span and NEPSY-II Comprehension of Instructions were positively correlated $r = .526, p < .05$, indicating better performance on the WISC IV digit span was associated with better performance on the language comprehension task. These correlations between the various tests of EF serve as a useful test of convergent validity.

**Language Comprehension**

Scaled scores were available for the full classical PKU sample at both time points (2001/2 and 2009). Mean scaled scores of the sample in childhood was 10.92 ($SD 1.65$), in adolescence mean performance had dropped to 8.15 ($SD 2.27$). As a group, children with classical PKU had deteriorated from just above normal performance on this task, dropping on average nearly a full standard deviation by adolescence. A paired samples t-test demonstrated that this loss of function was statistically significant $t(12) = 5.44, p = .000$.

A series of correlations were generated to test for biochemical markers associated with impaired performance at adolescence. One statistically significant correlation was observed for lifetime phenylalanine and comprehension $r = -.492, p = .044$. Other moderate correlations all $r > .4$ were observed for phenylalanine below 12 years; lifetime phenylalanine:tyrosine ratio; phenylalanine:tyrosine ratio below 12 years; and concurrent tyrosine (although these were not significant with $p$ values falling between .05 to .10).
Performance of the group was normal in early childhood, and correlations showed no effect of any biochemical marker on performance in 2001/2 (at mean age 5.9 years); no significant nor moderate ($r > 0.4$) correlations were observed.

**Inhibition and Impulse Control**

Response inhibition and total correct raw scores were correlated with children’s concurrent and lifetime phenylalanine and tyrosine levels in 2001/2. Two significant correlations were observed: a negative correlation between lifetime phenylalanine:tyrosine and total correct $r = -0.586, p = 0.018$ indicating lower lifetime phenylalanine:tyrosine was associated with better performance on this task; and a positive correlation between lifetime phenylalanine:tyrosine and response inhibition (error rate) $r = 0.560, p = 0.023$ indicating higher number of errors with increased lifetime phenylalanine:tyrosine.

Scaled scores to account for variations in age and sex were used in the 2009 correlational analyses to test for associations with biochemistry and performance on a similar Stroop task from the NEPSY-II. Inhibition and error scaled scores were used, therefore higher scaled scores represent better function.

Children with classical PKU demonstrated a mean scale score of 7 ($SD 2.34$) on the inhibition task and 6.46 ($SD 2.4$) on the error task. On average children with PKU performed at least one standard deviation below their age-expected norm (interestingly no individual child with classical PKU scored one standard deviation above the norm or higher on this task).

Four significant correlations were observed. Two positive correlations between inhibition and lifetime tyrosine $r = 0.49, p = 0.046$ and concurrent tyrosine $r = 0.52, p = 0.016$, indicating better inhibitory control was associated with higher
lifetime and concurrent tyrosine. Two negative correlations between error and lifetime phenylalanine:tyrosine ratio $r = -0.562, p = 0.023$ and phenylalanine:tyrosine ratio before 12 years $r = -0.529, p = 0.021$, indicating that better performance on the error task was associated with lower phenylalanine:tyrosine ratio over the lifetime and before age 12 years.

**Discussion**

*Rey Complex Figure Task*

As per previous studies, the RCFT proved to be a highly sensitive measure of deficit in this sample, with over half of the adolescent sample performing below the 5th percentile, and most of the “impaired” children falling well below the 1st percentile. The only association with biochemical markers was one in adolescence with concurrent tyrosine. No significant change in performance on the RCFT across time was observed. At both time points, children with PKU as a group scored just over one standard deviation below their age expected norm, and this performance did not appear to worsen over time. The RCFT demonstrated no association with other measures of EF in this sample, indicating that the copy trial at least, may be more a measure of visuospatial processing independent of underlying EF.

Only one previous study has investigated a possible biochemical mechanism of visuospatial performance in this population. Diamond and Herzberg (1996) tested children with PKU (under good phenylalanine control and of normal intelligence) against sibling and other controls on a visual contrast measure (spatial frequency). Children with PKU showed strong impairments (with PKU accounting for 70% of the variance in test performance). IQ was not
associated with the results in the PKU sample, but high phenylalanine:tyrosine ratio was. Diamond and Herzberg hypothesised that this visuospatial processing deficit was the result of lowered tyrosine creating less available dopamine required by the activity of the rapid-fire and rapid-turnover dopamine receptors in the retina (including those projecting to the frontal cortex). This resulted in poorer performance on their measure of visual contrast.

The current study found that visuospatial impairment was significantly associated with low concurrent tyrosine in this adolescent sample. Further, the copy trial of RCFT in this sample was not only a highly sensitive measure of deficit, but that deficit was not associated with EF impairment. Poor ability in copying the figure appear to stem from an as yet unknown problem, and does not appear to be related to other EF abilities measured. In contrast to usual findings of deficit in this population, only concurrent tyrosine was associated with this impairment.

Over half this sample in adolescence demonstrated very severe impairments in visuospatial processing (i.e., not mildly or moderately impaired, but falling well below the 5th and 1st percentiles), and this level of deficit may have serious consequences for daily functioning. For example, upon seeing the lowest scored RCFT by an adolescent male participant (Appendix D), the clinical neuropsychologist who double scored the results commented that she would be very concerned about this person’s capacity to drive safely. This severe level of impairment was associated with only concurrent tyrosine in this sample during adolescence. This is a curious result, in that this association was not evident in early childhood and needs to be viewed cautiously.
Given the severe deficit demonstrated by most of the children with PKU in this sample, it may be optimistic to believe that one fluctuation in one concurrent maker is responsible for this impairment, especially given concurrent biochemistry has shown no or weak associations with functioning in previous studies. It is plausible that multiple (or other) mechanisms may be involved e.g. white matter abnormalities, poor motor control, or an unknown variable not tested in this study.

**WISC IV Digit Span**

Children with classical PKU performed approximately half a standard deviation below the norm at both time points (in early childhood and adolescence). This finding is consistent with levels of intellectual deficit typically reported in this population, in that function is generally slightly lower than controls, norms, siblings or unaffected family members. No significant changes in performance were observed across time on the WISC IV digit span, suggesting that children remained on the same trajectory of slightly poorer function from mean age 7.2 years through to adolescence (15.2 years).

The WISC IV digit span did not detect statistically significant associations with biochemistry in our sample. However three out of four moderate correlations ($r > 0.4$) were observed between a high phenylalanine:tyrosine ratio and poorer performance, so statistical significance might be achieved within a larger sample.
Language Comprehension

Performance on the language comprehension task showed significant deterioration over time, with children as a group dropping from normal to sub-normal performance. No effect of phenylalanine or tyrosine on task performance was observed in childhood, yet effects of phenylalanine were evident (and possibly still emerging) by adolescence. This suggests that this language comprehension task is a) susceptible to damage in later years of development or b) susceptible only after very long term exposure (i.e., many years) to elevations in biochemistry caused by PKU. This finding also supports the Griffiths et al. (2000) hypothesis that language development in particular may be especially vulnerable to phenylalanine exposure.

The NEPSY-II language comprehension task showed no other statistically significant correlations with other EF measures (apart from the WISC IV digit span as previously reported), suggesting that it is primarily detecting comprehension abilities, with a possible secondary component of working memory.

This finding could be partly explained in terms of the development of rehearsal strategies children typically acquire as they age. Previous research has shown that children tend to move towards more working-memory based rehearsal strategies somewhere between the ages of 8 and 10 years (Lehmann & Hasselhorn, 2007). The fact that a moderate correlation was observed between children’s performance on the WISC IV digit span and this language comprehension task could point to a deficit in language-based rehearsal strategies as they emerge in middle childhood. This specific test may have been detecting both comprehension and working memory abilities, hence the strong difference in
performance across time i.e., the impairment may represent an additive effect of two separate impairments (comprehension and working memory).

This result supports the notion put forth in the introduction, that developmental changes across time may remain differentially susceptible to high phenylalanine concentrations. Whilst this research is primarily interested in exploring EF development and biochemical changes associated with EF, the result from this language comprehension task serves as a timely reminder that other functions remain at risk of phenylalanine exposure. This finding demonstrates a concerning loss of function over time in this sample, and this loss has been primarily influenced by long term exposure to high phenylalanine.

This finding also highlights the dangers of complacency when assessing children with PKU across time. As a group, this sample performed slightly above their age expected norm on this task in the early years of childhood, and no associations were evident with concurrent or lifetime measures of phenylalanine or tyrosine. However, this result has changed by adolescence, with sub-normal performance impacted by long term exposure to high phenylalanine (and possibly an emerging influence of high phenylalanine:tyrosine ratio). This highlights the brain’s differential sensitivity to damage during different stages of development, and the importance of repeating assessments over time, and assessing performance across a number of intellectual domains.

Finally, this result may also explain why previous assessments of language have been mixed in their findings (Janzen & Nguyen, 2010). Within this sample over time, language comprehension abilities have changed with age and phenylalanine exposure, going from slightly above average performance to sub-normal functioning; and from no association to biochemistry in childhood to a
clear association by adolescence. This supports the previous argument for more longitudinal developmental assessments in this population, to better untangle the inconsistencies from previous cross-sectional studies.

**Inhibition and Impulse Control**

A lack of available norms for the raw scores from the day night Stroop task in 2001/2 did not allow for a direct measure of the children’s change in performance over time. However, analysis of the classical PKU group in adolescence demonstrated impaired performance on both Stroop tasks (ability to inhibit a prepotent response, and total error rate). Children with classical PKU performed on average one standard deviation below their age norm, and no child with classical PKU performed well (i.e., one standard deviation or more above the norm) on this task. As per Diamond’s original hypothesis, performance on both tasks was clearly impacted by phenylalanine:tyrosine ratio at both time points (in early childhood and adolescence), and this study has also found an effect of tyrosine independent of phenylalanine in the adolescent stage.

This measure is confirmed as the most sensitive measure at both time points, from the tests selected for reassessment. The phenylalanine:tyrosine ratio and/or tyrosine were significantly associated with task performance on both occasions. This supports Diamond’s original hypothesis, that dopamine-specific EF domains are vulnerable to exposure to a high phenylalanine:tyrosine ratio (as well as low tyrosine independent of phenylalanine in this sample). Measuring children’s ability to perform a dual EF task: holding at least two rules in working memory whilst inhibiting a prepotent response (i.e., impulse control); is useful in ascertaining subtle levels of damage to dopamine-mediated EF. This, and similar
Stroop-type tasks, may therefore provide an early warning sign of dysfunction in both younger and older cohorts of children with classical PKU, and requires further exploration.

