

Fats in Mind

Effects of Omega-3 Fatty Acids on Cognition and Behaviour in Childhood

Ulrika Birberg Thornberg



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Ulrika Birberg Thornberg
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©Ulrika Birberg Thornberg
Department of Behavioural Sciences and Learning

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ABSTRACT

The aim of this thesis was to examine possible effects of omega-3 fatty acids on children's cognition and behavior. Longitudinal as well as cross-sectional comparisons were made among children with typical development and children with ADHD /at risk developing ADHD.

The specific purposes were to examine (1) breast-feeding in relation to cognition; (2) relation between long chain poly unsaturated fatty acids (LCPUFAs) in mothers breast-milk and children's cognition; (3) effects of EPA supplementation on cognition and behavior in children with ADHD; (4) if LCPUFAs have differential effects on working memory, inhibition, problem-solving and theory of mind (ToM).

The main conclusions were as follows; (1) duration of breast-feeding was positively correlated to children levels of intelligence (IQ); (2) LCPUFAs in breast-milk was related to children's ToM and IQ, the quotient DHA/AA, together with length of breastfeeding and gestation week explained 76% of the variance of total IQ; (3) subtypes of children with ADHD responded to EPA supplementation with significant reductions in symptoms, but there were no effects in the whole group with ADHD; (4) ToM ability was related to LCPUFAs, but not to any other cognitive measures as working memory, inhibition and problem-solving.

To conclude, these results indicate that fatty acid status in breast-milk at birth affect general cognitive function in children at 6.5 years of age, including ToM. Short-term intervention with omega-3 fatty acids does not affect cognition in children with ADHD, but improves clinical symptoms as assessed by means of teacher ratings. These results further indicate that hot executive function and social cognition may be an area of interest for future research.

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LIST OF PAPERS

Original publications

The present thesis is based on the studies listed below, and will be referred to in the text by their Roman numerals:

- I. Birberg-Thornberg, U., Karlsson, T., Gustafsson, P. A., & Duchén, K. (2006). Nutrition and theory of mind: The role of polyunsaturated fatty acids (PUFA) in the development of theory of mind. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 75, 33–41.
- II. Gustafsson, P. A., Duchén, K., Birberg, U., & Karlsson, T. (2004). Breastfeeding, very long polyunsaturated fatty acids (PUFA) and IQ at 6½ years of age. *Acta Paediatrica*, 93, 1280–1287.
- III. Gustafsson, P. A., Birberg-Thornberg, U., Duchén, K., Landgren, M., Malmberg, K., Pelling, H., Strandvik, B., & Karlsson, T. (2010). EPA supplementation improves teacher-rated behaviour and oppositional symptoms in children with ADHD. *Acta Paediatrica*, 99, 1540–1549.
- IV. Birberg-Thornberg, U., Gustafsson, P.A., Duchén, K. A. & Karlsson, T. (2011). Placebo controlled randomized study of PUFA (Poly Unsaturated Fatty Acids) as treatment for neurodevelopmental problems in 7 year old children and cognitive performance in relation to an age matched control group. (Manuscript).

ABBREVIATIONS

AA	Arachidonic acid, C20:4 n-6
ADHD	Attention Deficit Hyperactivity Disorder
ALA	Alphalinoleic acid, C18:3 n-3 = LNA
ANOVA	Analysis of variance
CD	Conduct Disorder
CPRS	Conners' parent rating scale
CTRS	Conners' teacher rating scale
CPT	Continuous Performance Test
CRF	Case Report Form
DGLA	Dihomo-gammalinoleic acid, C20:3 n-6
DHA	Docosahexaenoic acid, C22:6 n-3
DSM-IV	Diagnostical and Statistical Manual
EFA	Essential Fatty Acids
EPA	Eicosapentaenoic acid, C20:5 n-3
GLA	Gammalinoleic acid, C18:3 n-6
HUFA	Highly Unsaturated Fatty Acids
LA	Linoleic acid, C18:2 n-6
LNA	Alphalinoleic acid, C18:3 n-3 =ALA
LCPUFA	Long Chain Polyunsaturated Fatty Acids
ODD	Oppositional Defiant Disorder
NEPSY	Neuro Psychological Test Battery
PIQ	Performance IQ, from WISC-III
PUFA	Poly Unsaturated Fatty Acid
RCT	Randomised Controlled Trial
SDQ	Strenghts and Difficulties Questionnaire
SNAP-IV	DSM-based rating scale for ADHD and ODD
ToM	Theory of Mind
WISC-III	Wechsler Intelligence Scale for Children, 3 rd edition
WISC-IV	Wechsler Intelligence Scale for Children, 4 th edition
VIQ	Verbal IQ, from WISC-III

INTRODUCTION

There has been considerable interest in the effects of omega-3 and omega-6 fatty acids for human health lately. The research assessing omega-3 and omega-6 fatty acids in relation to brain development and brain function has increased considerably. Omega-3 fatty acid deficiency has been associated with neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD), dyslexia, and mood disorders (Bourre, 2005, 2006*a*; Freeman et al., 2006; Harbottle & Schonfelder, 2008; Richardson, 2006; Soh, Walter, Baur, & Collins, 2009).

Two families of fatty acids, omega-3 and omega-6 are of importance to health. They must be obtained through the diet, they cannot be synthesised by the body and are thus essential. Fatty acids are major structural components for the cell membranes in the whole body and are present in high concentrations in the central nervous system including the brain. The longest omega-3 and omega-6 fatty acids are most important for the brain, and they are together called long chain polyunsaturated fatty acids (LCPUFA). In the brain these long chain omega-3 fatty acids are particularly important for optimal nerve cell development and are also important for neurotransmitter release and cell signalling, and may therefore affect cognition and behaviour (Bourre, 2006*a*; Heinrichs, 2010; Innis, 2008).

In the present thesis, the potential relationships between fatty acids and cognition and behaviour in childhood are investigated. Four studies examine the relationship from two different angles. First, from a longitudinal perspective where fatty acid status in breast-milk at birth and in the blood of infants are examined in relation to cognitive performance (e.g., intelligence and theory of mind) at the age of 6.5 years. Second, two randomized placebo controlled intervention studies investigate whether omega-3 fatty acid supplements improve cognitive functions and decrease behavioural problems in school-aged children with ADHD or at risk of developing ADHD. In the two later studies, behaviours were assessed with rating scales for both parents and teachers. In one of these studies, an extensive cognitive test battery, assessing the children's working memory, inhibition, problem-solving, and Theory of Mind (ToM) was administered as well. All outcome measures were administered before and after treatment.

