Chapter 8

General Discussion
GENERAL DISCUSSION

With this thesis I attempted to find answers to research questions concerning 1) executive functioning, 2) behaviour/mental health and 3) social cognitive functioning and social skills that are represented by the PKU-COBESO study. The results of the previous chapters are summarized in Chapter 9. In addition to providing an overview of these issues in patients with PKU, this thesis also aimed to relate these COBESO outcomes to treatment guidelines. In this chapter I would like to answer the three main research questions that were posed in the General Introduction:

1. What should be the optimal treatment target for children with PKU (i.e. 0-12 years old) based on cognitive and behavioural outcomes? Is 240 µmol/L for children better than the currently most widely recommended upper target limit of 360 µmol/L?

2. What is the cognitive, behavioural and social profile of patients with PKU in different age periods?

3. Does the cognitive, behavioural and social profile of patients with PKU during adulthood relate to blood Phe levels of childhood and beyond?

Apart from answering these questions, some specific issues addressed in this thesis will be discussed in more detail and some foresights on directions for future research will be provided.

1. What should be the optimal treatment target for children with PKU (i.e. 0-12 years old) based on cognitive and behavioural outcomes? Is 240 µmol/L for children better than the currently most widely recommended upper target limit of 360 µmol/L?

Childhood (i.e. from birth until age 12) is an important sensitive period in life because the brain matures rapidly. Elevated Phe in this age period appears to have the most detrimental effect on brain development and subsequently on cognitive functioning, behaviour and social skills in adulthood (Chapter 2, 4, 5 and 6). There is a clear direction for guidelines in childhood. The new European treatment guidelines (van Spronsen et al., 2017) recommended Phe concentrations between 120 and 360 µmol/L as treatment target in the first 12 years of life. This recommendation in the European guidelines is supported by results from Chapter 4: patients with childhood Phe above 360 µmol/L had poorer working memory, specifically when a high working memory load was present, and had more depressive and somatic problems in adulthood than healthy control participants and in some tasks also compared to patients with childhood Phe below 360 µmol/L. Furthermore, this thesis examined 240 and 360 µmol/L as upper target level in childhood, because in Germany the target
limit of 240 µmol/L has been recommended for approximately 20 years (Burgard et al., 1999), despite the fact that this upper target limit is largely based on the results of one study in 14 adult patients between 17 and 24 years of age (Schmidt et al., 1994). The data presented in Chapter 3 of this thesis underscore that the upper target limit in childhood should be 360 µmol/L at maximum, but that a treatment target of 240 µmol/L may indeed be better for some aspects of cognitive outcome. The patients studied in Chapter 3 with Phe below 240 µmol/L had even better inhibitory control and cognitive flexibility than patients with Phe between 240-360 µmol/L, but this is only the first data comparing 240 µmol/L and 360 µmol/L as upper treatment target. Therefore, the evidence for 240 µmol/L as upper target limit in childhood is still scarce and further studies into this are required.

2. What is the cognitive, behavioural and social profile of PKU patients in different age periods?

The results presented in this thesis generally showed that PKU patients exhibit executive function impairments (Chapter 3, 4, 5, 7), internalizing mental health problems (Chapter 2, 4, 5) and social and social cognitive deficits (Chapter 2 and 6). More specifically, children with PKU showed deficits in inhibitory control, working memory and cognitive flexibility. Preliminary results showed that children did not exhibit mental health problems, but further analyses still have to be conducted for this age group. Regarding social outcomes, children with PKU had similar social cognition and skills as healthy counterparts.

The first analyses specifically with adolescent PKU patients demonstrated an absence of mental health problems, as reported by parents. However, social cognition was significantly poorer in adolescent patients compared to controls, while there was a trend towards poorer social skills. There were also indications for poorer executive functioning in young adolescent PKU patients (Chapter 3).

Executive functions were affected in adult PKU patients. They had poorer inhibitory control, working memory, especially with increasing task difficulty (or: higher working memory demands), sustained attention and cognitive flexibility. Internalizing behavioural problems were reported as well. They experienced more depressive problems and avoidant personality problems than control participants. Furthermore, adult PKU patients demonstrated clear deficits in social cognition and social skills compared to healthy individuals.