*Changes in Biochemical Markers Over Time*

The sample was too small to conduct repeated measures ANCOVA’s which would have provided an assessment of covariation with main effects e.g., whether the decline in function on the language task covaried with phenylalanine or phenylalanine:tyrosine. Therefore tests were restricted to correlational analyses to assess associations between test performance and phenylalanine/tyrosine. However, given neuropsychological test performance across time showed that either a) impaired performance in childhood that remained impaired in adolescence or b) normal performance in childhood that became impaired by adolescence; a series of paired samples t-tests were conducted to test whether, as a group, children with classical PKU demonstrated significant changes in their phenylalanine/tyrosine levels as they moved from early childhood to adolescence. As ASIEM guidelines allow a relaxation of phenylalanine restrictions at age 12 years, and the sample had a mean age of 13.95 years, significant differences across time in relation to phenylalanine levels were expected.

Paired samples t-tests showed significant increases between mean age of 5.9 years to 13.95 years in lifetime measures of: phenylalanine 343 umol to 438 umol, t(12) = -6.50, *p* = .000 and phenylalanine:tyrosine ratio 5.46 to 7.36, t(12) = -3.04, *p* = .01. A modest increase in tyrosine from 91.8 to 96.8 umol over the 8 year period was not significant.
Both phenylalanine levels and phenylalanine:tyrosine ratio significantly increased from 5.9 years to 13.95 years in our sample. Given the average age of the adolescent sample was 13.95 years, a mean level of phenylalanine across the previous year was conducted to ascertain if the teenagers were (on average) compliant with the recommended maximum of 500 umol post 12 years (ASIEM, 2005). The mean accumulated phenylalanine level across the previous year for this sample of children with classical PKU was 691umol ($SD$ 246.9; range 340 to 1266). This demonstrates that phenylalanine levels in this sample raised from those that (on average) were below recommended guidelines ($< 360$ umol; ASIEM) in early childhood to well above recommended guidelines by the time these children reached adolescence.

Similarly the phenylalanine:tyrosine ratio rose from 5.46 to 7.36 from childhood to adolescence. Given the modest increase in tyrosine levels, this increase in the ratio is largely a product of the sample’s rising phenylalanine levels across time. Only one report exists of what may constitute a safe level of phenylalanine:tyrosine ratio to protect EF development during childhood (phenylalanine:tyrosine below 6; Sharman et al., 2010). This analysis shows that on average this sample was only just below the safe cutoff suggested by Sharman, and by adolescence had moved above it. Although poorer compliance to diet is not atypical of this population, it highlights the problem of motivating teenagers with PKU to maintain ideal and safe levels via dietary control.

Summary

Longitudinal analyses of these 13 children with classical PKU over an eight year period has demonstrated two possible trajectories of EF impairment
associated with biochemical markers across time. 1) Normal function in early years of life but deficits evident by adolescence e.g., the language comprehension task or 2) deficits evident early in life that (on average) persist e.g., RCFT, WISC IV digit span and NEPSY-II Inhibition (Stroop) tasks.

Clear effects of phenylalanine and tyrosine have been observed. As hypothesized EF deficit was more strongly associated with phenylalanine:tyrosine ratio as well as tyrosine independent of phenylalanine in this sample. Elevations in phenylalanine:tyrosine ratio or lower levels of tyrosine early in life (i.e., by age seven years) appear to persist into adolescence. The EF performance of this group, did not significantly change over time, suggesting that level of impairment evident by age seven years, remains relatively stable. Taken in all, the longitudinal data testing EF function is suggestive of a possible sensitive period for EF impairment via high phenylalanine:tyrosine ratio or low tyrosine earlier in development. Whether or not this deficit could be reversed or improved with heightened tyrosine and/or lowered phenylalanine:tyrosine ratios after the age of seven years remains and important research question for the future. This result builds on the previous report of phenylalanine:tyrosine effect from Sharman et al. (2009), in that sample, phenylalanine:tyrosine ratio prior to age 12 years showed the strongest effects on subsequent EF during adolescence.

The two tasks in this longitudinal analysis that also related to non-EF functions, showed quite different vulnerabilities. The language comprehension abilities of this group significantly declined with age and exposure to higher levels of phenylalanine, indicating that phenylalanine control was important to protect general intellectual functions. Loss of this function occurred later in development, at some point between six and 14 years of age in this sample.
Normal task performance at age six years did not at all guarantee normal task performance at age 14 years, and exposure to higher levels of lifetime phenylalanine was associated with poorer performance by adolescence. This is suggestive of a sensitive period post age six years, and serves as a caution that normal performance in early years of life can be lost due to poor biochemical control by adolescence.

Task performance on the drawing test conversely, showed associations only with concurrent tyrosine in adolescence, and previous evidence has suggested that low tyrosine may have an impact in retinal function. The is the one finding inconsistent with all other results that suggest long term exposure is the most influential, and this effect was only observed at one time point (adolescence), therefore this specific result should be viewed very cautiously and investigated further.

A divergence of results (and biochemical influences) has also been demonstrated between the tests that clearly capture the separable (rudimentary) EF functions (e.g. working memory and inhibition) described by contemporary models of EF, compared to those tests that may be seen as mixed in terms of the pure EF components they purport to measure. As previously mentioned, test selection for these studies were constrained by historical limitations, however, future research may benefit from selecting tests that more tightly conform to a contemporary model of EF. This may further assist in the understanding the different developmental trajectories of these EF components (as suggested by Best & Miller, 2010), as well as a more specific idea of when their greatest vulnerability to biological insult may occur during development.
These results support the idea that loss of function in children with PKU can occur at any point in time from childhood through to adolescence, in response to (usually) long term exposure to high phenylalanine levels and/or low tyrosine. This longitudinal study has emphasized however, the importance of the brain’s relative sensitivity to developing impairments that is dependent on age of exposure, as well as type (of biochemical maker) and length of exposure.
CHAPTER 4

Study 2: Between Groups Executive Function Assessment

The study reported in Chapter 3 focused on the longitudinal development of EF impairment in children with PKU, and the relationship between those impairments and biochemical markers. Whilst this kind of study is important to further our understanding of the biochemical etiology of residual deficits in this population, the deliberate selection of instruments that are highly sensitive to specific domains of EF, may not fully inform a more realistic picture of the functional impact of residual impairments. As outlined in Chapter 2, coupling EF assessments that contribute to the theoretical basis of residual deficits of this disorder, alongside ecologically valid assessments that assess their functional impact, is an important advance, especially given the body of this research is likely to contribute to dietary guidelines. The use of ecologically valid instruments is thought to provide a better indication of how EF deficits manifest in terms of actual functioning, and thereby increase generalisability and representativeness of impairments reported (Burgess et al., 2006).

The functional impact of EF impairments in this population has previously been reported to be quite marked, with an increased risk of ADHD diagnosis and self reported lower rates of independence and problems in interpersonal relationships (Arnold et al., 2004; Simon et al., 2008). Therefore, an ecologically valid EF instrument was added to the longitudinal test battery to investigate between-groups differences in children with PKU compared to their siblings in
the 2009 time point only. The Behaviour Rating Inventory of Executive Function (BRIEF; Gioia et al., 2000) was selected, and has previously shown high sensitivity in this population (Antshel & Waisbren, 2003; Waisbren et al., 2010). The BRIEF assesses parent’s perception of behavioural indicators of EF function.

Hypotheses

Consistent with previous research it was expected that 1) the children with PKU would show greater impairments than their siblings on summary scales of working memory and global executive composite from the BRIEF; 2) EF impairment would be more strongly associated with phenylalanine:tyrosine ratio and/or tyrosine markers in line with the dopamine-deficiency theory of EF deficit in this population (Diamond et al., 1997).

Method

Participants

The 13 children with classical PKU as described in Chapter 3 were assessed for this between groups comparison.

Characteristics of the sibling sample.

Nine siblings (6 male; 3 female) were assessed for the between groups analyses. Their mean age at testing was 13.12 years; $SD$ 3.4 years; range 7.5 years to 17.58 years. Parents were asked to nominate up to two siblings closest in age to the child with PKU, who were also between the ages of 7 – 18 years as required by the BRIEF questionnaire. Parents were asked not to select siblings who had any known developmental delay or medical problem. One male sibling
was later excluded from the EF analyses after his mother reported he was receiving treatment for a severe working memory deficit (specific diagnosis was not reported). Five siblings were older than their sibling with PKU; and six were of the same gender.

**Materials**

The BRIEF provides an ecologically valid measure of the manifestation of EF deficits in daily life e.g., impact on school work, family functioning and social relationships. Since its publication in 2000, the BRIEF has been used widely in research applications to assess executive function in children with PKU (see Table 3), and other developmental disorders (Gioia, Isquith, Kenworthy, & Barton, 2002; Mahone et al., 2002; Mangeot, Armstrong, Covin, Yeates & Taylor, 2002). The BRIEF was used by the current research team in 2005/6 in a sample of younger children with ECT-PKU (Sharman et al., 2009; 2010a) and was found to be sensitive to EF changes associated with phenylalanine:tyrosine variations. The BRIEF was also recommended by the Waisbren et al., (2010) review of most appropriate test instruments for this population.

The BRIEF parent questionnaire contains 86 statements regarding the child’s behavior which parents are asked to rank on a “never, sometimes, often” basis to assess a range of executive functions. Such statements include “Tries the same approach to a problem over and over again even when it does not work”, “When given three things to do, only remembers the first or the last”. The BRIEF categorises EF into scales of: Inhibit; Shift; Emotional Control; Initiate; Working Memory; Planning and Organisation; Organisation of Materials; and Monitor. Scales are summed to provide overall indices of Behavior Regulation Index
(BRI), Metacognition Index (MI) and Global Executive Composite (GEC). The BRIEF contains checks for inconsistency (patterns of symptoms reported that are inconsistent with each other) and negativity (respondent is unduly negative towards child they are being questioned about). The BRIEF professional manual advises that data should be viewed as highly questionable if the recommended maximum number of inconsistency or negativity items is exceeded (Gioia et al., 2000). The Global Executive Composite (GEC; an overall score of EF impairment) as well as working memory subscale were used in the analyses. Working memory (WM) was chosen given the consistent findings that it remains the function most at risk in this cohort.