It is my hope that this work will contribute to the understanding of the role LCPUFAs may have for children's cognition and behaviour, both concerning children with typical development and children with disability in their cognitive and behavioural functioning. Fatty acids and medical issues will be discussed, although this is mainly a thesis in psychology and, consequently, the main focus is in the field of psychology.

The outline of the thesis is as follows. The thesis starts with a brief overview of cognition, executive functions, and ToM in children with typical development, before turning to children with ADHD and the cognitive functioning related to this disorder. Then, fatty acids and their role for the brain and for cognitive development will be discussed before reviewing clinical studies on omega-3. In the next section, the participants and methods used in the four studies are presented followed by summaries of the results. The main findings are highlighted followed by a discussion of methodological issues of importance to understand the results. Finally, future directions of research in this field and possible clinical implications are suggested.

COGNITION IN CHILDREN

The concept cognition refers in this thesis to the high-level cortical functions carried out by the human brain. In this thesis, several aspects of cognition are examined in relation to fatty acids. These aspects are intelligence, executive functions, working memory, and theory of mind, all discussed in this section. Definitions, theories and findings of these concepts are chosen in the light of the fatty acids studies included in the thesis and should therefore not be considered as attempts to be comprehensive. Typical tasks used to measure these abilities will be exemplified in this section, whereas the measures used in the thesis will be described in detail in the Empirical studies section.

Cognitive developmental psychology is a field of study in psychology focusing on development in terms of learning, memory, information processing, and language, among other aspects (Galotti, 2008; Sternberg, 2009). These mental processes are influential for our everyday functioning affecting our ability to perceive and attend to the world around us, and moreover, affect the ability to think, solve problems and make decisions. During childhood the individual undergoes dramatical changes in these mental processes.

Cognitive development is considered to proceed in almost the same way for all children with typical development, but there are also large differences in different domains. In this thesis I will use the term *children with typical development* to characterize children with a standard development without developmental deviations as a group.

Cognitive development is determined by a number of factors, including psycho-social and socio-cultural variables as well as biological factors (Galotti, 2008; Sternberg, 2009). Socio-economic status (SES) has stable relations with children's cognitive ability, academic achievement, and IQ (McLoyd, 1998; Noble, McCandliss, & Farah, 2007). Relationships with such confounding factors are typically strong and are observed throughout development, from infancy through adolescence and into adulthood. In longitudinal studies of the associations between fatty acids at birth, breast-feeding and subsequent cognitive growth confounding factors play an important role. For example, concerning breast-feeding there are frequent discussions whether the entire association with cognitive performance can be explained by confounding factors such as parental IQ, or are related to the nutrients in the milk (Der, Batty, & Deary, 2006; Jacobson, Chiodo, & Jacobson, 1999; Jacobson & Jacobson, 2002).

Recent imaging techniques show that brain regions associated with basic abilities, such as motor function and sensation, develop first. Brain regions that facilitate information integration, association areas, develop more slowly. Last to develop is the frontal and in particular the prefrontal cortex, which is involved in executive functions (Casey, Tottenham, Listen, & Durstone, 2005; Fuster, 2002). The frontal lobes, and thereby executive functions, thought to be localized there, are especially vulnerable for several reasons, first due to the slow rate of maturation and myelination, and, second, since it is the area that develops last. It is also dependent on the successful development of the structure and function of those regions previously developed (Fuster, 2002; Gouvier, Baumeister, & Ijaola, 2009).

Cognitive neuropsychology is the area in cognitive psychology that examines the relation between structure and function in the brain and different psychological aspects. A closely related area of research is the investigation of genetic and maturational underpinnings of cognitive abilities. The focus in neuropsychology is in studying cognitive effects of brain function and deviances in brain function (Bradshaw, 2001). Neuropsychological research indicates that cognitive abilities depend on the functions of several dissociable neural systems rather than being one single function (Diamond & Amso, 2008).

Neuropsychological testing offers a unique view into the functioning of the brain. These tests are often sensitive, but lack specificity, and therefore have neuropsychological testing (despite its history of brain localisation of cognitive functions, Bradshaw, 2001) been more and more focused on finding strengths and weaknesses of the children in order to plan effective educational and psychological interventions than localizing (Gouvier et al., 2009).

This very brief introduction to the multifaceted field of cognition is intended as an introduction to the specific areas of interest for this thesis and for fatty acid research. Researchers in the field of fatty acids have, until recently, mostly discussed cognition as levels of performance in standardized intelligence test batteries, but interest has turned to examine distinct cognitive abilities and executive functions as well (Chetham, Colombo & Carlson, 2006). Moreover, relatively little attention has been given to the selection of neuropsychological tests to detect effects of fatty acid intervention on cognitive functioning (Rosales, Reznick, & Zeisel, 2009; Sinn, Bryan, & Wilson, 2008).

Intelligence in children

Intelligence is examined in several of the studies included in this thesis. Intelligence refers to higher-levels of cognitive abilities such as problem solving, abstract thinking, learning and planning, and adapting to the environment (Sternberg, 1997).

Despite the broad definitions of intelligence as a construct, measurement of intelligence has focused on a quite narrow range of abilities, mostly intellectual abilities required for academic activities. Examining intelligence has historically been done by means of standardized scales such as Wechsler Intelligence Scales for Children (WISC, Wechsler, 1991, 2003) intending to assess different cognitive functions (Flanagan & Kaufman, 2009;

Zhu & Weiss, 2005). Wechsler combined different tasks and theories to develop this clinically useful, ecologically valid scale for measuring intelligence, which is one of the most used test batteries for assessing intelligence in children. Although there have been substantial support for the clinical utility, the Wechsler scales have endured a lot of criticism due to shortage of theoretical basis (Zhu & Weiss, 2005).

In studies of nutrition, standardized intelligence test batteries such as WISC-III and WISC-IV (Wechsler, 2003), Bayley Scales of Infant Development (BSID) and Kaufman Assessment Battery for Children (KABC), are frequently used (Chetham et al., 2006; Rosales et al., 2009). WISC are used in our studies, it was age-appropriate and included relevant sub-tests relevant for our purposes.

