CHAPTER 8
Summary and discussion
CHAPTER 8

SUMMARY OF MAIN FINDINGS

In this dissertation we aim to provide novel insights into childhood ADHD, by addressing a wide range of topics relevant to ADHD. Below, the main results from chapter 2-7 of this dissertation are summarised (see also Table 8.1 for an overview). Subsequently, findings are critically evaluated, and new avenues for future research are provided.

The main objectives of chapter 2 are to examine whether children with ADHD have decreased blood spot aromatic amino acid (AAA) concentrations, and whether blood spot AAA concentrations are related to symptoms of ADHD. AAAs, constituents of protein in foods, are involved in the biosynthesis of serotonin and dopamine, and therefore aberrant AAA concentrations may contribute to altered dopamine levels in ADHD (Oades, 2008), and to aberrant postsynaptic serotonin levels found in some individuals with ADHD (Oades, 2010). Given the central role of dopamine and serotonin alterations in ADHD (Oades, 2008), we hypothesise that decreased concentrations of tryptophan, tyrosine and phenylalanine in blood might contribute to the expression of ADHD symptoms. Based on the current literature, there is inconsistent evidence that AAAs are related to ADHD. The studies on this topic performed thus far are mostly outdated and hampered by methodological shortcomings (Baker et al., 1991; Bornstein et al., 1990; Comings, 1990; Hoshino, Ohno, & Yamamoto, 1985; Oades, Dauvermann, Schimmelmann, Schwarz, & Myint, 2010). We further explore whether abnormal blood spot AAA concentrations are related to decreased protein ingestion or by aberrant AAA excretion, as evidenced by increased urinary AAA concentrations. In the study 83 children with ADHD (75 percent males) and 72 typically developing (TD) children (51 percent males), aged 6 to 13 years, participated. AAA concentrations were assessed in blood spots and an 18-hour urinary sample. A nutritional diary was filled out by parents to calculate dietary protein intake. Parent and teacher questionnaires assessed symptoms of ADHD. In contrast to our hypothesis and some earlier studies on this topic (Baker et al., 1991; Bornstein et al., 1990; Comings, 1990), we did not find any differences in blood spot concentrations of tryptophan, tyrosine and phenylalanine between children with ADHD and TD children. In addition, below average (<16th percentile) blood spot AAA concentrations did not significantly increase the risk of being diagnosed with ADHD. In the combined sample of children with ADHD and TD children, no significant associations were found between AAA blood spot concentrations and ADHD symptoms. There was no difference between the ADHD and TD group in dietary protein intake or urinary AAA
concentrations, and AAA concentrations were not significantly related to protein intake and urinary AAA concentrations.

The objective of chapter 3 is to examine whether increased homocysteine concentrations are related to childhood ADHD. High concentrations of homocysteine can have detrimental effects on neurocognitive performance, by causing DNA damage, disturbed methylation, cell death or by altering the functioning of glutamate receptors (Mattson & Shea, 2003). A deficiency of folate or vitamin B12 leads to increased concentrations of homocysteine in the blood, since the conversion of homocysteine to methionine is dependent on the cofactors folate and vitamin B12 (Mattson & Shea, 2003). Homocysteine has been found associated with neurocognitive performance in neurodegenerative diseases (Teunissen et al., 2005), in the normal aging population (Garcia & Zanibbi, 2004), as well as in psychiatric populations (Dias, Brissos, Cardoso, Andreazza, & Kapczinski, 2009; Ford, Flicker, Singh, Hirani, & Almeida, 2013). Strikingly, the neurocognitive functions that seem to be related to homocysteine concentrations (working memory, interference control and attention) (Dias et al., 2009; Teunissen et al., 2005), are exactly those that are impaired in (subgroups of) children with ADHD (Mullane, Corkum, Klein, & McLaughlin, 2009; Tamm et al., 2012; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). Thus far, no studies have investigated the role of homocysteine concentrations in childhood ADHD. We hypothesise that children with ADHD have increased concentrations of homocysteine compared to TD children. We examine whether homocysteine concentrations in children with ADHD are (a) positively related to symptoms of ADHD, (b) negatively related to neurocognitive functioning in ADHD, which would lend further support to the role of homocysteine in ADHD, and (c) negatively related to intake of folate and vitamin B12, which would be informative for a dietary risk factor for ADHD. Homocysteine concentrations were assessed in blood spots of 55 children with ADHD and 54 TD children, aged 6 to 13 years. Parent and teacher questionnaires assessed symptoms of ADHD. Neurocognitive functioning was measured using the Digit Span Task, Grid Task and Flanker Task, targeting verbal and visuospatial working memory, interference control, variability in responding, and lapses of attention. Intake of folate and vitamin B12 was measured using nutritional diaries. In contrast to our hypothesis, we neither found a difference in homocysteine concentrations between children with ADHD and TD children, nor an association between homocysteine concentrations and symptoms of ADHD. Against our hypothesis, we did not find any evidence for the contribution of homocysteine to neurocognitive deficiencies in children.
with ADHD. Furthermore, we did not find significant associations between homocysteine and the intake of folate and vitamin B12, and children with ADHD did not have a lower dietary intake of folate and vitamin B12 than TD children.