Procedure

General procedure was as described in Chapter 3. Parents filled out the BRIEF questionnaire concurrent with neuropsychological test administration.

Scoring

As per the BRIEF test manual, raw scores were converted to t-scores to account for variations in age and sex. A t-score of 50 represents average function, with a deviation of 10 representing the equivalent of one standard deviation; higher t-scores indicate higher levels of impairment. A deviation of 15 (i.e., a t-score of 65 or above) is considered by the BRIEF scoring manual as clinically significant and in need of further investigation.
Data analyses

Two Independent sample t-tests were used to detect between groups differences in children with PKU compared to their siblings on WM and GEC; and one-tailed correlations to assess associations between parent report WM and GEC and nine biochemical variables in the PKU group.

Results

An independent samples t-test was conducted to test for significant differences in EF between children with classical PKU and their siblings. Table 5 presents the results.

Table 5

Differences in BRIEF T scores Between 13 Children with Classical PKU and Eight Sibling Controls

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKU – WM</td>
<td>60.1</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>Sibling – WM</td>
<td>56.0</td>
<td>12.2</td>
<td>( p = .511 )</td>
</tr>
<tr>
<td>PKU – GEC</td>
<td>56.1</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>Sibling – GEC</td>
<td>53.1</td>
<td>8.1</td>
<td>( p = .542 )</td>
</tr>
</tbody>
</table>

Note: WM – working memory; GEC – global executive composite

Table 5 shows that children with classical PKU were not reported to demonstrate significant differences compared to siblings on BRIEF t-scores in the scales of working memory and global executive function.
Biochemical Markers Associated with BRIEF T Scores

A series of correlations were generated to test for associations between biochemical markers in the classical PKU cohort and parent report of executive function impairment. As previously reported, measures from the BRIEF included global executive composite (GEC; an overall measure of EF); and working memory (WM). T-scores were used to account for age and sex variations. Biochemical markers included: phenylalanine levels; phenylalanine:tyrosine ratio and tyrosine levels (from birth to age 12 years; lifetime; and concurrent with test administration). One-tailed Pearson’s $r$ was used to assess significance in all correlations. Three significant correlations were observed: lifetime tyrosine and GEC ($r = -.546, p = .027$); tyrosine < 12 years and GEC ($r = -.500, p = .041$) and lifetime phenylalanine:tyrosine ratio and GEC ($r = .478, p = .049$). These correlations indicated that lower lifetime tyrosine and lower tyrosine prior to 12 years was associated with increased parent report of global EF impairment; and that higher lifetime phenylalanine:tyrosine ratio was associated with increased parent report of global EF impairment. WM showed no significant associations with biochemistry, likewise no measure of phenylalanine showed any significant associations with EF.

Associations Between BRIEF and Other Tests of EF

BRIEF WM and GEC were negatively correlated with WISC IV digit span $r = -.795; -.796, p < .01$ respectively, indicating poorer performance on the WISC IV digit span was associated with higher EF impairment as reported by parents using the BRIEF. BRIEF WM and GEC were negatively correlated with NEPSY Inhibition $r = -.504, p = .039; r = -.534, p = .030$ respectively, indicating poorer
performance on the NEPSY Inhibition tasks were associated with higher EF impairment as reported by parents. BRIEF WM and GEC were negatively correlated with NEPSY Error, $r = .705, p = .004; r = .641, p = .009$ indicating poor performance on the error task (impulse control) was associated with higher EF impairment as reported by parents. Neither BRIEF GEC nor WM were significantly correlated with the language comprehension task, NEPSY Comprehension of Instructions. No significant correlation was observed between either BRIEF scales and the Rey Complex Figure task, confirming the earlier observation that the children’s performance on RCFT copy trial did not appear to be related to EF in this sample. However, future research using the RCFT may benefit from other scoring methods also purported to measure components of contemporary models of EF such as the organizational strategy score (Anderson, Anderson, & Garth, 2001).

**Discussion**

In this sample, the BRIEF did not detect significant differences between children with classical PKU and their siblings; however three significant correlations between children with PKU and their phenylalanine:tyrosine ratio and tyrosine independent of phenylalanine were observed. The failure to a) find significant between-groups differences and b) significant correlations on the WM scale may be due to the small sample size. Parents may have shown a so-called “halo effect” (Collishaw, Goodman, Ford, Rabe-Hesketh, & Pickles, 2009), as a consequence of being asked to rate their own children. Whilst research into the differential ratings parents may give their children is scarce, future studies into
this area might be strengthened by including teacher ratings alongside parent ratings, as a check of convergent validity in reported EF impairment.

Correlations between tyrosine and EF impairment were in the expected direction (negative), in that lower levels of lifetime (and < 12 years) tyrosine were associated with higher levels of EF impairment. Although not all correlations between tyrosine and GEC/WM were statistically significant, all tyrosine correlations were in a negative direction, further indicating a trend towards low levels of tyrosine associated with increased EF impairment.

A high lifetime phenylalanine:tyrosine ratio was also found to correlate positively with GEC, in that the higher the ratio, the higher the level of parent-reported impairment. No correlations between phenylalanine on its own and EF were observed in this sample. This study has also found an association between tyrosine levels on their own (i.e., independent of phenylalanine) and EF.

In all, these results provide support for the dopamine hypothesis (Diamond et al., 1997). Phenylalanine exposure was not shown to be associated with EF deficit in this sample, rather, the strongest associations between biochemistry and reported EF, involved long term tyrosine deficit or high phenylalanine:tyrosine ratio. These data support the previous research by Luciana et al. (2000) and Sharman et al. (2009; 2010a) that found lifetime exposure to a high phenylalanine:tyrosine ratio was associated with EF deficits in adolescence. In all, both the longitudinal assessments (Chapter 3) as well as the parent report of EF, suggest that residual EF deficits observed in early and continuously treated children with PKU are more impacted by long term influence of those biochemical markers (phenylalanine:tyrosine ratio and tyrosine) that are hypothesised to result in dopamine insufficiency.
CHAPTER 5

Study 3: Early and Continuously Treated PKU and Mood

In the previous chapters, the relationship between PKU and cognitive function was explored. The purported mechanism underlying differences relates to changes in biochemistry caused by PKU, leading to dopamine insufficiency. Dopamine has also been linked to mood disturbance in the non-PKU population (Arnsten, 2006; Dremencov, Weizmann, Kinor, Gispan-Herman, & Yadid, 2006; Esposito, 2006; Sohlkhah et al., 2005; Tremblay et al., 2002; Tremblay & Blier, 2006). To elucidate this potential relationship further, this research has explored this link in the PKU population.

The psychosocial aspects of PKU may have been overlooked in favour of ensuring optimal intellectual development. However the emotional impact of low level, and persistent, depressive and anxiety symptoms may contribute to a “hidden disability” within this population (Gentile, Ten Hoedt, & Bosch, 2010, p. 64), affecting interpersonal relationships, attainment of independence and educational goals.

The relationship between PKU and mood arose from anecdotal reports of adults who presented with symptoms of depression, after they had chosen to relax their dietary intake of phenylalanine. These symptoms were said to resolve when the adult patients resumed correct diet and formula intake. Smith and Knowles (2000) conducted a review of 34 publications investigating potential links between anxiety and depression in early treated PKU. They found higher scores
on neurotic items and lower scores on anti-social/aggression items compared to population norms or controls. They also concluded that in general individuals with early treated PKU show higher rates of depression, anxiety and phobias.

Whilst adherence to diet is suggested to prevent or minimise these impacts (Gentile et al., 2010), no published reports identify which biochemical markers may be associated mood in the PKU population. Some evidence to support the possibility of neurotransmitter dysregulation via substrate deficit, resulting in psychological symptoms, comes from non-PKU studies. Trials of low tyrosine diets in chronically ill populations have resulted in unexpected side effects of depression and anxiety (Harvie, Campbell, Howell & Thatcher, 2002). Furthermore, tyrosine depletion studies in samples of healthy individuals have shown negative effects on both memory and anxiety (Grevet et al., 2002). Further to the substrate-deficit research, dopamine agonists (in a double-blind placebo trial) have been found to lower anxiety (Lawford et al., 1995).

Sharman (2006) conducted a screening test of depression using a sample of adolescents with ECT-PKU and sibling controls. Examination of the early and continuously treated PKU group revealed that long term exposure to high phenylalanine and low phenylalanine:tyrosine ratio was significantly associated with increasing numbers of depression symptoms endorsed by participants. These data provide preliminary evidence for biochemical association with mood disorder in the PKU population.

Why some people with PKU seem particularly susceptible to fluctuations in biochemistry resulting in mood disorder, and others not, remains unknown. It could be that individual variations in the blood brain barrier can exacerbate or minimize the brain’s exposure to phenylalanine. (Gizewska et al., 2003; Weglage
et al., 2002). Individuals with PKU who experience mood disorder may be genetically predisposed, and this is further exacerbated by exposure to the biochemical changes caused by PKU.

Relationship Between EF and Mood in PKU

The potential that EF and mood may be linked could arise from a number of possible relationships. Problems associated with EF may lead to feelings of inadequacy and frustration, creating subsequent emotional difficulties (Gentile et al., 2010). There is also a possibility that the neurotransmitter dysregulation caused by PKU affects the same underlying system (i.e., dopamine production) which in turn affects both EF and mood (Dremencov et al., 2006; Tremblay et al., 2002). This possibility is supported by previous research into the non-PKU population that has found neuropsychological outcomes caused by nutritional deficit e.g., studies have shown that other conditions such as anorexia nervosa and diabetes lead to reversible effects on both cognitive and psychological functioning (Duchesne, 2004; Hassan, Loar, Anderson, & Heptulla, 2006). Further, it is possible that EF impairment increases the risk of/or develop alongside of mood disorder in PKU via a currently unknown mechanism, and although dopamine is a plausible candidate mechanism, this needs to be explored further.

Hypothesised Model of Biochemical Impact on Depressive Symptoms in PKU

Two possible biochemical mechanisms appear most likely given the previous review:

1) Depression symptoms are impacted by concurrent/recent inflations of phenylalanine, which causes disruptions to the entire neurotransmitter
environment within the brain, leading to increased symptoms of depression.