Wechsler intelligence scale for children (WISC) has been published in several editions. The one used in this thesis is WISC-III (Wechsler, 1991). The WISC-III Full Scale IQ (FSIQ) is the overall composite score that estimates the child's general level of intellectual functioning and is the aggregation of the core subtest scores. The reliability is high and the overall internal-consistency coefficients are above .90. Concerning validity studies, Wechsler scales correlate highly with other intelligence scales and thereby seem to measure general intellectual ability (Flanagan & Kaufman, 2009). In WISC-III (Wechsler, 1991) the subtests so were organized into separate verbal and performance scales, and provided not only FSIQ but also Verbal IQ (VIQ) and Performance IQ (PIQ).

Known factors that influence IQ are parents' socio-economic status and parents' educational level (Davis-Kean, 2005; Tong, Baghurst, Vimpani, & McMichael, 2007). Other important factors are biological, like birth weight, gestation week and parental smoking during pregnancy (Rahu, Rahu, Pullman, & Allik, 2010). Gestational age have been reviewed both regarding children being born prematurely (Johnson, 2009) and children born at term (Yang, Platt, & Kramer, 2010) and has been found influential in regard to subsequent intelligence. Moreover, individual differences, and, especially motivation, have been discussed as variables that inflate the predictive value of intelligence (Duckworth, Quinn, Lynne, Loeberd, & Stouthamer-Loeberd, 2011).

The influence of genes and environment is bidirectional as the same developmental disadvantage lowers both IQ and academic performance. Also length of breast-feeding has shown to influence IQ (Anderson, Johnstone, & Remley, 1999; Oddy, Kendall, Blair, de Klerk, Silburn, & Zubrick, 2004), see the section on fatty acids and breast-milk for further discussion on breast-milk and its link to cognition. Accounting for factors known to influence intelligence is standard procedure in studies of relations between omega-3 fatty acids and cognition, as well as in this thesis.

Executive functions

The impact of fatty acids on executive functions is an area in its infancy. Executive dysfunction, however, is frequently discussed in relation to ADHD and to some extent also in omega-3 fatty acid treatment studies for children with ADHD.

Executive functions are closely related to cognition and describes a set of cognitive abilities that control and regulate other abilities and behaviours. There are different constructions, contributions and interpretations of this complex concept (Miyake, Friedman, Emerson, Witzki, Howerter, & Wagner, 2000; Kane & Engle, 2002). It was first connected to neuropsychological theories and localized to prefrontal cortex (Luria, 1966, 1980). Later these functions were organized into several cognitive domains; initiation, planning, inhibition, organizing behaviours, and fluency. Deficiencies in these cognitive functions were described as the “dysexecutive syndrome” (Baddely, 1986). Other definitions have been refined and involve viewing executive functions as the ability to initiate and stop actions, to regulate and modify behaviour, as well as to plan future behaviour (Barkley, 1997a, 1997b).

Most researchers today view executive function as an umbrella term that includes inhibition, working memory, monitoring skills, cognitive flexibility and planning (Lezak, Howieson, & Loring, 2004; Schneider, Schumann-Hengsteler, & Sodian, 2005). There are, however, different views, whether executive function should be seen as a unitary construct or a number of independent components (Berlin, Bohlin, Nyberg & Janos, 2004; Berlin, Bohlin & Rydell, 2003; Best & Miller, 2010; Miyake, Friedman et al., 2000).

Executive functions can be divided into hot versus cool executive functions (Hongwanishkul, Happaney, Lee, & Zelazo, 2005). Hot executive functions include motivation and affective aspects, and executive tasks require appraisal of the emotional aspect of stimuli (Zelazo & Müller, 2002b). Cool functions are more abstract and decontextualised aspects of cognitive abilities (Kerr & Zelazo, 2004). This view is supported by neuroanatomical findings where orbitofrontal parts of the cortex are more involved in hot executive functions, whereas the dorsolateral prefrontal cortex is more involved in cool executive functions (Hongwanishkul et al., 2005; Kerr & Zelazo, 2004). Although similar abilities may underlie both hot and cool executive functions and they seem to be part of the same system (Carlson, 2005), some findings indicate that hot executive functions develop relatively slowly (Prencipe, Kesek, Cohen, Lamm, Lewis, & Zelazo, *in press*).

Inhibition is often seen as a key component in executive functions. It is also a concept commonly discussed in relation to ADHD. Inhibition or inhibitory control can be seen as the ability to withhold an inappropriate action that is not relevant for the target or task at hand (Carlson, 2005). Dempster and Corkhill (1999) defined it as the ability to suppress irrelevant information during the execution of a plan. Inhibitory control deficits are often referred to as impulsivity. Impulsive behaviour can be seen as the behavioural response from a situation where one is not able to show inhibitory control. Impulsivity is also one of the central diagnostic features of attention problems (Fahie & Symons, 2003).

There are individual differences in executive functions affecting different social and cognitive aspects of development in children, for example school readiness (Diamond, Barnett, Thomas & Munro, 2007). Turning to more severe deficits they are associated with problem behaviours, for example, aggressive behaviour (Séguin, Parent, Tremblay, &

Zelazo, 2009), and also with a range of neurodevelopmental disorders, for example ADHD, conduct disorders and autism (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). These are all disorders frequently discussed in relation to fatty acids (Freeman et al., 2006; Harbottle & Schonfelder, 2008; Richardson, 2004, 2006; Soh et al., 2009).

During the last decade the research in executive functions has grown, especially the field of examination of interrelations between executive functions, working memory, verbal ability and theory of mind (see Schneider, Schumann-Hengsteler, & Sodian, 2005 for an overview). There have also been many attempts to find hierarchical models of the organization of executive functions often with working memory and inhibition in central positions (Miyake, Friedman, et al., 2000; Kane & Engle, 2002).

Engle and Kane (2004; Engle, 2002; Kane & Engle, 2002) are among the most influential proponents for developing the idea of a common mechanism behind working memory and inhibition. They have developed a two-factor theory of cognitive control (Engle & Kane, 2004), in which they argue for a central core of working memory capacity that they call executive attention. This core function consists of a mechanism that keep the goal active in memory and a simultaneous inhibition function that keep irrelevant prepotent actions away. Theoretical definitions of different aspects of executive function are needed, to be able to choose valid and reliable neuropsychological tests for studying possible effects of omega-3 fatty acids in children. A closer description of working memory follows in the next section.