In chapter 4 we aim to examine whether neurocognitive profiles can be distinguished in children with ADHD and TD children. Thus far three studies applied community detection procedures to distinguish neurocognitive subgroups of individuals with ADHD, all showing distinct neurocognitive profiles (Fair, Bathula, Nikolas, & Nigg, 2012; Mostert et al., 2015; Van Hulst, De Zeeuw, & Durston, 2015). However, since all three studies used different selections of neurocognitive measures, profile characteristics differed across studies. This violation of measurement invariance, due to the selection of different neurocognitive constructs or similar constructs assessed by different instruments, limits the possibility to derive final conclusions regarding the number and type of neurocognitive profiles being core to ADHD. Another aim of the current study is to address the clinical value of neurocognitive profiling, by examining whether neurocognitive profiles are related to problems often found co-occurring in ADHD, including externalising, social and academic problems in children with ADHD. Neurocognitive data of 81 children with ADHD and 71 TD children were subjected to confirmatory factor analysis. Neurocognitive functioning was measured using the Digit Span Task, Grid Task, Flanker Task and Children’s Emotion Recognition Task, targeting verbal and visuospatial memory, verbal and visuospatial working memory, interference control, processing speed, variability in responding, lapses of attention and facial emotion recognition. Factor analysis resulted in a well-fitting model that consisted of six latent factors: memory, interference control, processing speed, variability in responding, lapses of attention and emotion recognition. The resulting factors were used for community detection in the ADHD and TD group. The results showed four neurocognitive subgroups in children with ADHD, each representing a distinct neurocognitive profile, with one subgroup characterised by poor interference control, one by slow processing speed, one by poor emotion recognition, and one by increased attentional lapses and fast processing speed. Three of the neurocognitive subgroups in the ADHD group were also observed in the TD group, with children with ADHD showing generally weaker neurocognitive performance compared to TD children. More specifically, children with ADHD showed weaker neurocognitive performance than TD children on one to four factor scores within each of the profiles. In the TD group no subgroup with a profile characterised by fast processing speed and increased attentional lapses was found. Our results showed no significant differences between any of the
neurocognitive subgroups in the ADHD sample on problems often found co-occurring with ADHD, including externalising, social and academic problems.

The main goal of chapter 5 is to gain more insight into sleep disturbances in children with ADHD, using objective measures of sleep quality and quantity. A meta-analysis of studies using subjective measures of sleep quality (questionnaires filled out by parents) shows that children with ADHD have higher bedtime resistance, more sleep onset difficulties, nocturnal awakenings, difficulties with arising in the morning and sleep–disordered breathing compared to controls, although for all results considerable heterogeneity was reported across the studies (Cortese, Faraone, Konofal, & Lecendreux, 2009). A recent meta-analysis on sleep studies using actigraphy to objectively measure sleep, showed as well that non-medicated children with ADHD have increased sleep onset latency and decreased sleep efficiency, although, again, results were inconsistent (De Crescenzo et al., 2016). The fact that evidence for sleep problems in children with ADHD is inconsistent, might be explained by confounding influences of comorbid internalising and externalising problems, and low socioeconomic status (SES) in studies performed thus far. In chapter 5 we study sleep quality and quantity using actigraphy in 63 medication-free children with ADHD and 61 TD children, aged 6 to 13 years. Our results showed that medication-free children with ADHD did not differ from TD children in sleep quality and quantity. ADHD symptoms were not related to any of the sleep parameters within the ADHD sample. We did not find a moderating role of low SES on the association between ADHD symptoms and sleep disturbances. Moderation analyses in the ADHD group showed interaction effects between ADHD symptoms and internalising and externalising behaviour and between ADHD symptoms and externalising behaviour on total sleep time, time in bed and average sleep bout duration. However, interactions could not be easily interpreted when comparing sleep patterns in ADHD samples with and without (sub) clinical levels of comorbid psychiatric symptoms. Nevertheless, our findings indicate a complex interplay between ADHD symptoms and comorbid psychiatric symptoms, which might at least partly explain the heterogeneity within the current literature on sleep quality and quantity in ADHD.