2) Depression symptoms are impacted specifically by higher phenylalanine:tyrosine and/or lower tyrosine which decrease dopamine in the brain, leading to symptoms of depression. If this is the case, symptoms of depression should also show an association with symptoms of EF impairment, in that participants with impaired EF will be more likely to report higher levels of depressive symptoms.

Analysis of the biochemical relationship with depressive symptoms in this population should also help to determine whether depressive symptoms in individuals with PKU are a result of the disorder, or the treatment. It is reasonable to suggest that an individual with a lifelong health condition requiring restrictive dietary practices may experience depressive symptoms as a consequence of their perceived health problems, or the stringent nature of the treatment. If this is the case, correlations with biochemistry should show no relationship, or may even be inverse (i.e., stricter dietary control leads to lower phenylalanine and phenylalanine:tyrosine, but higher levels of depressive symptoms due to the perceived oppressive nature of the treatment).

Purpose of Study

The study’s aims were to, first, conduct a between groups analysis of depression symptoms in a cohort of adolescents with PKU and their siblings (controls). Second, to test for associations between depressive symptoms in adolescents with PKU and their biochemical markers (lifetime, childhood and concurrent: phenylalanine; phenylalanine:tyrosine and tyrosine). Third, to
establish whether any relationships existed between depressive symptoms and EF in this sample.

Hypotheses

Minor elevations in depressive symptoms in children with PKU compared to their siblings were expected. Depressive symptoms were hypothesised to be associated with changes in biochemistry, although given the lack of evidence in this area, this investigation was largely exploratory.

Data Analyses

An independent samples t-test was conducted to assess between groups differences on the total t-score of depressive symptoms in children with PKU compared to siblings. Correlations assessed the relationship between depressive symptoms and nine biochemical variables (concurrent; lifetime; < 12 years – phenylalanine; tyrosine; phenylalanine:tyrosine ratio).

Method

Participants

Participants were the 13 children with PKU and eight sibling controls as described in Chapter 3.

Materials

The Children’s Depression Inventory (CDI; Kovacs, 1992) is a 27-item parent report questionnaire adapted from the Beck Depression Inventory that takes approximately 10 minutes to complete. The CDI measures five subscales of
depression (negative mood; interpersonal problems; ineffectiveness; anhedonia; negative self-esteem) as well as a total depression score. The CDI has some important strengths in terms of its ability to capture information required by this study design.

CDI reliability is high, and its validity is likewise high in western samples of children. Knight, Hensley, and Waters (1988) found the CDI significantly discriminated between children with clinically diagnosed depression and non-depressed children in an Australian sample. Fundudis et al. (1991) found reasonable validity in a British sample of children with a misclassification rate of 25%. The current research team used the CDI in a pilot sample of children with ECT-PKU and the depression scales from this instrument were able to achieve significant correlations with biochemical variables. The CDI has been used previously in samples of chronically ill children to determine relationships between biological, cognitive and mood factors (Hassan et al., 2006; Whittemore et al., 2002). The parent form of the CDI was used for this study, which has been demonstrated to correlate strongly with both the children’s and teacher’s version of the instrument (Wierzbicki, 2006).

*Executive function measures.*

As previously reported in Chapters 3 and 4: BRIEF (working memory and global executive composite scales, NEPSY-II Inhibition (Stroop) task, WISC IV digit span (working memory).
Procedure

General procedure was as described in Chapter 3. The parent report questionnaire was filled out by the accompanying parent or carer concurrent with test administration.

Data Analysis

Raw scores were converted to t-scores to account for variations in age and sex. A t-score of 50 is considered average, with deviations of 10 representing one standard deviation from the age-expected norm. An independent samples t-test was used to assess significant differences in depressive symptoms measured via t-scores, between children with PKU and their siblings. The second set of analyses tested for correlations between biochemical markers and depressive symptoms (Pearson’s $r$) within the PKU group only. Finally, correlations between reported symptoms of EF and depressive symptoms were generated.

Results

Depressive Symptoms in Children with PKU and Sibling Controls

The mean t-score from the CDI for children with PKU was 48.92 ($SD$ 8.69) compared to siblings 43.88 ($SD$ 5.98), both groups scored within the normal range, and although children with PKU demonstrated slight elevations in terms of number of depressive symptoms compared to their siblings, the difference between children with PKU and their siblings was not significant $t(20) = 1.503, p = .149$. 
Correlations between depressive symptoms and biochemistry.

To investigate any relationship between depressive symptoms and biochemistry in the PKU group, a series of correlations were generated, with the total t-score of depressive symptoms tested against phenylalanine levels, tyrosine levels and the phenylalanine:tyrosine ratio (concurrent; lifetime; < 12 years). Table 6 displays the results of significant correlations.

Table 6

**Significant Correlations (Pearson’s r) Between Depressive Symptoms (CDI T-score) and Biochemical Markers in Children with ECT-PKU**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lifetime</th>
<th>&lt; 12 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine</td>
<td>-.584*</td>
<td>-.544*</td>
</tr>
<tr>
<td>Phenylalanine:tyrosine ratio</td>
<td>.693**</td>
<td>.681**</td>
</tr>
</tbody>
</table>

One tailed correlations; *= p <.05; **=p<.01

No significant correlations were observed between phenylalanine levels and depressive symptoms; nor concurrent biochemical markers and depressive symptoms. Table 6 shows a strong relationship between long term exposure to either low tyrosine or a high phenylalanine:tyrosine ratio and increased depressive symptoms in this sample.

Associations Between Depressive Symptoms and EF

To test for associations between reported depressive symptoms and EF impairment, correlations were generated using t-scores from the CDI as well as t-scores from the BRIEF (GEC and working memory scales). Correlation between the parent report CDI and parent report BRIEF GEC was \( r = .74, p<.01 \); and
BRIEF WM \( r = .708, p < .01 \). Significant correlations between EF (as measured by the test administrator) and parent report of depression were as follows: WISC IV digit span \( (r = -.504, p = .04) \); NEPSY-II Inhibition (Stroop) tasks (inhibit \( r = -.495, p = .043 \); error \( r = -.693, p = .008 \)). As expected correlations for the direct tests of EF were in the negative direction as better (rather than impaired) EF performance was measured against increasing depressive symptoms.

**Discussion**

Between groups analyses showed no statistically significant differences between children with ECT-PKU and controls in depressive symptoms. However, it is important to note that depression in children is relatively rare with prevalence estimates of between 2 – 3% (Australian Bureau Statistics, 2006), coupled with a small sample size, this is not an ideal sample to test in terms of depression symptoms. Longitudinal analyses may subsequently determine whether these minor elevations in depressive symptoms in children with early and continuously treated PKU are signs of an emerging mood disorder as they age.

Further examination of the PKU group in relation to depression and metabolic markers showed significant and strong correlations with long term exposure to low tyrosine levels or a high phenylalanine:tyrosine ratio. Neither phenylalanine on its own nor concurrent biochemistry showed an association with depressive symptoms in this sample. However, the sample size issue was small, and our results viewed cautiously suggest further exploration of the association between all biochemical markers with depressive symptoms.

These data also lead to more than one possible interpretation. It could be that tyrosine or phenylalanine:tyrosine ratio association with depressive
symptoms, along with the observed overlap in depressive symptoms and EF deficit, is suggestive of the same underlying system (i.e., dopamine) being responsible for the etiology of both EF deficits and depressive symptoms in this sample. It could also be argued that symptoms of EF deficit and depression show significant overlap (e.g. lack of concentration) so the test instruments are detecting common problems. Or that EF impairment may lead to depressive symptoms via psycho-social mechanisms in that impaired EF may lead to feelings of inadequacy and frustration. Further, the daily tasks of managing a chronic illness could also by hypothesised to lead to minor mood impairments in this population.

Summary

Results from this study suggest the biochemical changes associated with poor dietary control may result in impaired EF as well as minor increases symptoms of depression, but not to clinically significant levels in this sample. Longitudinal analyses, or cross-sectional analyses within a larger and older sample, are needed to confirm these findings. Although our results indicate tyrosine and phenylalanine:tyrosine ratio as the markers most strongly associated with depressive symptoms, the small number of participants in our sample warrant caution in the interpretation of our results. As the phenylalanine:tyrosine ratio demonstrated stronger association than tyrosine on its own, further studies may find that phenylalanine alone may show a significant association as well within larger samples.

Our study has shown however, that long term exposure to high phenylalanine or low tyrosine demonstrates the stronger relationship across most
neuropsychological outcomes tested. The relative influence of concurrent or short
term fluctuations in phenylalanine or tyrosine levels has usually been shown to be
weak or absent in our sample. This study has supported previous reviews of
mood disorders in this population, in that minor increases in depressive symptoms
may be a feature of PKU (Gentile et al., 2010; Smith & Knowles, 2000). This
study has extended previous investigations by demonstrating that long term
biochemical changes associated with poor dietary control was significantly and
strongly correlated with these increases in depressive symptoms in this sample.
CHAPTER 6

Study 4: Hyperphenylalaninemia

Four children (two sibling pairs) with hyperphenylalaninemia were included in Dr Jones’ original dataset. As per the rationale outlined in the introduction, including these four children as part of the classical PKU sample for analyses was not considered appropriate as this condition results in substantially lower levels of phenylalanine (although still higher than the normal population). There are little published data regarding treatment and outcome of children with this condition, and anecdotal reports suggest treatment is highly variable (ranging from monitoring only, to some restrictions of protein in diet and supplemental formula; Arnold, 2009). Neuropsychological outcomes in this population have been reported to range from normal, to showing mild EF deficit, that is, milder than children with classical PKU, but slightly impaired to compared to controls (Gassio et al., 2005; Smith et al., 2000).

As only four children were available for testing, the data from these four children are presented here in a largely descriptive format. The metabolic dietician advised all four children were on a protein restricted diet (but much less restricted than the classical PKU diet) and prescribed enough PKU formula to ensure intake of 1g protein per kilogram of body weight per day.
Method

Participants

Two sibling pairs (three male; one female) with hyperphenylalaninemia were assessed in 2001/2 and again in 2009, mean age 12.45 years (SD 1.12), on the same measures as previously reported. Biochemical characteristics of these four children are presented in Table 7.