One problem with assessment of executive function is that global executive tasks have low reliability (Miyake, Emerson, Friedman, 2000). Tasks are only valid tests of executive function when the stimuli in them are new, as soon as a task has been performed it become somewhat automatized (Burgess, 1997; Phillips, 1997). Even a child with modest general abilities can, with training, solve difficult tasks and perform well. When the task is performed again it will be in a qualitatively different way (Rabbitt & Lowe, 2000). Another difficulty is the complexity of executive tasks, it is very difficult to isolate a single aspect of the performance but tasks often tap several aspects (Hughes & Graham, 2007).

The first difficulty regarding finding reliable, valid and sensitive measures of executive function is the continuum between an action being controlled and being automatised (Hughes & Graham, 2007). With this I mean that the process underlying the performance on a novel task will be gradually changing from controlled to automatic over time. Retesting thus, does not tap executive function at the same extent as the first time when the performance was new (Lowe & Rabbitt, 1998). Moreover, even a small difference in task demands can make the individual to return to a controlled action again. Therefore executive tasks often have poor test-retest reliability (Miyake, Emerson, et al., 2000).

To summarize, there are different views on executive functions, but most researchers agree that they involve, working memory, inhibition, planning, updating and cognitive flexibility. Working memory is of special importance for this thesis and will be further dealt with below. Working memory is also closely related to ADHD and often considered

a core deficit in ADHD (Barkley, 1997b; Martinussen, Hayden, Hogg-Johnson, & Tannock, 2005; Pennington & Ozonoff, 1996).

Working memory

Working memory abilities are discussed and measured in several of the studies in this thesis. Working memory refers to the capacity to store and manipulate information for a short period of time (Baddeley, 1986; Conway, Jarrold, Kane, Miyake, & Towse, 2008). It is important to separate short-term memory from working memory. Short-term memory is often described as a simple storage buffer, where the capacity is based on practiced skills and strategies, such as rehearsal and chunking. Working memory on the other hand is more complex and involves some kind of processing of information and consists of a storage component as well as an attention component (Engle, 2002).

The function of working memory is to maintain memory representations during distraction, processing or attentional shift (Baddeley & Hitch, 1974). There are two major perspectives on working memory described in this section; Baddeley's component model and the processing model with proponents like Kane and Engle (2002).

The component model focuses on separate components for storing and processing information from different modalities. Baddeley and Hitch (1974) originally divided the working memory system into three components and in a revision of the model (2000), a fourth component was added. The central executive is a domain-general component responsible for the control of attention and processing, which is involved in a range of regulatory functions including the retrieval of information from long-term memory. The temporary storage of information is thought to be mediated by two domain-specific stores, i.e., the phonological loop, which provides storage of verbal material, and the visuo-spatial sketchpad, which deals with the maintenance and manipulation of visual and spatial representations. The fourth component integrated in the model is the episodic buffer with the purpose to temporarily integrate spatial, visual and phonologic information (Baddeley, 2000).

The other major perspective, the processing model, deals with the working memory capacity of the whole system and focuses on function. Engle and Kane (2004; Engle, 2002; Kane & Engle, 2002) hold working memory as a limited-capacity ability closely related to executive attentional selection. It also includes the ability to maintain information online and to activate some information or plans over others, termed *updating*. Moreover, it is closely related to anterior attention networks and inference control (Posner & Rothbart, 2007).

Working memory capacity is crucial for maintaining focused behaviour in different situations of life (Alloway, Gathercole, Kirkwood, & Elliott, 2009). Difficulties in learning (i.e., reading, spelling and math) are for example related to working memory deficits (De Jong, 1998; Gathercole & Alloway, 2008). Some insight into why working memory constrains learning has been provided by observations of children with low working memory capacity in the course of their regular classroom activities (Gathercole & Alloway, 2008).

Low results on working memory tasks have further been related to high levels of inattention (Martinussen & Tannock, 2006). Systematic evaluations of the behavioural characteristics of children with low working memory results have been conducted (Alloway et al., 2009). Conners' teacher rating scales (CTRS, Conners, 2005) were used to assess the children's inattentive, hyperactive and oppositional behaviour. Children with low scores on working memory tasks had an "extremely high risk of making poor academic progress and 'have a highly distinctive profile of inattentive behaviour and forgetting that disrupts their classroom functioning'" (Alloway et al., 2009, p. 619).

Neuropsychologists often add *planning* to the working memory domain. Planning is the ability to mentally organize a series of actions in temporal sequence. This ability probably also requires other skills, such as reasoning and attention, and is often described in visuo-spatial terms (Pennington & Ozonoff, 1996).

The origin of working memory variability is still unknown; but it appears to be unaffected by environmental influences, such as parental educational level and economical background (Alloway, Gathercole, Willis, & Adams, 2004). There is, on the other hand, strong evidence for heritability (Kremen et al., 2007), therefore it has been suggested that it is not possible to increase working memory capacity. However, there are indications that working memory capacity can be increased by intensive training (see e.g., Klingberg et al., 2005; Lundqvist, Grundström, Samuelsson, & Rönnerberg, 2010;).

Theory of mind

Another component of cognition, more specifically of social cognition, is Theory of mind (ToM). In two of the four studies in this thesis we have examined ToM ability in children in relation to omega-3 fatty acids. ToM involves the ability to think about mental states such as emotions, beliefs and intentions in one self as well as in others. This ability enables understanding and reasoning about as well as predicting what other people know and how they will act (Astington, 1993; Premack & Woodruff, 1978; Sodian, 2005).

Research interest in ToM has largely been addressed to the idea that impairment in social cognition is responsible for the core deficits in autism (Baron-Cohen, Leslie, & Frith, 1985; Happé, 1993). Later, relations between ToM and other neurodevelopmental disorders such as ADHD (Sodian & Hülken, 2005) and communication disorders (Sundqvist & Rönnerberg, 2010), have been discussed as well.

There appears to be a reciprocal relation between ToM abilities and interpersonal relations. ToM seems to influence social skills and may help to establish good relationships with peers as well as important adults (Hughes & Leekam, 2004). Competence in ToM may also affect academic achievement, through the child's ability to understand what is expected from him or her in a teaching situation (Kloo & Perner, 2008).