In chapter 6 we aim to provide paediatric reference values for blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine. Over the past decades, the use of blood spot samples to examine amino acid concentrations has increased, in particular in newborn screening (Fingerhut & Olgemöller, 2009; Zytkovicz et al., 2001).
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Collecting blood spots, by means of a finger prick, is less invasive for children than taking venous blood samples, and the dried blood spot technique is sufficiently robust and stable for diagnostic purposes (Chace, Sherwin, Hillman, Lorey, & Cunningham, 1998; Kand’ár & Žáková, 2009; Rashed et al., 1997). In addition, blood spots can be assessed at home, as they can be stored at room temperature and be sent by regular mail. Analysis of amino acid concentrations in blood of children is often used in clinical practice for diagnostic purposes and to make decisions regarding treatment (Lepage et al., 2006). Adequate reference values are required to determine which amino acid concentrations should be considered abnormal, and are thereby essential for clinical decision-making. There are some reference values published on blood spot amino acid concentrations in infants, required in newborn screening (Rashed et al., 1997; Zytkovicz et al., 2001). However, to our current knowledge, there are no normative values available for blood spot concentrations of amino acids in primary school-aged children. In chapter 6 dried blood spots were obtained in a community sample of 104 healthy children, aged 6 to 12 years (52 percent males). Blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine were determined by positive electrospray liquid chromatography–tandem mass spectrometry. We examined whether age and gender affected amino acid concentrations, to take the potential effects of age (Held, White, & Pasquali, 2011; Lepage, McDonald, Dallaire, & Lambert, 1997; Van Beynum et al., 2005; Venta, Prieto, & Alvarez, 2002) and gender (Jung & Adeli, 2009) on individual differences in amino acid metabolism into account. Neither age nor gender had an impact on amino acid blood spot concentrations in our community sample. Therefore, reference values for blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine could be presented for the whole group of primary school-aged children. Reference values were established, based on the 5th and 95th percentile, representing the central ninety percent of the sample distribution, which is common in a community sample (Refsum et al., 2004). Furthermore, the 10th and 90th percentiles were presented, which are more stable measures in a modest-sized normative sample, as used here (Lepage et al., 1997).

Finally, in chapter 7 we aim to examine developmental effects on facial emotion recognition in primary school-aged children. The development of facial emotion recognition during childhood has received much scientific interest, although results are somewhat inconsistent on the extent and range of the change in emotion recognition ability (Herba & Phillips, 2004). A potential explanation for differences across studies
into the developmental pathway of facial emotion recognition during childhood, is that the development is emotion-dependent (Mancini, Agnoli, Baldaro, Ricci Bitti, & Surcinelli, 2013; Rodger, Vizioli, Ouyang, & Caldara, 2015). Furthermore, gender and IQ may act as moderators of emotion recognition, as there is evidence for a small female advantage in facial emotion recognition in children (McClure, 2000), and for an effect of IQ on facial emotion recognition (Buitelaar, Van der Wees, Swaab-Barneveld, & Van der Gaag, 1999). The inconsistencies across studies may also be explained by the use of different methodologies; while some paradigms used static pictures with a high expression intensity (e.g., Lawrence, Campbell, & Skuse, 2015; Mancini et al., 2013), others used morphed pictures, in which the intensity of emotional expressions was manipulated (e.g., Herba, Landau, Russell, Ecker, & Phillips, 2006; Thomas, De Bellis, Graham, & LaBar, 2007). Inconsistencies in the literature of the development of facial emotion recognition in children might also be caused by the use of pictures of adult faces versus child faces. In most studies performed thus far in children, tasks were based on pictures of adult faces, using the well-validated Ekman-Friesen Pictures of Facial Affect (e.g., Herba et al., 2006; Lawrence et al., 2015). However, children may perform better in recognising facial emotions expressed by children than in recognising those expressed by adults, due to the so-called own-age bias (Hills & Lewis, 2011; Proietti, Macchi Cassia, & Mondloch, 2015). In children, the ability to recognise emotions in other children’s faces is particularly important in social interaction with peers (Nowicki & Mitchell, 1998). The aim of chapter 7 is to examine the effects of expression intensity, emotional condition, age, gender and IQ on facial emotion recognition in primary school-aged children. For this purpose the Morphed Facial Emotion Recognition Task (MFERT) was constructed, consisting of photographs of children’s faces depicting four basic emotions (anger, fear, happiness and sadness). High-intensity expressions (100 percent) were morphed with neutral expressions, resulting in 240 stimuli, varying in emotional intensity (10 to 100 percent). The MFERT was assessed in a community sample of 75 children, aged 6 to 12 years (45 percent males). Results showed that for all emotional conditions emotion intensity has a linear effect on accuracy. Furthermore, we found that accuracy is highest for happy expressions, followed by angry and frightened expressions. Accuracy is lowest for sad expressions. Age is related to emotion recognition at some intensity levels. Girls have higher accuracy at middle-intensity emotional expressions than boys, but not at low- and high-intensity expressions. The results showed no relation between IQ and facial emotion recognition.
Table 8.1. Summary of the main findings of this dissertation