Table 7

Biochemical characteristics of Four Children with Hyperphenylalaninemia – Means and Standard Deviations (in brackets)

<table>
<thead>
<tr>
<th>Phenylalanine</th>
<th>Tyrosine</th>
<th>Phe:tyr ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifetime</td>
<td>227 (20.7)</td>
<td>85 (17)</td>
</tr>
<tr>
<td>&lt; 12 years</td>
<td>225 (21.7)</td>
<td>85 (16.8)</td>
</tr>
<tr>
<td>Concurrent</td>
<td>295 (48)</td>
<td>80 (23.4)</td>
</tr>
</tbody>
</table>

Materials and Procedure

All tests were administered as described in Chapter 3.

Results

Table 7 shows the children with hyperphenylalaninemia maintaining phenylalanine levels substantially below ASIEM maximum recommended guidelines (up to 360 umol). Further, concurrent phenylalanine levels for this sample in adolescence were less than half that of the classical PKU sample.

A series of independent samples t-tests were conducted to compare mean biochemistry of children with classical PKU and children with
hyperphenylalaninemia and significant differences were detected: lifetime phenylalanine, \( t(13.3) = 4.95, p = .000 \); phenylalanine < 12 years, \( t(13.5) = 4.5, p = .001 \); concurrent phenylalanine \( t(13.9) = 5.27, p = .000 \).

Performance on Neuropsychological Tests in Adolescence

All four children with hyperphenylalaninemia performed within one standard deviation of the normal age-expected norm on the Rey Complex Figure Task [RCFT]. Table 8 presents the means, SD’s and range for all other neuropsychological tests conducted in adolescence. The BRIEF results are represented in t-scores (average = 50; 10 = one standard deviation; higher t-scores represent higher levels of impairment); the WISC IV digit span; NEPSY II language comprehension task and inhibition (Stroop) tasks are represented as scaled scores (10 = average; 3 = one standard deviation; higher scores represent better function).

Table 8

| Neuropsychological Performance of Four Adolescent Children with Hyperphenylalaninemia |
|----------------------------------|--------|--------|-----------|
|                                  | Mean   | SD     | Range     |
| BRIEF GEC                        | 47.25  | 7.4    | 38 - 56   |
| BRIEF WM                         | 51.75  | 9.4    | 43 - 63   |
| WISC DS                          | 11.25  | 0.5    | 11 - 12   |
| NEPSY CI                         | 12     | 1.8    | 10 - 14   |
| NEPSY Inh                        | 9      | 1.4    | 7 - 10    |
| NEPSY error                      | 10.75  | 2.2    | 8 – 13    |
Table 8 shows that no child with hyperphenylalaninemia fell more than 1.3 $SD$'s below their age-expected norm on any task; and at least one child performed a $SD$ or more above the norm on the language comprehension and Stroop tasks.

Independent samples t-tests were used to assess significant differences between the children with hyperphenylalaninemia and classical PKU and significance results were: RCFT (as measured by $SD$’s from the age expected norm) $t(11) = -2.95$, $p = .013$; NEPSY-II language comprehension $t(15) = -3.07$, $p = .008$; NEPSY-II error $t(15) = -3.17$, $p = .006$. What these tests confirm, is that the most sensitive measures in the children with classical PKU, showed strong differences compared to the children with hyperphenylalaninemia. These tests demonstrate that performance on the RCFT and NEPSY-II tests were all normal within the hyperphenylalaninemia group, although clearly sub-normal within the classical PKU group.

A paired samples t-test was conducted on the NEPSY-II comprehension task as it was the test that showed a clinically and statistically significant decline from childhood to adolescence in the classical PKU group. In the hyperphenylalaninemia group, mean performance on the comprehension task in 2001/2 was a scaled score of 12.75 ($SD$ 1.7) and in 2009, a scaled score of 12 ($SD$ 1.8), this minor drop between early childhood and adolescence was not significant $t(3) = .878$, $p = .44$. In contrast to the classical PKU group, the hyperphenylalaninemia group demonstrated nearly one standard deviation above
average performance on the language comprehension task, and they remained slightly above average through to adolescence.

A question may be raised as to whether children with hyperphenylalaninemia demonstrate normal performance due to the genetic variation of their disorder compared to children with classical PKU; or whether their improved results can in fact be attributed to lesser exposure to harmful biochemistry across their lifetime. In this sample of 13 children, one child with classical PKU was observed to have maintained phenylalanine levels and phenylalanine:tyrosine ratio similar to those observed in the hyperphenylalaninemia group. This child’s newborn screening result convincingly indicated classical PKU: 1630 umol phenylalanine; 95 umol tyrosine; phenylalanine:tyrosine ratio over 17. However, by the age of 12 years, this child had maintained an average lifetime phenylalanine level of 226 umol and a phenylalanine:tyrosine ratio of 3.9. His was by far the best biochemical control observed in the classical PKU group. The child’s mother reported that he was home-schooled, which may explain how she was able to exert a strong level of influence in terms of dietary control (to keep the phenylalanine low) and regular formula intake (to keep the tyrosine high). Home-schooling may have also minimized temptation arising from daily peer pressure to fit in with his peer group. This child also demonstrated a neuropsychological profile similar to the children with hyperphenylalaninemia. Out of the neuropsychological subtests reported in this PhD thesis, he was within one standard deviation of his age-expected norm for eight tests; one standard deviation above the norm for the WISC IV digit span; and one standard deviation below the norm for the NEPSY-II Inhibition (error sub-test result), although this was the same child with the eye
condition as previously mentioned in the RCFT test; and as the NEPSY-II Inhibition (Stroop) tasks score visual ability, caution would be suggested in interpreting this result.

The next best biochemical control observed from any child in the classical PKU group was an 11 year old male whose lifetime phenylalanine was an average of 260 umol but whose lifetime phenylalanine:tyrosine ratio was 5.3. By way of contrast, his neuropsychological results showed residual impairments (in the RCFT; BRIEF and NEPSY-II inhibition task), indicating that low phenylalanine on its own had not been successful in preventing subtle neuropsychological impairments.

Although this is could at best be described as only a case study analysis (and children with classical PKU who can maintain such low levels of phenylalanine in concert with such low levels of phenylalanine:tyrosine are very rare), it does support the possibility that the difference in neuropsychological outcomes between the children with classical PKU and hyperphenylalaninemia is genuinely a function of their exposure to harmful biochemistry across their lifetime. Consistent with the previous phenylalanine:tyrosine experimental studies, comparison of these two children with classical PKU provides a useful example of how maintaining low phenylalanine on its own may not be enough to be enough to protect children from developing residual impairments. The best neuropsychological outcomes in this group have been observed in the children with hyperphenylalaninemia and the one child with classical PKU who all maintained very low phenylalanine (< 300umol) in combination with low phenylalanine:tyrosine (<4) across their lifetimes.
Discussion

Comparison of the classical PKU group to the hyperphenylalaninemia group demonstrates normal neuropsychological profiles at both periods of time (early childhood and adolescence). The three most sensitive tests in the classical PKU group (RCFT, NEPSY-II language comprehension and inhibition/error tests) were performed within completely normal ranges in the hyperphenylalaninemia group; and the one task that showed significant decline across time in the classical PKU group (language comprehension), showed no decline in the hyperphenylalaninemia group.

These findings provide an important comparison group in terms of test administration and robustness of test performance over time. Given these results, it would be hard to contend that differences across time or impairments observed in the classical PKU group were influenced by differences in test administrators, test administration procedure, or the effect of time alone.

These four children with hyperphenylalaninemia have demonstrated normal function across all neuropsychological measures, and have shown no decline in function over time. Even the most sensitive neuropsychological instruments for the PKU population have failed to detect any discernable differences between these children and their age-expected norm. Several significant differences were detected however in terms of biochemistry and neuropsychological test performance between the children with classical PKU and hyperphenylalaninemia. This exercise has also provided a test of neuropsychological test robustness, and confirmed that deficits observed in the classical PKU group were not evident in the hyperphenylalaninemia group across
the eight year gap; suggesting that the neuropsychological assessments are reliable.

Whether or not children with hyperphenylalaninemia should be actively treated for their condition with diet and formula is not answered based on such small numbers in this study. However, these children (in this acknowledged small sample) were tested on the most sensitive measures available to detect impairments in the PKU population, and they demonstrated no neuropsychological deficits whatsoever. Furthermore the children with hyperphenylalaninemia significantly outperformed the children with classical PKU on the most highly sensitive measures of neuropsychological deficit in this population. Interestingly, the one child with classical PKU who was also able to keep his lifetime phenylalanine below 300 umol and phenylalanine:tyrosine ratio at a similar level to the children with hyperphenylalaninemia (< 4) likewise showed normal neuropsychological function; and no decline across time. This finding is perhaps a step toward providing data to establish at what point these children might require active treatment. Arnold (2009) has suggested that if phenylalanine on unrestricted diet remains below 360 umol in the hyperphenylalaninemia population, no treatment should be required, and this study supports that assertion (although Arnold makes no comment about phenylalanine:tyrosine ratio). In all, this study demonstrated no discernable differences in neuropsychological function, having used highly sensitive test instruments in this population, when lifetime phenylalanine was maintained below 300umol in combination with a lifetime phenylalanine:tyrosine ratio lower than four, in children with both hyperphenylalaninemia and classical PKU.
CHAPTER 7

Study 5: Tyrosine Monitoring and Treatment: Current Clinical Practice

The four studies described thus far have provided data that largely supports the dopamine-deficiency hypothesis of the etiology of residual neuropsychological impairments in early-treated PKU. As tyrosine is a precursor to dopamine, and tyrosine levels have shown an independent (of phenylalanine) relationship with residual EF deficits in this sample, this may suggest a potential role for future monitoring and possibly treatment of tyrosine levels in children with PKU. Tyrosine is easily available in Australia (can be purchased ‘over the counter’ in health food stores), or can be made available to families via prescription from their treating metabolic physician. However, despite the emerging evidence regarding the potential role of tyrosine in the development of neuropsychological deficits in children with PKU, the extent to which the phenylalanine:tyrosine ratio and/or tyrosine levels are routinely assessed and/or treated in this population of children is largely unknown. There are some emerging reports that treatment with tyrosine (over and above that contained in PKU formula) is being used as an adjunct therapy to improve the functioning of children with PKU who display EF deficits. For example, Posner, Gorman, and Nagel (2009) describe the remission of ADHD symptoms in a child with PKU after treatment with supplemental tyrosine. However, the use of tyrosine supplementation for this purpose has not been strongly or universally supported by the current evidence (e.g. see Cochrane Review: Poustie & Rutherford, 1999). Given this possible gap between current theoretical understanding and actual practice at the clinical level, the purpose of the following survey was to collect a
brief snapshot of tyrosine monitoring and treatment in children receiving
treatment for PKU in metabolic clinics.