The development of ToM is considered to start early, probably already at birth (Perner, 2000; Premack, 1991; Sodian, 2005). Young children first understand aspects of desires and intentions (Bartsch & Wellman, 1989). Infants can display expectations about actions

of others, and 18-month-old children are able to show understanding of intention (Meltzoff, 1995; Meltzoff, Gopnik, & Repacholi, 1999) as is evident from experiments showing that preverbal children engage in helping an adult achieve a goal (Warneken & Tomasello, 2006). The ToM abilities improve markedly between age three to seven (Astington, 1993; Wellman, Cross, & Watson, 2001). The false-belief paradigm examines the understanding that people can have beliefs that contradict reality and that they will act in accordance with their own beliefs in certain kinds of tasks (Wimmer & Perner, 1983).

Even though children at these ages are able to describe representational mental states or beliefs of others, distinct from their own, their repertoire of mental concepts, such as desires and perceptions, are still limited (Saxe et al., 2004). At the age of 7 or 8 years children start to show increased use of personality traits and begin to be aware that people have stable dispositions that help to predict future behaviours across different situations. By adulthood, ToM has developed to the point where it is used in various social contexts, such as those involved in interpreting faux pas and humour (Hughes & Leekam, 2004).

At the same time as children's performance on ToM tasks improves the most, i.e., at 3-5 years of age, they also improve their performance on executive function tasks dramatically. The relation between these two entities has also been shown in several studies (e.g., Carlson & Moses, 2001, and see Moses, Carlson, Sabbagh, 2005, for a review). One suggestion is that executive abilities play a critical role in the development of ToM, and working memory as well as inhibition are particularly involved (Moses et al., 2005).

Language and quality of communication between the child and its parents and peers are closely related to ToM (Hughes & Leekam, 2004). This relation is complex and a number of factors are involved. Witnessing apparent emotional interactions within the family can positively influence development of ToM (Lagattuta, Wellman, & Flavell, 1997). Moreover, language has been proposed to have a mediating role between social relations and ToM (Hughes & Leekam, 2004). A link between number of peers in children's networks and the children's mentalization ability was for example demonstrated in children who used augmentative and alternative communication (AAC) and thus had limited capacities to communicate with peers (Sundqvist & Rönnberg, 2010).

Investigations of the development of ToM have implicated a role for the frontal lobes in ToM in children (Rowe, Bullock, Polkey, & Morris, 2001; Gallagher, Happé, Brunswick, Fletcher, Frith, & Frith, 2000). The same regions have also been implicated in executive functioning (Shallice, 2001). Functional neuroimaging, neurophysiology, and brain lesion studies have suggested a network of brain regions associated with ToM. These regions include the temporo-parietal junction, the superior temporal sulcus, and the temporal poles. Prefrontal cortex has also frequently been showing importance for ToM abilities (Knight & Stuss, 2002; Stuss, Gallup, & Alexander, 2001). Furthermore, an EEG study demonstrated that dorsal medial prefrontal cortex and the right temporo-parietal junction was important for development of ToM in preschool children (Sabbagh, Bowman, Evraire & Ito, 2009).

To conclude this section on cognition in children, studies have conceptualised executive functions as related systems involving multiple processes and that are interrelated and interdependent. Despite the relative consensus of the theoretical definition of executive function it is much more difficult to establish an operational definition of executive function. Such an operational definition would be needed to design proper and reliable investigations of possible effects of omega-3 PUFA on executive functions.

ADHD IN CHILDREN

The ADHD diagnosis

Attention Deficit Hyperactivity Disorder (ADHD) is considered a neurodevelopmental disorder (Bradshaw, 2001), which according to the guidelines of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) is defined as problems of attention, hyperactivity and impulsivity. In this thesis two of the studies were conducted with children diagnosed with, or at risk developing, ADHD.

An ADHD diagnosis is currently established by using symptom-based descriptions of problem behaviours. The DSM-IV defines the disorder according to two behavioural areas: hyperactivity-impulsivity and inattention, with nine possible symptoms each. The child needs to meet six out of nine criteria to fulfil the diagnosis. Some of the symptoms must have been present before the age of 7, and they should be present in two or more settings; moreover, the symptoms need to have considerable impact on everyday functioning. In the present thesis, children at risk developing ADHD are included in the last study. The label *neurodevelopmental immaturity* is used interchangeable, as a description of these children with unspecific symptoms of neuropsychological dysfunction, in form of inattention and inhibition difficulties, restlessness or impulsivity and motor deviances, for example clumsiness. The symptoms are above cut-off for reaching an ADHD diagnose, but children have not been diagnosed by a clinician. These symptoms have furthermore given rise to considerations about possibilities to manage to start school at the same age as their peers.

In DSM-IV, there are three different subtypes of ADHD. The hyperactive/impulsive subtype (ADHD-HI) is characterized by hyperactivity and impulsivity, the predominantly inattentive subtype of ADHD (ADHD-I) by inattention, and the combined subtype (ADHD-C) is characterized by hyperactivity/impulsivity together with symptoms of inattention. There is disagreement concerning whether the predominantly inattentive subtype is a separate subtype of ADHD (Barkley, 1998) or if it is an entirely different disorder (Lahey & Applegate, 2001; Milich, Balentine, & Lunaham, 2001). For example, qualitative differences have been found regarding the attentional deficits in the subtypes, which indicate that the inattentive subtype may be a separate disorder (Milich et al., 2001). The incidence of these subtypes is estimated to 6.8% for all types of ADHD together, 0.6% ADHD-HI, 2.9% of ADHD-C and 3.2% for ADHD-I (Nigg, 2006).

There is a high prevalence of co-morbid conditions, often with other neurodevelopmental disorders. Common co-morbid diagnoses are; conduct disorder (CD), oppositional defiant

disorder (ODD), Tourette's syndrome (TS), and anxiety and mood disorders (Angold et al., 1999; Kadesjö & Gillberg, 2001; Rasmussen & Gillberg, 2000). Furthermore, ADHD are also associated with reading disability (RD), particularly regarding the inattentive symptoms (Maedgen, 2000) and nearly one fourth of the children with ADHD also have some kind of specific learning disability (Nigg, 2006). Children with ADHD usually have functional impairment across multiple settings at home, in school, and in peer relationships and often show interpersonal problems influencing their everyday life (Maedgen, 2000). Some of the difficulties continue into adulthood and difficulties regarding academic performance and less career success are common (Wilens, Prince, & Biederman, 2008).