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Participants</th>
<th>Measures</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>83 children with ADHD</td>
<td>• Blood spot concentrations of tryptophan, tyrosine and phenylalanine</td>
<td>• No group differences in blood spot concentrations of tryptophan, tyrosine and phenylalanine</td>
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<tr>
<td></td>
<td>72 TD children</td>
<td>• Parent- and teacher-rated ADHD symptoms (SWAN)</td>
<td>• AAA concentrations not related to ADHD symptoms</td>
</tr>
<tr>
<td></td>
<td>(Study 1)</td>
<td>• Urinary concentrations of tryptophan, tyrosine and phenylalanine (18-hour sample)</td>
<td>• No group differences in protein intake and urinary AAA concentrations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Dietary protein intake</td>
<td>• Blood spot AAA concentrations not related to protein intake and urinary AAA concentrations</td>
</tr>
<tr>
<td>3</td>
<td>55 children with ADHD</td>
<td>• Blood spot concentrations of total homocysteine</td>
<td>• No group differences in homocysteine blood spot concentrations</td>
</tr>
<tr>
<td></td>
<td>54 TD children</td>
<td>• Parent- and teacher-rated ADHD symptoms (SWAN)</td>
<td>• Homocysteine concentrations not related to ADHD symptoms or to neurocognitive functioning</td>
</tr>
<tr>
<td></td>
<td>(Study 1)</td>
<td>• Verbal working memory (Digit Span Task)</td>
<td>• No group differences in intake of folate and vitamin B12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Visuospatial working memory (Grid Task)</td>
<td>• Intake of folate and vitamin B12 not related to homocysteine concentrations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Interference control, variability in responding and lapses of attention (Flanker Task)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>81 children with ADHD</td>
<td>• Verbal memory and verbal working memory (Digit Span Task)</td>
<td>• Four distinct neurocognitive subgroups in ADHD sample; one characterised by poor interference control, one by slow processing speed, one by poor emotion recognition, and one by increased attentional lapses and fast processing speed</td>
</tr>
<tr>
<td></td>
<td>71 TD children</td>
<td>• Visuospatial memory and visuospatial working memory (Grid Task)</td>
<td>• Three distinct neurocognitive subgroups in TD sample, closely resembling profiles of first three subgroups in ADHD group</td>
</tr>
<tr>
<td></td>
<td>(Study 1)</td>
<td>• Interference control, processing speed, variability in responding and lapses of attention (Flanker Task)</td>
<td>• No subgroup characterised by increased attentional lapses and fast processing speed in TD sample</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Facial emotion recognition (CERT)</td>
<td>• Children with ADHD weaker neurocognitive performance than TD children on one to four factor scores within each profile</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Parent- and teacher-rated externalising behaviour (DBDRS)</td>
<td>• No differences between four neurocognitive subgroups in ADHD sample on externalising, social and academic problems</td>
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<tr>
<td></td>
<td></td>
<td>• Social acceptance and rejection (sociometric data)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• Parent- and teacher-rated social problems (CBCL/TRF)</td>
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<tr>
<td></td>
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<td>• Reading comprehension, spelling and mathematics (Pupil monitoring system)</td>
<td></td>
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</tbody>
</table>
### Table 8.1. Continued