Many studies focused on children with PKU and have demonstrated impairments in executive functions such as inhibitory control, (working) memory and attention (Anderson et al., 2007; Antshel & Waisbren, 2003; Huijbregts, de Sonneville, van Spronsen, Licht, & Sergeant, 2002; White, Nortz, Mandernach,
Huntington, & Steiner, 2001). Two meta-analyses showed that PKU patients from all
ages had problems with information processing speed, planning, inhibitory control,
working memory and flexibility (Albrecht, Garbade, & Burgard, 2009; DeRoche &
Welsh, 2008), while these types of executive function deficits were also reported
specifically in adolescents and adults with PKU (Channon, Mockler, & Lee, 2005;
Moyle, Fox, Arthur, Bynevelt, & Burnett, 2007). This thesis confirmed that PKU
patients of different ages have more or less similar cognitive profiles (see Figure 1
for an overview of the analysed COBESO outcomes per age group), as assessed with
the same instruments in different age groups. Note that the cognitive functioning in
adolescent patients of the COBESO study has not been analysed yet and that mental
health was also not extensively examined in these PKU children and adolescents
of the COBESO sample. Still, the same cognitive profile would be expected in
adolescents with PKU, as both children and adults showed impairments in EF
and therefore it is likely that PKU patients in adolescence also exhibit these same
problems, which some studies already have shown (Albrecht et al., 2009; Channon
et al., 2005; DeRoche & Welsh, 2008; Moyle et al., 2007). Furthermore, the cognitive
functions develop from childhood onwards and when deficits emerge early in
development, it will be more difficult to overcome and to solve these problems over
time and therefore this may remain an area of concern even in older PKU patients.
However, effect sizes seemed to be smaller in adults compared to younger patients
(Anderson et al., 2007; Huijbregts et al., 2002; Huijbregts et al., 2002). There are also
studies showing the opposite (Moyle et al., 2007), which may have been caused by
differences in patient characteristics (e.g. higher historical Phe levels in participants
of studies showing larger deficits in adulthood compared to childhood). In order
to resolve these inconsistencies, future studies into potential differences between
patients of different ages should take into account as many patient characteristics
as possible, thereby always including historical Phe levels. The literature showed
that PKU patients in all age groups experience internalizing behavioural problems
(Smith & Knowles, 2000): depressive mood, anxiety, physical complaints and social
isolation in children and adolescents (Cappelletti et al., 2013; Weglage et al., 2000),
and anxiety and withdrawal in PKU adolescents and adults (Manti et al., 2016). In
this thesis internalizing mental health problems were evident in adult patients with
PKU, but not observed in PKU children and adolescents of the COBESO study. In
contrast, social deficits were emerging in adolescent PKU patients and progressed in
adulthood. The social domain has not been examined in previous studies, but should
clearly be part of research into the behavioural phenotype associated with PKU.
Figure 1. Overview analysed COBESO outcomes per age group

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<tr>
<th>PKU patients ≤ 12 years</th>
<th>CO</th>
<th>BE</th>
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<tr>
<td>Clinical</td>
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<th>BE</th>
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<td>Borderline</td>
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<td>Clinical</td>
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<th>PKU patients ≥ 18 years</th>
<th>CO</th>
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<td>Normal</td>
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CO = cognitive/executive functions; BE = behaviour/mental health; SO = social cognitive functions and skills. *Preliminary analyses
Note: not all COBESO outcomes are examined yet in all age groups

3. Does the cognitive, behavioural and social profile of PKU patients during adulthood relate to blood Phe levels of childhood and beyond?

Overall, the most frequently observed finding was that patients with high Phe concentrations in childhood had poorer cognitive and behavioural outcomes (Chapter 5) and poorer social functioning in adult life (Chapter 6). Phe concentrations from adolescence onwards were also associated with poorer cognitive outcome, although effects were smaller (Chapter 5), while this was not observed for mental health (Chapter 5) and social functioning (Chapter 6). The effect of adolescent Phe concentrations was specifically examined in this thesis. An increase of Phe between childhood (assessment at time point 1) and young adulthood (time point 2) was related to poorer cognitive outcome (inhibitory control and cognitive flexibility) in adulthood (Chapter 5). Adult depressive and somatic problems were related to lifetime Phe next to childhood Phe (Chapter 5). However, only childhood Phe was related to ADHD problems. Antisocial personality problems, which had not been reported before, were associated with Phe concentrations throughout life. Also, all age groups (children, adolescents and adults with PKU) had similar Phe concentrations in childhood, but over time Phe concentrations increased. The COBESO problems in adulthood could therefore not only be explained by childhood Phe, but also by Phe concentrations thereafter.