Evaluation for ADHD is commonly made in child psychiatric clinics and consists of interviews with the parents and often includes intelligence testing, including cognitive profiles (Nigg, 2006). Behavioural questionnaires, such as Conner's rating scales for parents and teachers (CPRS, CTRS, Conners, 2000) and often some kind of computerized tests of attention, Continuous Performance Test (CPT) are also standard procedures in evaluation of ADHD in children (Naglieri, Goldstein, Delauder, & Schwebach, 2005)

There is an agreement among most researchers that the aetiology of ADHD is a multifaceted phenomenon. Biological factors are often seen as the primary factor underlying ADHD, but social factors such as child rearing, punishment and family stress have been considered as contributing and interacting factors (Nigg, 2006). A large number of genetic studies have shown interaction between different genes and between genes and environment (Castellanos & Tannock, 2002). Neurological and genetic factors are suggested to be the major contributors. ADHD have been discussed in terms of neurodevelopmental immaturity. In this view, supported from neuro-anatomic evidence, the child is considered to suffer from a developmental delay regarding neurological maturation, which results in behaviours deviant from their chronological age (Rubia, 2007; Shaw et al., 2007).

Functional anatomy and neural circuitry includes frontostriatal circuit, posterior cortices, limbic regions, and the cerebellum as well (Mostofsky, Cooper, Kates, Denckla, & Kaufmann, 2002; Sowell et al., 2003). The deviations involve a 10-12 % smaller size in some brain areas, for example, right frontal regions, basal ganglia and corpus callosum. Several studies have also shown that the cerebellum is smaller in children with ADHD (Nigg, 2006). Biochemical abnormalities such as dopamine and noradrenergic neurotransmitter systems deficiency are also apparent, although not conclusive (Arnsten, 2009; Schuck & Crinella, 2005). In the search for underlying causes of ADHD researchers still have not found any biological marker discriminating children with ADHD, although identifying such endophenotypes is of importance. Endophenotype is defined as a quantifiable characteristic thought to mediate between neurobiology and environmental factors (Castellanos & Tannock, 2002).

In search for an underlying cause of ADHD fatty acid deficiency have also been discussed, and is a topic further elaborated on in this thesis. Omega-3 fatty acids are also discussed regarding treatment of ADHD, and several trials have been conducted for

evaluation of treatment with fatty acid supplements in children with ADHD. Today, the most widely used treatments are psycho-stimulants or psychosocial treatments, often Cognitive Behavioural Psychotherapy, CBT (Van der Oord, Prins, Oosterlaan, & Emmelkamp, 2008).

ADHD and medication

Many children with ADHD are medicated with different psycho-stimulant medications. Methylphenidate and dextroamphetamine are two of the most common types, which have proved to be efficient in mitigating core behavioural symptoms of ADHD, as confirmed in a large number of randomized, placebo-controlled studies (Faraone & Buitelaar, 2010; Solanto, Arnsten, & Castellanos, 2001). A short review of this medical treatment is made because treatment with omega-3 fatty acid is sometimes seen as a treatment alternative to psycho stimulant treatment, moreover, treatment effects are commonly compared with the effects of psycho-stimulants.

Stimulants as methylphenidate and dextroamphetamines act by blocking dopamine and norepinephrine reuptake and the increased amount available at the synapses increase synaptic catecholamines and thereby affect neural circuits in the frontal lobes, basal ganglia and cerebellum (Biederman & Faraone, 2005). Among children with ADHD-C most respond well to medication and experience a reduction in behavioural symptoms, which begins almost immediately (Castellanos & Tannock, 2002; Van der Oord et al., 2008).

Comparisons of psychosocial treatment with methylphenidate and a combined condition with both psychosocial and medical treatment in a meta-study shows that all three treatment are effective in reducing core ADHD symptoms, but psychosocial treatment is less effective than psycho-stimulantia and the combined psychosocial and medication condition. Psychosocial treatment was defined as behavioural or cognitive behaviour therapy. None of the treatment types helped to improve academic performance. Psychosocial treatment is equally good for improving social behaviour and reducing comorbid ODD or CD symptoms as medication alone, or the combined medication psychosocial treatment (Van der Oord et al., 2008).

There is a general agreement in literature that methylphenidate improves cognitive abilities in children with ADHD (Barnett et al., 2001; Pietrzak, Mollica, Maruff, & Snyder, 2006). Improvements were demonstrated in planning/cognitive flexibility and attention/vigilance and inhibitory control in a large number of studies, and improvement in visuo-spatial working memory and divided attention in a lower amount of the reviewed studies (Barnett et al., 2001; de Jong et al., 2009; Pietrzak et al., 2006).

Investigations with neuroimaging techniques, such as electroencephalogram (EEG), event related potentials (ERP), or functional magnetic resonance imaging (fMRI), to find underlying mechanisms of medication in children with ADHD, indicates that catecholamines and prefrontal as well as anterior cingulate cortices are sites of actions for this type of medication (Pliszka, 2007).

ADHD and cognition

In addition to the problems with inattention, impulsivity and hyperactivity per se, cognitive deficits and executive deficits are common in ADHD. Children with ADHD are found to be impaired in working memory, divided attention and inhibitory tasks. These deficits are also central in neurocognitive models of ADHD (Barkely, 1997b; Castellanos & Tannock, 2002; Pennington & Ozonoff, 2006; Sonuga-Barke, 2005) and are some of the symptoms associated with ADHD, for example impulsiveness and inattention, are thought to arise from these cognitive deficits (Berlin, Bohlin, & Rydell, 2003; Castellanos & Tannock, 2002).

Children with ADHD often demonstrate deficiencies in some other cognitive abilities as well, i.e., fine and gross motor coordination (Kadesjö & Gillberg, 2001), verbal fluency (Grodzinsky & Diamond, 1992), and self-regulation of emotion (Berlin, Bohlin, Nyberg, & Janols, 2004; Maedgen & Carlson, 2000). Furthermore, children with ADHD have been demonstrated to be less sensitive than children with typical development to delayed rewards and to reinforcement, thus cognitive deficits will probably affect reinforcement processes further (see review Luman, Oosterlaan, & Sergeant, 2008).