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Participants</th>
<th>Measures</th>
<th>Main findings</th>
</tr>
</thead>
</table>
| 5       | 63 children with ADHD  
61 TD children (Study 1) | - Time in bed, total sleep time, nocturnal motor activity, sleep onset latency, morning arising latency, average wake bout duration and average sleep bout duration (actigraphy)  
- Parent- and teacher-rated internalising behaviour (CBCL/TRF)  
- Parent- and teacher-rated externalising behaviour (DBDRS) | - No group differences in any actigraphic measures  
- ADHD symptoms not related to sleep quality or quantity  
- Interaction effects between ADHD symptoms and internalising and externalising behaviour on time in bed, total sleep time and average sleep bout duration, which could not be easily interpreted |
| 6       | 104 children from a CS (Study 1) | - Blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine | - Age not related to blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine  
- No gender differences in blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine  
- Reference values provided, using the 5th, 10th, 90th and 95th percentile |
| 7       | 75 children from a CS (Study 2) | - Facial emotion recognition of angry, happy, frightened and sad expressions, across 10 intensity levels (MFERT)  
- IQ (WISC-III) | - For all emotional conditions a positive linear effect of expression intensity on accuracy  
- Accuracy higher for happy expressions than for angry and frightened expressions. Recognition of sad expressions lowest  
- Age positively related to facial emotion recognition at some middle- and high-intensity levels  
- Girls higher accuracy than boys at middle-intensity levels, but not at low- and high-intensity levels  
- IQ not related to facial emotion recognition |

**Notes.** AAA, aromatic amino acid; ADHD, attention-deficit/hyperactivity disorder; CBCL, Child Behavior Checklist; CERT, Children’s Emotion Recognition Task; CS, community sample; DBDRS, Disruptive Behaviour Disorder Rating Scale; MFERT, Morphed Facial Emotion Recognition Task; SWAN, Strengths and Weaknesses of ADHD-symptoms and Normal Behaviour rating scale; TD, typically developing; TRF, Teacher Rating Form; WISC-III, Wechsler Intelligence Scale for Children.
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GENERAL DISCUSSION

This dissertation has yielded several important insights into childhood ADHD. The main insight is that the findings described in chapter 2 and 3 of this dissertation imply that abnormalities of tryptophan, tyrosine, phenylalanine and homocysteine in blood are not involved in the aetiology of ADHD. We therefore failed to support the idea that these amino acids may act as biomarkers for ADHD, and could not expand our understanding of the aetiology of ADHD. As was argued in chapter 1 in this dissertation, ADHD is a clinically heterogeneous disorder. Finding a single or unitary aetiology of ADHD is therefore highly unlikely. However, we do not rule out the possibility that amino acid abnormalities contribute to the presence of ADHD symptoms in certain more homogenous subgroups of children with ADHD. For instance, it might be that only children with ADHD and severe deficiencies in executive functioning (EF) have altered tyrosine and phenylalanine concentrations, as an altered dopamine functioning in the prefrontal cortex and the striatum is thought to impair executive functions, including sustained attention and interference control in ADHD (Del Campo, Chamberlain, Sahakian, & Robbins, 2011; Oades, 2008). The suggestion of tyrosine and phenylalanine alterations in children with ADHD and severe EF deficiencies is further supported by a meta-analysis that showed evidence for the association between phenylalanine alterations (which result in tyrosine deficiencies) and EF impairment (Albrecht, Garbade, & Burgard, 2009). Likewise, it might be that only children with ADHD and comorbid autism spectrum disorder (ASD) have increased homocysteine concentrations, as previous studies showed an increased prevalence of hyperhomocysteinemia in children with ASD (Paşca et al., 2006; Puig-Alcaraz, Fuentes-Albero, Calderón, Garrote, & Cauli, 2015). This implies that amino acid alterations are not related to ADHD itself, but to associated problems often found in ADHD. Our results emphasise the complexity of exploring single aetiologial risk factors in a disorder that is characterised by a large heterogeneity. Studies into aetiological risk factors for ADHD therefore require large sample sizes, enabling to perform analyses in more homogenous subgroups of ADHD.

There is growing interest in biomarkers in child psychiatry, aimed at providing a biologically guided diagnosis of psychiatric disorders and at gaining insight into biological factors that may moderate treatment response. One of the reasons underlying this interest is the criticism that current diagnostic procedures for ADHD, mainly based on subjective measures (questionnaires, interviews, observation), are insufficiently reliable and valid
(Faraone, Bonvicini, & Scassellati, 2014). Furthermore, there is hope that biomarkers could identify children at risk of developing psychiatric disorders prior to the onset of severe behavioural problems (Singh & Rose, 2009). Analysing amino acid concentrations in blood spots of children would be a relatively cheap and non-invasive addition to diagnostic procedures, in contrast to measuring biomarkers obtained through, for instance, neuroimaging techniques and electroencephalography. However, thus far, no biological markers have been found for ADHD that have been proven to be of any clinical utility, due to low sensitivity and specificity (Scassellati, Bonvicini, Faraone, & Gennarelli, 2012). Our results show that the same holds for tryptophan, tyrosine, phenylalanine and total homocysteine, implying that, based on the available evidence, these amino acids should not be considered as promising biomarkers for ADHD.