The meta-analysis of Albrecht et al. (2009) showed that the strength of the negative association between blood Phe and effect sizes for speed of performance decreased with age, indicating that in older patients the relations between Phe and cognitive outcome measures are weaker but remain present (see also Brumm et al.,
2004). Other studies with (young) PKU adults also showed a lack of, or inconsistent associations between cognitive outcomes and Phe in different age periods beyond childhood (Channon et al., 2005; Christ, Huijbregts, de Sonneville, & White, 2010; Luciana, Sullivan, & Nelson, 2001). Taken together, all these results support the notion that strict control of Phe levels during childhood is imperative.

Studies on mental health in exclusively adult PKU patients are scarce and often include adolescents as well. Nevertheless, these studies have demonstrated some relationships with Phe. Concurrent Phe was related to mood swings in patients (ranging from a childhood until age 35) (Anjema et al., 2011), lifetime Phe was associated with anxiety and stress in 15 to 25 year old patients (Clacy, Sharman, & McGill, 2014), while another study did not find associations between Phe and mental health in young adults (12-44 years) (Manti et al., 2016). However, the inclusion of adolescent patients in these previous studies makes it difficult to compare the results with adult PKU patients in this thesis.

Overall, the results of this thesis indicated that elevated childhood Phe concentrations have the strongest effects on COBESO outcomes in adulthood, but that Phe concentrations after childhood still had some influence as well. Phe concentrations from adolescence onwards should therefore still be the focus of new research. Also, without strong evidence to the contrary one should be very cautious in relaxing the treatment regimen after childhood.

**FUTURE DIRECTIONS**

There are still many gaps in the knowledge of PKU. The precise cause of the deficits in executive function, mental health and social outcomes is still unknown. Where can we capture the origin of the derail, so what causes the disruption of a normal development? And what can we do about it? These are still outstanding questions. Suggestions for future studies and advice for daily practice and treatment are provided below.

**Examining metabolic parameters in relation to COBESO**

In this thesis the relationship between deficits in EF, mental health and social abilities and Phe levels throughout life were examined. Despite the fact that a number of associations was demonstrated, Phe concentrations may not tell the whole story. Among factors that might also influence the outcomes of interest may be metabolic parameters other than Phe.
Treatment parameters

Next to the upper (but also lower) target limit, there may be other Phe-related indices that have a stronger relationship with outcome measures of interest than Phe itself. This thesis briefly addressed variability of Phe and the Phe to Tyr ratio (Chapter 3), while other studies have also determined their importance and relationship with cognitive outcome (Anastasoaie, Kurzius, Forbes, & Waisbren, 2008; Luciana et al., 2001; Sharman, Sullivan, Young, & McGill, 2010). For instance, a lifetime Phe:Tyr ratio above 6 has been associated with clinically impaired EF, although this was examined in only 11 adolescent patients (Sharman et al., 2010). Therefore a larger sample size should be used to examine whether a Phe:Tyr ratio of 6 is a proper upper target limit. There are also no indications yet regarding upper target levels for Phe variability. Only a strong relationship between Phe variability and cognitive outcome has been determined (Anastasoaie et al., 2008). Future studies should continue to investigate the relative importance of these alternative Phe-related parameters (i.e. Phe variability, Phe:Tyr ratio, Tyr) and try to determine upper target levels for these indices as well, so they can be used in clinical practice.

Neurotransmitter shortages: Research with other metabolic disorders

As mentioned earlier in Chapter 1, low concentrations of the neurotransmitters dopamine and serotonin may be consequences of inadequately treated PKU. Both neurotransmitters are important for cognitive functioning, mental health and social skills. Although the direct connection between our outcome measures and these neurotransmitters were not investigated, it is possible that the deficits reported in this thesis are caused by low dopamine (Christ et al., 2010) and serotonin levels (Gellynck et al., 2013; Kiser, Steemers, Branchi, & Homberg, 2012). Future studies should consider the possibilities to incorporate these metabolic measurements or their derivatives and more importantly to examine these in relation to cognitive, behavioural and social outcomes.