Studies have found differences between the cognitive deficits in the three types of ADHD, i.e., both the inattentive subtype and the combined type demonstrate impairments of attention. The combined subtype however is in particular characterized by distractibility (Huang-Pollock et al., 2006) and the inattentive subtype by a slow cognitive tempo and start-up difficulties as well as lack of initiative (Lahey & Appelgate, 2001; Milich et al., 2001). Therefore, the nature between these two types has been discussed as qualitatively different. Children with the inattentive subtypes often have more internalizing symptoms, than the other subtypes. Further, symptoms of inattention predicted results in performance on both verbal and visual-spatial central executive tasks, while symptoms of hyperactivity/impulsivity did not. These findings were independent of age, verbal cognitive ability, and reading and language performance. They are also consistent with data implicating neuropsychological impairments in ADHD (Martinussen & Tannock, 2006).

Most cognitive deficits in children with ADHD are related to executive functions and in particular working memory, as discussed further below.

Intelligence in children with ADHD

Studies of intelligence in mixed groups of children with ADHD have generally shown lower mean IQ scores among these children than the normative mean (Doyle, Biederman, Seidman, Weber & Faroane, 2000; Barkley, 1990), even if others found no differences (MTA Cooperative group, 1999). The association between ADHD and IQ is relatively weak and corresponds to 2-8 IQ points (Dennis et al., 2009). WISC is by far the most commonly used test battery for assessing IQ in children with ADHD (Ellenberg & Kramer, 2008).

There are different opinions whether children with ADHD actually differ from other children regarding intelligence, for example studies seldom differentiate between subtypes

of ADHD or from co-morbid learning disorders (Ellenberg & Kramer, 2008). Furthermore, among the researchers that found lower IQ in children with ADHD many of them will refer this to low scores in some of the subtests (Schuck & Crinella, 2005). In the Wechsler scales these subtests are representing tasks more incident to executive functioning as attention and processing speed, e.g., in WISC-III a depressed ACID profile, Arithmetic, Coding, Information, and Digit span (Anastopoulos et al., 1994; Schuck & Crinella, 2005). These subtests are also among the subtests with the lowest g-load in the scales and in the new version, WISC-IV, they are not considered as measuring the global cognitive functioning but rather the supportive functions (Flanagan & Kaufman, 2009; Wechsler, 2003). Inattentive symptoms and IQ are related, the higher the levels of symptoms of inattention, the lower the IQ (Schuck & Crinella, 2005).

The close relationship between performance on IQ tests and specific deficits related to ADHD is important to consider when choosing tests to estimate IQ level in ADHD research. This close relation is indeed valid for the cognitive construct of executive functions.

ADHD and executive functions

Children with ADHD have been assumed to exhibit difficulties with executive functioning (e.g., Barkley, 1997a, 1997b; Pennington & Ozonoff, 1996) and this is also one of the most prominent neuropsychological theories of ADHD. Deficits in executive functions are often considered as core features of ADHD, and abilities of particular interest have been working memory, inhibition, or a more general vulnerability in executive control (Pennington & Ozonoff, 1996; Seidman, Biederman, Faraone, Weber, & Ouellette, 1997).

The hypothesis of executive deficits as core features of ADHD has received substantial support; most investigations have found that children with ADHD perform poorly on some executive tasks but not on others (see reviews; Pennington & Ozonoff, 1996; Sergeant, Geurts, & Oosterlaan, 2002; Geurts, Verte, Oosterlaan, Roeyers, & Sergeant, 2004). The deficits found are most commonly in the domains of working memory (including both spatial and verbal working memory), sustained attention and response inhibition. The extent of these deficits seems to vary from child to child (Nigg, 2006).

Several studies have shown relations between difficulties with inhibitory control and problems with inattention and hyperactivity (Berlin & Bohlin, 2002; Berlin, Bohlin, & Rydell, 2003; Thorell, Bohlin, & Rydell, 2004; Pennington & Ozonoff, 1996). Both the inattentive subtype and the combined subtype of ADHD, show inhibitory deficits relative to children with typical development, but in the stop-signal task children with the inattentive subtype are slow and show a cautious response style that probably has an impact on inhibitory functioning (Adams, Derefinko, Milich, & Fillmore, 2008). Further, they seem to have deficits in using feed-back from rewards and punishment for modulating their behaviour in order to improve their performance on inhibition tasks (Luman et al., 2008). Impulsiveness is often linked to the prefrontal cortex (Dalley, Mar, Economidou, & Robbins, 2008; Fuster, 2002), and, as described earlier, usually understood as a lack of response inhibition. This indicates that difficulties to withhold a previously rewarding response, will eventually lead to impulsive behaviour (Barkley,

1997b). In line with the response inhibition theory, the review by Pennington and Ozonoff (1996) also concluded that children with ADHD show deficits in motor inhibition.

Dopamine activity in the frontal lobes has been related to ADHD (Arnsten, 2009; Schuck & Crinella, 2005) and may have a direct impact on executive functions. Fatty acids have also been associated with dopamine activity in the same areas (Chalon, Vancassel, Zimmer, Guilloteau, & Durand, 2001), and is implicated as one of the explanatory models of why improvement can be seen in fatty acid supplementation studies.

Working memory in children with ADHD

In two of the studies in this thesis, measures of verbal and visuo-spatial working memory are included to investigate if supplementation with fatty acids has any effect. Working memory is discussed frequently in ADHD research; however, the findings have, up to this date, been inconsistent. An influential review has had great impact on the view of working memory deficits in children with ADHD (Pennington & Ozonoff, 1996). The overall conclusion in their review is that ADHD is associated with planning deficits (as assessed with different tower tests) but not with any kinds of deficits in verbal working memory.

More recently two meta-analyses reviewed Pennington and Ozonoff's research from 1996 (e.g., Martinussen et al., 2005; Willcutt et al., 2005). Both investigated verbal and spatial short-term and working memory. They also tried to evaluate the IQ effects and to control for co-morbid disorders such as learning disabilities. In their metaanalysis, Martinussen et al. (2005) came to the conclusion that there is a deficit in working memory for children with ADHD and that the deficits seen in working memory were larger for visuo-spatial than for verbal domains. Regarding verbal working memory Martinussen et al. (2005) found 16 studies with an moderate effect size ($d = 0.47$) for short-term memory and ($d=0.43$) for working memory, while Willcutt et al. (2005) (who used different criteria for inclusion), showed a moderate effect size ($d = 0.54$) for working memory in five of nine published studies. Thus, verbal working memory seems to be mildly affected in contrast to the results by Pennington and Ozonoff (1996).

Turning to spatial working memory, significant group differences with large effect size ($d = 0.72$) were found in five of six studies (Willcutt et al., 2005) while Martinussen et al. (2005) identified nine relevant studies with a larger effect size ($d = 0.85$) for spatial short-term memory. Spatial short-term memory therefore seems to be largely affected in children with ADHD.