The findings that blood spot concentrations of tryptophan, tyrosine and phenylalanine are not decreased and that blood spot concentrations of homocysteine are not increased in children with ADHD, argue against certain nutritional interventions in ADHD. During the past years some studies have examined the effects of AAA supplementation in children and adults with ADHD, with inconsistent results (Nemzer, Arnold, Votolato, & McConnell, 1986; Reimherr, Wender, Wood, & Ward, 1987; Wood, Reimherr, & Wender, 1985; Zametkin, Karoum, Rapoport, Brown, & Wyatt, 1984). The apparent lack of AAA deficiencies in ADHD might explain the conflicting results of amino acid supplementation on reducing ADHD symptoms (Hurt, Arnold, & Lofthouse, 2011). Likewise, our results do not provide support for nutritional interventions with folate (Ghanizadeh, Sayyari, & Mohammadi, 2013) or vitamin B12 (Ghanizadeh et al., 2013; Patel & Curtis, 2007) in children with ADHD, as childhood ADHD is not related to increased homocysteine concentrations or decreased intake of folate and vitamin B12. This implication is in line with a growing consideration that, in case of interventions based on nutritional supplements, effects may be limited to ADHD patients with nutritional deficiencies (Hurt et al., 2011).

Another insight that is provided by this dissertation, is that associated problems in ADHD might not be related to ADHD itself, guided by our finding of no sleep disturbances in medication-free children with ADHD. Associated problems in ADHD might be epiphenomena, caused by other factors characteristic of ADHD, including stimulant medication use and comorbid psychiatric conditions. For instance, we hypothesised that stimulant medication use may mediate sleep disturbances in children with ADHD,
as previous studies have reported that methylphenidate has a (direct) negative impact on sleep quality as compared to placebo (Corkum, Panton, Ironside, MacPherson, & Williams, 2008; Schwartz et al., 2004). Therefore, children with ADHD who participated in our study into sleep disturbances (chapter 5), had to withdraw from stimulant medication use when sleep was monitored. This approach may have contributed to our finding that ADHD is not associated with any sleep disturbances. Our findings imply that methylphenidate use may have confounded results of earlier studies that showed an association between ADHD and sleep disturbances (Yoon, Jain, & Shapiro, 2012). Furthermore, we found interactions between ADHD symptoms and internalising and externalising problems in the association with sleep problems, indicating a moderating role of comorbid psychiatric conditions on the association between ADHD and sleep disturbances. We hypothesise that the same may hold for the association between ADHD and social problems and academic underachievement; we suggest that factors characteristic of ADHD (including comorbid psychiatric conditions) increase the risk of social problems and academic underachievement in children with ADHD. For instance, social problems in ADHD might be mediated through or moderated by comorbid ASD (Van der Meer et al., 2012) or comorbid externalising behaviour (Becker, Luebbe, & Langberg, 2012). Academic underachievement, on the other hand, might be mediated through or moderated by low SES (Sjöwall, Bohlin, Rydell, & Thorell, 2017). Our results of chapter 4 indicate that neurocognitive profiles did not seem to mediate or moderate the association between ADHD and both social problems and academic underachievement. This lack of findings emphasises the need to explore other factors than neurocognitive deficiencies that mediate or moderate the association between ADHD and associated social and academic problems. For instance, further research might focus on other environmental factors, including family and parenting factors (Becker et al., 2012), to disentangle the true risk factors for associated social and academic problems in ADHD. This may enhance early detection of children with ADHD who are at risk of (multiple) other problems that impair daily life functioning.

Furthermore, the results of this dissertation emphasise yet again the heterogeneity of ADHD. For instance, the results described in chapter 4 point out that there are multiple distinct neurocognitive subgroups in ADHD, each characterised by other neurocognitive strengths and weaknesses. Furthermore, the great variance found in many domains (ADHD symptoms, externalising behaviour, internalising behaviour, ASD symptoms, sleep disturbances, academic performance and social functioning) in chapter 4 and
marks the great individual differences between children with ADHD. While some children with ADHD in our sample were characterised by problems in only one domain, others were hampered by a wide range of associated problems. The great heterogeneity evokes to apply an individual approach in treatment of children with ADHD. Currently, clinical guidelines propose a standard approach to treatment of children ADHD (e.g., American Academy of Pediatrics, 2011). For instance, stimulant medication use is the first-choice treatment for children with severe ADHD symptoms. However, there is a large group of children with ADHD for whom treatment with methylphenidate is not effective (10 percent), or for whom treatment with methylphenidate is not more effective than placebo (additional 13 percent) (Greenhill et al., 2001; Vitiello et al., 2001). Moreover, there is evidence for idiosyncratic dose-response curves for methylphenidate (Greenhill et al., 2001), implying that for each individual the dose-response curve can take other forms; a higher dose does not lead to a greater reduction in symptoms in all children (Konrad, Günther, Heinzel-Gutenbrunner, & Herpertz-Dahlmann, 2005). The effects of methylphenidate may also differ across outcomes, as a particular dosage may be most effective in reducing impulsiveness, while another dosage is most effective in reducing concentration problems (Konrad et al., 2005). Taken together, pharmacological treatment of children with ADHD should be tailored and evaluated on an individual basis. For instance, it is recommended to use double-blind placebo-controlled titration when stimulant medication is advised for children with ADHD (MTA Cooperative Group, 1999). This form of titration enables detecting non-responders and placebo-responders, and may enhance prescription of optimal individual dosages.