Method

Participants

In total, 20 clinics from 12 countries responded to the survey. Clinics were given the option to identify from which country they were responding. Those respondents who chose to identify their country of origin were located in Australia, Austria, Canada, Ireland, Italy, New Zealand, Spain, Sweden, Switzerland and the United States of America. Surveys were completed by the consultant metabolic physician (n=17) or metabolic dietician (n=3). The median number of children managed by each clinic was 60 (Mean = 105); with a range of 12 – 500.

Materials

A 20 question web-based survey was developed to capture information about current clinical practice in the measurement and treatment of tyrosine levels in children with PKU, as well as any anecdotal information from clinics as to the perceived usefulness of tyrosine screening and treatment (Appendix B). This questionnaire was developed primarily to capture information about tyrosine monitoring practices, although information about current phenylalanine monitoring practices was also collected as a comparison base. Clinics were also asked open-ended questions regarding their opinions of the veracity of tyrosine treatment, as well as their current protocols in assessing neuropsychological development.
The questionnaire was developed by the PhD candidate in consultation with supervisory staff, as well as the metabolic physician and nurse based at the Royal Children’s Hospital metabolic clinic. In order to maximise response rate the questionnaire was deliberately kept brief (20 questions); and included a number of “check box” options so that the average time to complete the questionnaire in full could be minimized.

Procedure

The survey was emailed directly to all metabolic clinics across Australia and New Zealand where the research team is based. 50% of clinics in this region responded. The same survey was also sent to the Society for Study of Inborn Errors of Metabolism National President/Secretary of each member nation requesting that the survey be forwarded to the clinics in their region that managed children with PKU.

Results

Current Phenylalanine-Screening Protocols

Questions regarding phenylalanine and tyrosine screening were split into three age groups according to ASIEM guidelines (Infants 0 – 12 months; Children 1 – 12 years; Adolescents 12 – 18 years). Clinics were asked to indicate their recommendations to patients regarding frequency of phenylalanine-screening and the actual frequency of phenylalanine-screening for each age range.

Agreement between clinics in recommended frequency of phenylalanine-screening was high. During infancy, 80% of clinics both recommended, and reported adherence to, phenylalanine-screening every 1 – 2 weeks, the remaining
20% of clinics recommended and received monthly phenylalanine-screens. A drop off in recommended screening frequency was reported during childhood with 25% of clinics recommending phenylalanine-screening every 1 – 2 weeks, whereas the majority (75% of respondents) decreased recommended screening to monthly. The beginning of attrition from recommended screening frequency in some patients began during childhood, with 40% of patient estimated to provide blood samples less frequently than requested. In adolescence, 80% of clinics recommended monthly phenylalanine-screening, the remaining 20% requested quarterly phenylalanine level checks. In practice however, the clinics estimated that on average, only 30% of adolescents adhered to the recommended frequency of phenylalanine-screens.

Forty-two percent of clinics nominated either their own country’s guidelines or international guidelines as their primary reference point regarding “safe” phenylalanine levels recommended to patients. Fifteen percent of clinics cited their “own guidelines” or “clinical experience” as their reference point. The remaining clinics listed the cut-offs they used but did not nominate from where those figures were derived (i.e. formal guidelines). One clinic acknowledged they were unsure of the reference and another noted that they disagreed with the National Institutes of Health (NIH, 2000) consensus statement regarding appropriate phenylalanine levels.

Clinicians were also asked to nominate which biochemical markers they believed best predicted cognitive outcomes in children with PKU, clinicians were able to select more than one option (i.e., these results do not add up to 100%). Fifty-five percent of clinicians stated they believed that lifetime phenylalanine levels best predicted cognitive outcomes in children with PKU; 55% nominated
phenylalanine levels prior to age 12 years; 5% stated phenylalanine:tyrosine ratio and 15% nominated that both phenylalanine and tyrosine across childhood were important to a similar degree.

Tyrosine Monitoring/Treatment

Frequency of tyrosine screening was highly variable, both within countries and internationally. Tyrosine screening at least monthly has clearly increased during the last 5 years, with over 80% of clinics now routinely screening tyrosine levels in infancy. Figure 1 shows the comparison of tyrosine screening practices from over 5 years ago, compared to the during the last 2 years.

Figure 1. Percentage of clinics reporting monthly tyrosine screening as a function of child age, across the previous 2 years compared to over 5 years ago.
Twenty-five percent of clinics reported that they supplemented paediatric patients with tyrosine over and above that contained in PKU formula if their tyrosine levels dropped below “normal” levels. Clinics that recommended optimal tyrosine levels to patients stated they did so, on the basis of: the normal reference point from laboratory; published data; and clinical concerns regarding neurotransmitter function.

Paediatric patient presentations that were described as requiring supplemental tyrosine treatment included those with persistently low tyrosine levels and younger children with optimal phenylalanine levels. One clinic nominated 1 gram tyrosine per day as a practical start, other clinics nominated the “smallest amount” or “whatever” was necessary to shift the tyrosine level back into normal range. The only contraindications to tyrosine supplementation noted were if the patient was allergic to tyrosine (sic), or if dietary control was already good.

*Reported Side Effects/Benefits of Tyrosine Supplementation*

Those clinics who used supplemental tyrosine reported no noticeable adverse effects; likewise most reported no clinical improvement apart from one physician who noted that tyrosine supplementation appeared to improve mood if the patient had previously exhibited signs of depression. One clinic made the observation that in their experience, if PKU formula was adhered to and phenylalanine levels within guidelines, tyrosine regulation was not necessary.
Neuropsychological Data

Fifty percent of clinics reported routine assessments of cognitive function and/or psychological function during childhood. The other 50% reported no routine assessments. Assessments were mostly global measures of IQ and conducted prior to school entry.

Discussion

This practice survey provides a snapshot of current clinical practice, primarily to ascertain current protocols regarding tyrosine screening and treatment in children with ECT-PKU and any anecdotal information from clinics regarding their experience. Whilst some caution in interpreting these data is warranted because of the survey response rate, and results may not generalize to all clinics, the findings suggest that tyrosine screening and treatment practices are highly variable both internationally and within countries. This finding is consistent with recent phenylalanine practice surveys (van Spronsen et al., 2009).

As previously mentioned, this variation in monitoring may have impacted the research agenda, with few clinics having the data (e.g., lifetime phenylalanine:tyrosine ratio) necessary to assess the relative importance of exposure to type of biochemical marker and neuropsychological test performance. This may explain why some researchers continue only test biochemical markers that have previously been shown to be less likely to show an association with neuropsychological function (e.g., concurrent phenylalanine), as that may be the only data available.

Half the clinics surveyed did not routinely assess cognitive or neuropsychological function in their patients, those who did tended to chose test
instruments of global assessments of IQ. Tests using specific measures of EF were rare, which is important at this is known to be the primary deficit now observed in children early and continuously treated for PKU. No clinic who did not routinely assess function commented on why they did not include regular neuropsychological examination as a part of their service. Given the wide divergence in the countries surveyed, failure to offer routine assessments of intellectual or neuropsychological function, may be a product of differing levels of resources and availability of specialist staff to perform this service.

When clinics do assess neuropsychological function, they do not typically examine the most at-risk functions that are known to be associated with early and continuously treated PKU. Instead, most clinics preferred global measures that will give a good indication of severe deficit, but may miss more subtle impairments. This supports the point raised in Brumm and Grant’s (2010) recent review, which suggested outcomes in this population are not being maximized. If clinics are not routinely testing for residual impairments, there will be little impetus to explore or change treatment protocols which may assist in remediating such impairments.

Conversely, whilst the veracity of tyrosine supplementation to a) restore poor tyrosine levels to normal or b) lower the phenylalanine:tyrosine ratio has yet to be convincingly demonstrated, a significant minority of metabolic clinics from our practice survey were pursuing this as a treatment strategy for patients. Coupled with the Posner et al. (2009) case study of tyrosine supplementation to remediate ADHD symptoms in a child with PKU, this treatment strategy represents the other end of the spectrum in managing children with PKU. As some clinics are already utilising extra tyrosine supplementation as a treatment
strategy, further research is urgently needed to determine if, and to what extent, manipulating tyrosine levels back to normal or to improve the phenylalanine:tyrosine ratio, is advisable as an additional treatment strategy for patients with PKU.

A final point to be drawn from this data, is the level of confusion that must be experienced by parents and patients with PKU given this serious divergence in practice. Alongside the variations in international guidelines in terms of phenylalanine guidelines, a child or family with PKU born in different countries, or even within different provinces of one country, may be receiving very different advice and treatment as to guidelines regarding dietary management practices. This survey has demonstrated that advice to people/families with PKU can vary from guidelines regarding phenylalanine control alone, right through to phenylalanine control along with tyrosine supplementation above that contained in PKU formula (under a variety of different conditions).

This lack of consistency in advice runs the risk of creating a lack of confidence in the guidelines presented to patients and their families, and may potentially contribute to diminished adherence to the dietary controls prescribed. This survey also demonstrated a reduced level of compliance to even basic phenylalanine-monitoring practices as a function of the children’s age (the older they get, the less compliant they become), indicating that the Levy and Waisbren (1994) finding of apathy and attrition from diet remains as relevant today as it did over a decade ago.

This continued lack of agreement among physicians, clinics and countries may serve to diminish confidence in the medical advice given to individuals with PKU, and may push them towards selecting treatments that have greater personal
appeal, but are not necessarily in their best interests. Whilst various professional bodies representing clinicians (e.g., the ASIEM and their counterparts internationally) have worked to come to a consensus on guidelines, Table 2 clearly showed that even at the most basic level of how much phenylalanine is too much, and over what period of time, continues to draw debate. This issue has not gone without notice previously, with van Spronsen and Burgard (2008) calling for more cohesion in the various guidelines dependent on age, along with functionally relevant advice for people with PKU. The fact that tyrosine guidelines are currently absent, and actual clinical practice so diverse, this may further lead to suboptimal outcomes for patients.
CHAPTER 8

The Final Word

It is perhaps somewhat unusual to end a thesis with the point of view of participants. However, it seems only fair to allow the children to have the last say on how they perceive their condition to impact upon their lives. As described in Chapter 3, the PhD candidate developed a brief Screening Questionnaire (Appendix A) to be administered prior to the standardised neuropsychological tests. This questionnaire was developed as somewhat of an “ice-breaker” and rapport building exercise, and gave children the opportunity to discuss their impressions about their cognitive abilities, and whether they felt they were impacted at all by the biochemical changes associated with PKU. The questionnaire started with general questions such as “what grade are you in at school” and then moved to ascertain any difficulties with schoolwork; cognitive abilities and changes perceived by the children in response to changes in concurrent biochemistry (e.g., “do you feel different if your phenylalanine levels are high?”). The following section outlines the primary themes of the children’s perception of their metabolic disorder and how it impacts their lives. Children with hyperphenylalaninemia are presented first.