Knowledge of other metabolic disorders that are characterized by deficiencies in the metabolism of one of the other LNAAs, for example Hereditary Tyrosinaemia Type 1 (HT1), might enhance the overall picture of associations between the behavioural phenotypes and pathophysiological mechanisms involving cerebral protein and energy metabolism as well as neurotransmitter deficiencies. One study demonstrated high Tyr in cerebrospinal fluid (CSF) and reduced serotonin turnover in three HT1 patients. Even though these patients also showed cognitive and behavioural impairments, the direct relationship with neurotransmitter deficits could not be determined as this was a descriptive study (Thimm et al., 2011). Another study of our research group demonstrated that HT1 patients exhibit similar cognitive and social impairments compared to patients with PKU (van Ginkel et al., 2016):
there as well, it remains to be determined whether this is associated with LNAA and neurotransmitters. Combining forces with researchers of other inherited metabolic disorders with abnormal LNAA in CSF/brain and similar disease manifestations as in PKU, can improve the understanding of associations between LNAA, neurotransmitters and outcomes in the domains of cognitive and social functioning and mental health.

**Brain research**

As has been described in the General Introduction (Chapter 1), an alternative or additional explanation for the observed cognitive deficits in PKU could be the presence of white matter damage (Anderson et al., 2007; Christ et al., 2010), which seems to be caused by elevated Phe (Dyer, 1999). There are a few studies that have examined brain activity of patients with PKU. However, brain research in combination with cognitive assessments is scarce and may be a topic for future research. Methods such as functional magnetic resonance imaging (fMRI), magnetic resonance spectroscopy (MRS), correlated spectroscopy (COSY) and diffusion tensor imaging (DTI) seem very useful, but should not be carried out in isolation. Integrative accounts of LNAA/neurotransmitter levels, data from structural and functional neuroimaging, from neuropsychological testing and interview and questionnaire data would be ideal. Techniques that might be particularly useful in PKU research are MRS (for proper use of MRS in PKU see Kreis, Zwygart, Boesch, & Nuoffer, 2009) and COSY (see Waisbren et al., 2016), which can both accurately measure brain Phe levels.

**Clinical relevance**

The issue whether the results are clinically relevant should be addressed more extensively in future studies. This thesis included control participants to determine whether patients with PKU differ from the healthy population regarding the COBESO outcomes. For most instruments, norm references were also available. However, effect sizes give a better indication for clinical relevance. According to Cohen’s rules of thumb (Cohen, 1988), effect sizes in the medium to large range were observed for executive functions (Chapter 4) and social (cognitive) skills (Chapter 6), and small to large effect sizes for problems in mental health (Chapter 4, see also Figure 1 where the strength of effect sizes is expressed in normal, borderline and clinical ranges). For the adult patients, clinical relevance (i.e. large effect sizes) was particularly evident when patients with PKU with lifetime or childhood Phe levels ≥360 µmol/L were compared to healthy controls, with medium to large effect sizes for IQ, for a number of executive functions and for depressive behaviour (Chapter 4).
Chapter 8

Treatment guidelines

In order to increase the strength of the statements on treatment guidelines in PKU, further studies on neurocognitive, behaviour and social development and functions are necessary in children, adolescents and adults with PKU. There are indications for each age period but proper evidence is lacking. Particularly for adolescents and adults it remains unclear what the optimal Phe upper target limit should be, while even not discussing the lower target Phe limit. However, this thesis demonstrated that Phe concentrations at any time during life have an influence on cognitive, behavioural and social outcomes in adulthood, stating that treatment should not only be continued after childhood but that treatment for life is indeed required. More research on treatment targets for each age group is needed. A larger collaboration of (international) clinics, thus including a larger study sample, while using a longitudinal study design, is recommended for future research.

Tetrahydrobiopterin (BH$_4$)

The effect of BH$_4$ on cognitive and behavioural outcomes has been examined only briefly in this thesis by comparing BH$_4$ and non-BH$_4$ users. It seemed that BH$_4$ has a positive effect, even when all other conditions (i.e. childhood Phe, lifetime Phe, age, IQ) were similar for non-BH$_4$ and BH$_4$ users, as described in Chapter 4 of this thesis. If that is really the case, the question arises whether BH$_4$ should be administered to all patients irrespective of whether they are BH$_4$ responsive or not. However, those patients who are BH$_4$ responsive are also those with a milder form of PKU, and are therefore often better off either way compared to patients with classical PKU, although in this thesis Phe levels and biochemical phenotype were taken into account and were similar for the BH$_4$ and the non-BH$_4$ group. Moreover, one study in PKU mice has demonstrated that BH$_4$ has a direct positive effect on brain neurotransmitters (Winn, Scherer, Thony, & Harding, 2016) and another study showing improvements on aspects of executive functioning in BH$_4$ responsive PKU patients who were given BH$_4$ compared to a placebo group (Burton et al., 2015). These studies show that BH$_4$ might improve cognitive outcome irrespective of the effect on blood Phe levels. Still, the influence of BH$_4$ on cognitive, mental health and social outcomes, regardless of the effect on Phe levels, needs more thorough investigation in future studies. This can be done with an experimental study in which non-BH$_4$ and BH$_4$ users could be assessed while controlling for variables such as biochemical phenotype, age and IQ. At time point 1 both groups should be assessed, after which the non-BH$_4$ group is administered BH$_4$ for a certain period of time, e.g. 3 months, after which both groups are re-examined at time point 2. Also, a non-BH$_4$ group not given BH$_4$ could be included as control group. Such experimental design could demonstrate whether
the non-BH$_4$ group improves after administering BH$_4$ compared to the (non-)BH$_4$ users.