For other interventions for ADHD an individual approach is warranted as well, and it is recommended to examine which children with ADHD benefit most from the intervention of interest. For instance, it has been shown that the presence of comorbid anxiety in children with ADHD moderates treatment response, as children with anxiety disorders showed relatively stronger response to behavioural intervention than those without anxiety disorder did (MTA Cooperative Group, 1999). Therefore, clinicians may consider a different approach for children with ADHD and anxiety, from an approach for children with ADHD and other or no comorbidities (Hinshaw, 2007). An individual approach when it comes to the selection of the most adequate treatment for children with ADHD may enhance treatment outcomes. However, currently there is insufficient scientific evidence to adjust clinical guidelines for treatment of ADHD to individual characteristics. Therefore, more research is warranted to detect which factors mediate or moderate
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treatment response in children with ADHD, which may help to apply an individual approach in treatment of children with ADHD.

The heterogeneity in ADHD also evokes to expand the diagnostic procedures in children. When presented with a child with ADHD symptoms, it is recommended to perform a broad screening of associated problems during the diagnostic procedures. Using screening instruments that cover multiple psychiatric conditions, as well as functional impairments, may lead to better and earlier detection of children with ADHD who are at risk of severe problems.

RESEARCH AGENDA

Even though the results described in the current dissertation, being mainly null-findings, rule out some lines of research into the aetiology of ADHD and into treatment of ADHD symptoms (such as nutritional interventions targeting amino acid abnormalities), they also raise a number of research questions and themes for future research.

In chapter 2 and 3 we explored whether amino acid abnormalities are related to childhood ADHD. One of the potential underlying mechanisms for aberrant amino acid concentrations in blood could be a decreased intake of protein, folate or vitamin B12. Our lack of finding amino acid abnormalities in ADHD can therefore partly be explained by a lack of dietary deficiencies in our childhood ADHD sample. We might question whether amino acid abnormalities would be more prevalent in malnourished children. Decreased dietary intake of protein, folate and vitamin B12 is more likely in children living in low SES environments. We suggest to further explore the risk of dietary deficiencies for ADHD in children living in low SES environments (Liu & Raine, 2006). As our ADHD sample consisted of only a small subsample of children with a low SES (n=14), our study was underpowered to explore the effects of low SES on dietary intake and, subsequently, on amino acid concentrations. We hypothesise that in a sample of children with ADHD and a low SES the effects of dietary deficiencies on ADHD symptoms are larger than already established for some micronutrients, including zinc (Toren et al., 1996), folate (Durá-Travé & Gallinas-Victoriano, 2014), iron (Konofal, Lecendreux, Arnulf, & Mouren, 2004), and omega-6 fatty acids (Ng, Meyer, Reece, & Sinn, 2009). In case specific deficiencies are found in children with ADHD from low SES families, the effects of nutritional interventions for these children could be explored. Indeed, beneficial effects...
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of multivitamin/mineral supplementation and essential fatty acids have been suggested larger in children with ADHD who have dietary deficiencies (Hurt et al., 2011).

Another avenue that could be explored in future studies, is to focus on the effects of prenatal nutritional deficiencies, as we expect that nutritional deficiencies are more detrimental during the early stage of prenatal development. For instance, a recent study showed negative effects of low prenatal folate levels on brain volume, language, learning/memory and visuospatial processing in six to eight year old children (Ars et al., 2016). This line of research may also provide more insight into the association between low SES and ADHD. Many studies have shown that low SES appears to be a risk factor for ADHD (Willcutt, 2012). However, it is not clear which factors associated with social disadvantage contribute to the onset of ADHD. It has been suggested that social disadvantage involves poor prenatal nutrition and increased pre- and post-natal toxicant exposure, which can have a detrimental impact on brain development of children (Nigg & Craver, 2014). Therefore, we suggest to further explore the effects of nutritional deficiencies in pregnant women, in relation to low SES, on later behavioural functioning of children, in a longitudinal study.