No child with hyperphenylalaninemia stated that they were struggling at school or had any difficulties completing their schoolwork. Children with hyperphenylalaninemia nominated Health and Physical Education, French, math (n = 2), art, English as their best subjects at school; and English, math (n = 2), science, “shapes” (sic) and “don’t really know…algebra?” as their worst subjects
at school. Three children with hyperphenylalaninemia indicated no discernable
difference in their functioning if their phenylalanine levels were high, and the
remaining one child did not understand the question.

Out of the 13 children with classical PKU, two reported that they were
having difficulties with school work: “a bit difficult”; “not so easy, takes a bit of
time to work it out”. Ten children out of 13 endorsed math as the subject they
found most difficult: “definitely math”; and no child with classical PKU endorsed
math as their best subject. No other patterns were apparent in terms of other most
favoured or least-favoured subjects from children with classical PKU.

Nine out of 13 children with classical PKU reported feeling different if
their phenylalanine levels were high. Eight children nominated difficulties in
concentrating/focusing/being distracted: “I can’t concentrate at all… don’t
remember anything…harder to take info in”; “I seem to tune out and not be
motivated”; “I go off with the fairies”; “I start to feel a bit weird, in the head…
like really dizzy sometimes”; “can’t keep myself on the problem”. Two
mentioned mood variations: “I get angry”; “Mum reckons I get angry and
moody”. One noted tiredness/lack of energy.

These comments from the children themselves add to the findings of their
neuropsychological profiles as tested in this PhD research. A difficulty with math
in particular in this population has previously been reported (Schmidt, Burgard, &
Rupp, 1996) and is understandable given the deficits in working memory
associated with PKU. In contrast, no child with hyperphenylalaninemia said they
found the work at school difficult, and two endorsed math as their best subject;
and two as their most difficult subject.
To determine any patterns in reported deficits as a result of fluctuating phenylalanine levels, the children’s perceptions and their actual test results were further investigated. Ten out of the 13 children with PKU showed some level of deficit more than 1.5 $SD$’s below their age-expected norm. Out of the ten children with classical PKU who demonstrated this level of impairment, eight said they felt different if their phenylalanine levels became high, two said they noticed no difference. Out of the three children with PKU who demonstrated neuropsychological profiles within 1.5 $SD$’s of their age expected norm, two stated they felt no difference if their phenylalanine levels became high; and one stated that she did (focus-distraction). Whilst the majority of children with PKU endorsed feeling different if their phenylalanine levels were high, their ability to discern a difference did not appear to be especially related to actual test performance on neuropsychological instruments.

Overall, the majority of children with classical PKU endorsed feeling or thinking differently as a result of biochemical variations caused by PKU; and a similar pattern in their reported difficulties with schoolwork (math problems) reported by this group. In contrast, children with hyperphenylalaninemia endorsed no impact of their condition, and no consistent patterns in terms of their preferences for school work. The children with classical PKU held a perception of the functional impact of their disorder that was not always in agreement with parent report, or test scores of their neuropsychological function.

Nine out 13 children with PKU (compared to zero children with hyperphenylalaninemia) stated they could “tell” if their phenylalanine levels were high, and reported symptoms largely clustered around concentration and difficulty completing schoolwork as a result. This is at odds with the bulk of
neuropsychological research that, for the most part, has been unable to detect differences in function as a result of short term fluctuations in phenylalanine levels. It could be the variations experienced by individuals with PKU are too minor to be reliably detected by the type of neuropsychological test instruments that are typically used to detect cognitive decline. It may be that more sensitive tests typically used to detect more minor and fluctuating variations in ability/behavior are needed to explore this issue further e.g., similar to tests used to assess concentration/mood in response to increasing alcohol intake. It could also be argued that this is not a genuine feature of PKU, but perhaps something that has been suggested to the children by their parents or treating health professionals. Nonetheless, this is a curious area of divergence between neuropsychological evaluations of these children, and the child’s perception, and should be followed up in future research.
CHAPTER 9

Summary and Conclusions

This PhD research has extended a number of findings in relation to the neuropsychological development of children with PKU; the impact this condition has on their daily functioning; as well as issues related to the monitoring and treatment in this population. Whilst the current treatment protocols for PKU have undeniably lead to vastly better outcomes for this population, the results of the studies from this PhD confirm that there is certainly scope for improvement. It is timely that a very recent call has been made for a new review of the NIH consensus statement regarding the treatment guidelines for people with PKU.

Enns, Koch, Brumm, Blakely, Suter and Jurecki (in press) state:

Despite the remarkable success of public health programs that have instituted newborn screening and early introduction of dietary therapy for PKU, there is a growing body of evidence that suggests that neurocognitive, psychosocial, quality of life, growth, nutrition, bone pathology and maternal PKU outcomes are suboptimal. The time may be right for revisiting the 2000 NIH Consensus Statement in order to address a number of important issues related to PKU management, including treatment advancements for metabolic control in PKU, blood Phenylalanine variability, neurocognitive and psychological assessments, routine screening measures for nutritional biomarkers, and bone pathology.

Certainly the data from this PhD should contribute usefully to the development of a new NIH consensus statement. Similar to previous studies, this PhD has found that residual neuropsychological deficits are evident in this
population, including in those children who have maintained a sound level of phenylalanine control. At an individual level, ten out of the 13 children with PKU in this sample, showed clinically significant levels of residual deficit in at least one neuropsychological test, and most children reported that their condition impacted on their intellectual function, mood or perceived energy levels.

Longitudinal developmental assessments in this population are rare and our results have highlighted some important considerations for future research. Keeping a focus on the most at risk cognitive ability (executive function) has yielded clear evidence of suboptimal functioning. Test selection for this PhD included different measures of EF to best capture the profile of EF deficit, via a range of domains (parent report; visuospatial; language; and tasks involving inhibition and error suppression). Only one instrument chosen in this PhD research (the Rey Complex Figure copy task) was an unreliable measure of EF in this sample.

The longitudinal analyses demonstrated that in general EF deficits were evident early (by age seven years) and persisted into adolescence. This finding of early impairment in terms of EF is consistent with previous research (Antshel & Waisbren, 2003; Diamond et al., 1997; Malloy-Diniz et al., 2004), however this PhD has extended those findings to suggest that whilst such impairments persist, they do not worsen. This result may be suggestive of a critical period, in that the damage done to EF in this population is occurring the early years of life. Whether or not such damage is reversible (if tyrosine was increased and phenylalanine:tyrosine ratios were subsequently lowered) is an important future research question, and needs to be investigated further. Given reversibility of structural white matter abnormalities has been described previously in this
population (Cleary et al., 1995; McCombe et al., 1992), it is plausible that this residual EF deficit may show improvement if the hypothesized dopamine deficiency that underpins function is remediated. Consistent with previous research, EF in this group was anywhere from half a standard deviation to a full standard deviation below their age-expected norm (for review see DeRoche & Welsh, 2008), depending on the specific instrument used to measure impairment. The dual EF task (NEPSY-II inhibition) a Stroop-type task, showed the greatest sensitivity in separating out the children within the PKU group according to different levels of impairment, and may prove to be a useful test instrument to capture emerging deficits at young ages in this population.

These results provide support for the dopamine-deficiency hypothesis, in that the phenylalanine:tyrosine ratio and/or tyrosine levels independent of phenylalanine were found to be most influential in the development of EF impairment in this population (including the ecologically valid parent report measure of EF used alongside direct assessment of EF for the longitudinal assessments). Long term exposure (i.e., lifetime levels) was consistently found to have the strongest impact on EF development. Whilst the phenylalanine:tyrosine ratio impact on EF has been previously documented (Diamond et al., 1999; Luciania et al., 2001; Sharman et al., 2009; 2010a), this is the first time an effect of tyrosine independent of phenylalanine on EF impairment has been reported. This is not to say that phenylalanine level control is unimportant, but now that individuals with PKU (to a greater extent) have their phenylalanine levels under control, tyrosine has also been shown to be important in protecting EF development.
Further to the previous point, this sample’s phenylalanine control was poorer than the Sharman et al. (2009) sample, and as hypothesized, the phenylalanine:tyrosine effect in this sample was shown to be weaker. That is, as the phenylalanine raises, the impact of phenylalanine:tyrosine and/or tyrosine becomes less influential. In the Sharman et al (2009) sample, where children had on average, maintained levels within current Australian guidelines, the correlations observed between phenylalanine:tyrosine and EF were in the magnitude of $r \sim 0.8$. In the sample under investigation for this PhD research, where children on average demonstrated lifetime phenylalanine levels higher than recommended ($> 400$ umol), the phenylalanine:tyrosine/tyrosine effect on EF has weakened to $r \sim 0.4$. In general what has been observed, is that as the phenylalanine crossed the threshold recommended by current Australian guidelines, the impact of phenylalanine:tyrosine and/or tyrosine is lowered or obscured.

The impact of higher phenylalanine leading to poorer neuropsychological function is an important reminder that phenylalanine control remains the mainstay of treatment for children with PKU, and that the impact of phenylalanine:tyrosine and/or tyrosine may prove weaker and possibly even ineffectual in children if their phenylalanine is not under sound control. However, in accordance with recent reviews (Enns et al., in press; Van Spronsen, 2010) calling for a revision of current guidelines to maximize potential in this population, managing the phenylalanine:tyrosine ratio (via extra tyrosine supplementation) may prove a useful adjunct therapy to protect EF development in children who are maintaining good phenylalanine level control. In short, keeping phenylalanine low is clearly the best harm minimization strategy in this population; whilst increasing tyrosine
to lower the phenylalanine:tyrosine ratio may prove useful to maximize potential in children who are consistently engaging in good harm minimization efforts in the first place.