**Training executive functions and social skills**

When thinking about the deficits in the cognitive, mental health and social domains, treatment guidelines can be optimized to improve and to prevent these impairments. However, more can be done and the following question arises: how can we improve cognitive, behavioural and social outcomes of patients with PKU? There are training modules available for people with deficits in executive functioning (e.g. Klingberg, Forssberg, & Westerberg, 2002) and social skills (Barrera et al., 2017; Spence, 2003), and these modules may be suitable for PKU patients as well and may help to improve their COBESO outcomes. Targeting executive functions and social skills may be sufficient to also change internalizing behaviour, as one can feel more confident after participating in a training module (Spence, Donovan, & Brechman-Toussaint, 2000). To determine whether these training modules improve the cognitive, behavioural and social functioning of patients with PKU (in spite of treatment and treatment adherence), an experimental study could be performed with two or more (to determine long-term effects) assessment points, including an experimental group and a patient control group that should again be matched on biochemical phenotype, age, IQ and BH$_4$ use.

**Psychological evaluation and consultation**

Current visits to the clinic often consist of an appointment with the physician and dietician. Patients may however also benefit from cognitive, behavioural and social assessments. Vockley et al. (2014) have already suggested psychological testing at various ages and the European guidelines also recommended neurocognitive evaluations at age 12 and 18 due to life phase changes, with further psychological assessments when necessary (van Spronsen et al., 2017). Furthermore, patients with PKU should be more informed thoroughly about COBESO consequences of elevated Phe. This thesis provided direct evidence that high Phe in childhood has a (clinically relevant) impact on cognitive and behavioural outcome in adulthood. With regular psychological assessments in relation to metabolic outcome, patients could almost instantly see the direct effects of high or low Phe on EF, mental health and social skills. However, implementation of even more regular assessments than currently recommended would be very difficult, at least in the short term. One step towards making this more feasible is to develop easy and suitable instruments that are not time consuming. For this thesis the outcome of the ‘user-friendly’ BRIEF questionnaire on EF was compared to that of the computerized ANT (also measuring EF). Results showed that different aspects of EF were measured by these instruments,
which of course does not disqualify either instrument, but does show how difficult
it is to find an instrument that is both easy to use and captures the exact weaknesses
that were shown to be typical in (selected, e.g. high Phe) PKU patients. More
research is needed to select valid and proper instruments for daily practice. Next to
understanding patients’ cognitive functions, it is important to monitor their quality
of life. A PKU-specific health related quality of life (HRQoL) questionnaire has been
developed (Bosch et al., 2015) and recommended by the European Phenylketonuria
Group (van Spronsen et al., 2017). Patients generally had a good HRQoL, but the
emotional impact and management of PKU had a negative effect on their lives. There
is already a Dutch web based instrument available for several diseases including
PKU, assessing quality of life (‘KLIK’ = Kwaliteit van Leven in Kaart, i.e. quality of
life mapped).

Finally, not only educating patients with PKU but also educating their family,
friends, school or work, is very important and should be part of psychological
consultation. Awareness and understanding of the consequences of PKU within
patients’ social networks may enhance social support for patients, which in turn
may affect their wellbeing and mental health. The Dutch PKU Society already took
initiative to create information pamphlets, originally for PKU children who often
give a speech/presentation at school about PKU, and short video’s (containing
interviews with professionals) that explain different issues in PKU (to be found on
YouTube). These pamphlets and video’s may be useful for other people as well and
can be made into a tool for educating people about PKU.
REFERENCES


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with and without parental involvement. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 41*(6), 713-726.


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