For future dietary intervention studies in ADHD, it is recommended to establish a thorough justification of the intervention of interest, by providing a sound theoretical framework on the working mechanisms underlying the supposed intervention effects. Such a theory on working mechanisms is crucial to understand why an intervention may be beneficial and to whom the intervention should be applied. For dietary interventions in ADHD, theories may be built on nutritional deficiencies in children with ADHD, in case of nutritional supplements being provided as intervention. Theories may also be built on allergic responses to nutrients occurring naturally in food (among which eggs and peanuts) or to artificial ingredients (including artificial colours) in children with ADHD, in case of elimination diets. The initial plan for the current dissertation was to focus on the treatment effect of nicotinamide (part of vitamin B3) supplementation in children with ADHD who suffer from a tryptophan deficiency. The main postulations for a randomised controlled trial with nicotinamide supplementation were that (a) a significant subgroup of children with ADHD would suffer from decreased tryptophan concentrations, and (b) nicotinamide supplementation would improve upon the uptake and transport of tryptophan, resulting in an increase of tryptophan concentrations. During the preparation of the research protocol, we realised that there was insufficient theoretical justification.
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for tryptophan abnormalities in children with ADHD, as well as for the beneficial effect of nicotinamide supplementation on the uptake and transport of tryptophan. Given the inconsistent evidence in the current literature for tryptophan abnormalities in children with ADHD, we decided to investigate in a carefully set-up study whether there was evidence for the postulation of tryptophan abnormalities in children with ADHD. As our results showed that this was not the case (described in chapter 2), further studies into nicotinamide supplementation in children with ADHD were not conducted. This dissertation illustrates that experimentally testing the postulations underlying a novel intervention, is fruitful prior to carrying out a large randomised controlled trial. Also for current nutritional interventions in ADHD, including restricted elimination diets, it would be interesting to explore the main postulations regarding the underlying working mechanisms. For instance, it is unlikely that an allergic mechanism is involved in the efficacy of restricted elimination diets (Pelsser, 2011), which invokes to explore which factors may explain beneficial effects of elimination diets. Without a sound theoretical framework, alternative explanations, including a biased perception of parents (Sonuga-Barke et al., 2013), become more plausible to explain the effects of a certain intervention that requires a large investment of participants and their parents.

A last suggestion for future research is to explore the predictive validity of neurocognitive deficiencies in children with ADHD. While our results described in chapter 4 point out that children with ADHD generally show weaker neurocognitive performance compared to TD children, these deficiencies do not seem to mediate the association between ADHD and current externalising behaviour, academic achievement and social functioning. Furthermore, there is limited predictive validity of neurocognitive functioning for persistence of ADHD (Van Lieshout, Luman, Buitelaar, Rommelse, & Oosterlaan, 2013), and no evidence for predictive value of neurocognitive functioning for the emergence of nicotine dependence or substance use disorders later in life in individuals with ADHD (Groenman et al., 2015). One might therefore question whether it is useful to perform clinical neurocognitive assessments in children with ADHD, which may be highly time-consuming. It has been suggested that children with ADHD outgrow neurocognitive deficiencies, as EF deficiencies that were found in childhood ADHD, were not found in the follow-up of that sample in adolescence (Thissen et al., 2014). It may be, however, that a subsample of children with ADHD and neurocognitive deficiencies continues to be impaired in terms of neurocognitive functioning later in life. For instance, it was found that also in adults separate neurocognitive profiles could be detected, with individuals...
with ADHD performing worse on neurocognitive measures than controls (Mostert et al., 2015). Unfortunately, it was not investigated whether the different cognitive profiles in adults with ADHD showed different functional impairments. We recommend to further explore the predictive validity of neurocognitive profiles for future functioning, using a longitudinal study design. If certain neurocognitive profiles prove to increase the risk of future academic underachievement, this may provide more insight into which deficiencies should be targeted in cognitive interventions and which children may benefit most from these interventions. Currently there seem to be no far transfer effects of cognitive interventions on academic performance in children with ADHD (Rapport, Orban, Kofler, & Friedman, 2013). However, when cognitive interventions target the neurocognitive deficiencies that are largest in some subgroups of children with ADHD, effects on academic performance may emerge. Predictive validity of neurocognitive profiles would thereby justify the role of clinical neurocognitive assessment in diagnostic procedures in children with ADHD.


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