One longitudinal result inconsistent with the bulk of EF assessments in this sample, was their performance on the language comprehension task. This task is thought to display properties consistent with a combination of comprehension and working memory abilities, and significantly declined between childhood and adolescence. This result highlights the dangers of complacency in neuropsychological assessments in this population. Normal function at mean age six years declined to suboptimal performance by mean age 14 years in our sample; and this decline was significantly associated with lifetime exposure to phenylalanine. Normal performance in childhood did not guarantee normal performance in adolescence in this sample. This result suggests neuropsychological assessments should continue throughout development to determine if functioning has remained intact. It also serves a warning that normal function can be lost due to poor dietary adherence, possibly later in development than is typically accepted. This result may also shed light on why cross sectional studies continue to show such divergence in findings. This sample at age six years was normal, and no influence of phenylalanine/tyrosine on this task was evident. However the same sample’s performance at age 14 years was subnormal and demonstrated a clear impact of long term exposure to phenylalanine as being associated with that decline in function. This result suggests that neuropsychological assessments need to be repeated across the lifespan to accurately capture the developmental trajectory of this population. Otherwise there is a risk, based on the usual cross-sectional designs, that a dangerous
misassumption could be made that development in a particular domain is within normal ranges; shows no relationship with biochemistry; and is therefore no longer at risk. It is also possible that abilities other than EF, with different maturational features (e.g., visuospatial ability, language development) are susceptible to phenylalanine/tyrosine changes, but at different points in time.

The one longitudinal result that is perhaps hardest to conceptualise within the other findings from this PhD was children’s performance on the visuospatial task, the RCFT copy trial. Over half of the children with PKU in this sample demonstrated a severe level of impairment, a level at which everyday visuospatial functioning (such as reading maps, plans, or drawing simple diagrams) would most likely be compromised. Associations with biochemical markers were weak, with only one significant correlation between impaired performance and concurrent tyrosine in adolescence found. This has raised the question as to whether low tyrosine (and therefore impaired dopamine function) may be impacting other organs e.g., retinal function. However, it may be optimistic to believe that such a severe level of deficit could be the result of such a temporary fluctuation in one biochemical marker given this has consistently not been found to be the case in looking at other brain-based functions. It is possible we have missed other influences, including white matter abnormalities, or currently unknown/unmeasured variables. Given the ease and low cost of extra tyrosine supplementation, a template to assess visuospatial performance alongside concurrent tyrosine levels within an experimental, double blind placebo study, may be a useful future endeavour.

This PhD also investigated the potential role of biochemistry on mood. Depressive symptoms were within normal ranges in the PKU group but were
significantly associated (positively) with both a) elevated phenylalanine:tyrosine ratio and b) EF impairment. This raises an interesting possibility that either the same biochemical system may be responsible for minor mood impairments (dopamine dysregulation) or that problems with EF give rise to depressive symptoms in this population. Whilst higher rates of mood disorders in this population have been previously documented (Smith & Knowles, 2000), this is the first time to the author’s knowledge that a potential biochemical and/or cognitive mechanism has been demonstrated to be associated with symptom expression. Given this possible overlap in symptom expression and etiology, treatment options for mood disorder in this population may need adjustment to take into account these influences. In all however, this result regarding mood in this sample with children with PKU intriguingly raises more questions than it answers, and needs further exploration within older cohorts and larger samples.

Results from our international practice survey (Sharman et al., 2010b) indicated a high level of variability in terms of monitoring and treating tyrosine levels in this population. As this PhD has suggested that the phenylalanine:tyrosine ratio may be especially important in terms of EF development, research regarding phenylalanine:tyrosine ratio management in this population should be a priority in the development of future guidelines to people with PKU. The future role of tyrosine monitoring or management in this population is an important consideration given children with PKU still fail to meet expectations in terms of achieving their best possible intellectual and psychosocial outcomes. Whilst IQ has risen to within normal ranges under current treatment protocols (Burgard, 2000), a number of young adults report psychosocial or functional impacts of their disorder that impact negatively on
their career progress and interpersonal relationships (Gentile et al., 2010; Simon et al., 2008). As a group, the children with PKU themselves tended to report impacts of their condition (i.e., noticing if their phenylalanine levels were high) on their concentration, mood and energy levels. Children also tended to report problems with mathematics in particular at school.

Whilst this PhD research has focused on performance of the children with PKU as a group, individual variations were evident in this sample; raising the question as to whether biochemical variations may be more or less detrimental given a range of other individual factors (e.g., genetic predispositions, social disadvantage). This is a limitation of this PhD research, and studies into this condition generally. A common complaint from patients with PKU is that their individual needs are not necessarily taken into account in current management strategies that tend to recommend a broad base adherence to general guidelines. For example, we had one child in our sample who demonstrated no detectable neuropsychological impairments despite a lifetime phenylalanine level nearly double that recommended and a phenylalanine:tyrosine ratio over 10. As mentioned in the introduction, these cases are well known and most likely represent an individual who has been fortunate enough to inherit a protective genetic difference, such as poor phenylalanine transfer across the blood brain barrier. Is it therefore reasonable to suggest this child comply with current guidelines? Conversely we had a child (results presented in Chapter 6) whose phenylalanine levels had been maintained well below current guidelines; but whose phenylalanine:tyrosine was 5+; who displayed signs of EF impairment. Was this a case of bad luck i.e., the child was already genetically predisposed to EF impairment; or is this child especially vulnerable to suboptimal tyrosine
levels? Regardless of the etiology, treatment of these two children could reasonably be argued to allow for some flexibility (relaxation of guidelines for child one; trial of tyrosine supplementation for child two) to better improve their functioning or lifestyle. At present, with no consensus on the veracity of tyrosine management or optimal programme of neuropsychological assessment across time, these decisions of patient management practices are being left to individual clinics, and no doubt impacted by resourcing and availability of specialist staff. As previously discussed, this level of divergence regarding best practice options for patients will also add to confusion and a loss of confidence in following recommendations and adherence to treatment guidelines.

What does all of this mean for a child with ECT-PKU learning to manage their condition? EF deficits represent a significant neuropsychological impairment for children who need to be vigilant and predict outcomes for their behavioural choices in terms of dietary management. A potential cycle, depicted in Figure 2, may be particularly associated with dietary compliance in children with EF impairment.

![Figure 2. Dangers of temptation in the PKU diet](image-url)
Possible methods to interrupt this destructive cycle may include regular screening of cognitive variables or mood in order to provide personal feedback to people with PKU that demonstrate detrimental changes in behavior or mood alongside long term increases in metabolic markers. This sort of individualized feedback may be more useful to present to children/adolescents with PKU in order to motivate them to keep their dietary compliance within guidelines. Brief intervention programs along these lines have been trialled with success with people struggling with alcohol abuse (Anderson & Larimer, 2002; Ehrlich, Haque, Swisher-McClure & Helmkamp, 2005), so it may be that a treatment program aimed around delivering personal feedback to people to encourage them to make healthier choices would better succeed in boosting confidence in current dietary regimes recommended in this population as well.

This PhD research has extended previous findings into the impact of phenylalanine/tyrosine on neuropsychological development in a rare longitudinal study. By tracking children’s development over an eight year period, from early childhood through to adolescence, these studies have demonstrated a number of important considerations in terms of the interpretation of past research; useful recommendations for future research; and development of new treatment guidelines in this population. It has been demonstrated that the divergence in findings from previous cross sectional designs may have missed emerging deficits, primarily because the brain demonstrates relative sensitivity to biochemical changes throughout development (including the possibility of critical or sensitive periods, potentially later in development than previously thought). Also, the selection of non-sensitive neuropsychological instruments (e.g., global IQ measures) and/or weakly associated biochemical markers (e.g., concurrent
phenylalanine) has further lead to the likelihood of missing or minimizing residual deficits evident in this population. This PhD research is the first to the author’s knowledge to find a relationship between tyrosine levels (independent of phenylalanine) and EF deficit and this finding supports the dopamine-deficiency hypothesis of EF impairment. Furthermore, evidence of an effect of tyrosine independent of phenylalanine in contributing to EF deficit will contribute usefully to new treatment guidelines that may assist in minimizing residual deficits.

Although the relationship between phenylalanine/tyrosine and depressive symptoms has been long suspected, this PhD research is the first to the author’s knowledge to demonstrate this in children/adolescents with PKU. This PhD has provided data as to the variability in actual practice and perceived veracity of tyrosine treatment within clinics across the world.

PKU is a disorder that provides a lens through which the complex relationships between brain biochemistry and neuropsychological function can be further examined. Data from these studies supports previous PKU research that suggests neuropsychological function is relatively robust against temporary fluctuations in biochemistry that disrupt neurotransmitter function. However, long term exposure to harmful levels of biochemistry, during potentially sensitive periods of brain development can impair selective functions. Further research is warranted to better understand the level, timing and length of exposure and subsequent impacts on brain development.

Whilst it is irrefutable that good phenylalanine control is of paramount importance in preventing unnecessary brain damage in children with PKU, there are unanswered questions as to why children with “treated” PKU, and especially those children with well controlled phenylalanine levels, continue to be at risk of
developing selective neuropsychological deficits. There is a potential that a biochemical mechanism involving dopamine insufficiency could account for residual EF impairments in the PKU population. Since Diamond’s first proposal of this hypothesis, relatively few experimental studies have been conducted to test this link. Problems with small sample sizes, availability of lifetime data, separation of PKU groups according to phenylalanine exposure and age, and different sensitivities test instruments have all combined to confound a clear delineation of which biochemical change, at what point in brain development, are most likely responsible for residual neuropsychological deficits in this population. Individual differences may further compound or ameliorate these impacts, and at present, treatment for this disorder persists along a general “one size fits all” approach.

Further investigation of these biochemical impacts across time, and their expression among different individuals may help further our understanding of the etiology and development of other childhood disorders, especially those involving EF impairment such as ADHD. Children with PKU provide us with a unique natural experiment, to ascertain the relative influence of neurotransmitter dysregulation within the developing brain, and the neuropsychological sequelae that result from such disruption. Continued research into this population across the lifespan, will not only lead to better outcomes for individuals with PKU; but also provides us with a rare insight to better appreciate the intricate complexities of the neurophysiology of the developing brain.
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