Concerning planning, 26 relevant studies were included in which results varied depending on the measures used. The strongest effects were found for Tower of Hanoi with four of six studies showing significant group differences. For Tower of London, three of six had significant effects. However, Porteus mazes and Rey-Osterrieth complex figures showed only weak effects. So these effects are notable but if ADHD is related to deficits in the central executive function of spatial working memory deficits should be large in the spatial planning task (Willcutt et al., 2005).

The conclusions is that deficits in working memory were much larger for visuo-spatial than for verbal domains. One reason for this can be that spatial tasks tend to involve the

right hemisphere to a higher degree than do verbal tasks, and the right hemisphere has been implicated to be involved in the ADHD pathophysiology (Giedd, Blumenthal, Molloy, & Castellanos, 2001). For an extensive discussion of possible reasons for this difference, see Nigg (2006).

Almost all prior studies have used simple reverse span tasks (Martinussen et al., 2005). These tasks may not measure working memory, since they do not include both storage and processing design of the type recently suggested to be used in the working memory literature. Therefore, studies are needed that examine both working memory and cognitive suppression (updating) (Engelhart et al., 2008). On the other hand, suggestions have been made that since children's memory span is shorter, it may be that also the forward condition of a span task tap processing (since effortful processing is needed even at relatively simple tasks) and therefore becomes a measure of working memory rather than short-term memory. This might also be true for other populations with low memory capacity (Towse & Cowan, 2005).

One explanation suggested to why children with ADHD have difficulties with working memory is that the neuropsychological correlates associated with ADHD (as described earlier) in certain neurotransmitters, e.g., dopamine, and in neuroanatomical deviances, especially in the frontostriatal system coincide with the neural substrates that are connected with working memory (e.g., Frank, Scheres, & Sherman, 2007). Stimulant medications do increase dopaminergic activity, and medicated children with ADHD often respond well regarding working memory deficits (for a review, see Pietrzak et al., 2006). A comparison between children with ADHD with or without psycho-stimulant medication and an age-matched control group demonstrated that children with ADHD who received psycho-stimulants performed as well as the control group, whereas the un-medicated children with ADHD were impaired regarding spatial working memory (Barnett et al., 2001).

Theory of mind in children with ADHD

ToM ability has been associated with executive function both in typical and atypical child development (Carlson & Moses, 2001). Both ToM and executive dysfunction may be indicators of meta-cognitive deficits underlying peer and social problems in children (Fahie & Symons, 2003).

There are relatively few studies about ToM abilities in children with ADHD. In a study with several diagnostic groups of children, those with ADHD demonstrated lower second-order false-belief performance than children in the control groups (Buitelaar, Van Der Wees, Swaab-Barneveld, & Van Der Gaag, 1999). In a study comparing executive function and second-order false beliefs in preschool children (4–6 years of age) at high and low risk of developing ADHD. Children with high risk had lower scores on executive function tasks, but the groups did not differ regarding false-belief tasks (Perner, Lang, & Kloos, 2002).

More advanced ToM reasoning abilities as in second-order false belief or social understanding seem not to differ between children with ADHD and a control group of

children with typical development; however, the former tended to neglect mental states when high inhibitory demands were needed. This may imply that children with ADHD can understand mental states but are unable to use them correctly in demanding situations (Sodian & Hülksen, 2005).

To summarize the section on ADHD, studies indicate that children with ADHD as a group have relatively weak performance on various cognitive tasks. That is the case for tasks of verbal learning (particularly encoding), working memory (in particular visuo-spatial working memory), planning and organization, complex problem solving, and response inhibition (Pennington & Ozonoff 1996; Seidman, 2006). Scores on intelligence tasks are often lower but may depend on the cognitive deficits in attention and working memory per se. ADHD is associated with some neuropsychological correlates, both deviations in certain neurotransmitters (f.ex. dopamine) and also with anatomical deviations particularly in the frontostriatal system. These deviations coincide with the neural substrates that are connected with working memory and are discussed as the explanation to why children with ADHD have difficulties with working memory.

FATTY ACIDS AND COGNITION

Dietary fatty acids

There are two groups of essential polyunsaturated fatty acids (PUFA), the n-6 (omega-6) fatty acids and the n-3 (omega-3) fatty acids. The omega-6 fatty acids come primarily from plants and the omega-3 come primarily from fish (Innis, 2008). Both omega-3 and omega-6 fatty acids are essential for health, and the highly unsaturated fatty acids, which have longer molecular chains (LCPUFA) of each group, are highly active and concentrated in the brain (Bourre, 2006; Heinrichs, 2010). By definition, LCPUFAs are those with 20 or more carbon atoms, in this thesis this term is used, even if the term highly unsaturated fatty acids (HUFA) and very long polyunsaturated fatty acids (VLCPUFA) are occurring in the literature as well. The omega-3 and omega-6 series are presented with their trivial names as well as their biochemical names, (lipid numbers), in Figure 1.

Omega-6 fatty acids primarily from vegetable oils	Omega-3 fatty acids primarily from fish oils
LA C18:2 n-6 Linoleic acid <i>Precursor for the omega-6 series</i>	LNA C18:3 n-3 Alfa-linolenic acid <i>Precursor for the omega-3 series</i>
GLA C18:3 n-6 Gamma-linolenic acid (also called LNA n-6)	SDA C18:4 n-3 Stearidonic acid
DHGLA C20:3 n-6 Dihomo-GLA	ETA C20:4 n-3 Eicosatetraenoic acid
AA C20:4 n-6 Arachidonic acid	EPA C20:5 n-3 Eicosapentaenoic acid
C22:4 n-6	DPA C22:5n-3 Docosapentaenoic acid
C22:5 n-6 Osbond acid	DHA C22:6n-3 Docosahexaenoic acid

Figure 1. Omega-3 and omega-6 fatty acid series.

A number of nutritional and genetic studies have indicated that the human diet has changed since the Paleolithic period (Old Stone Age, dated to about 2.5 million to 20000 years ago), and especially during the last century. It has changed both in type and amount of fatty acid intake (Heinrichs, 2010; Innis, 2008). The diet during the Paleolithic period contained about equal amounts of omega-6 and omega-3, and it has been suggested that this balanced diet played a role in the enlargement of the brain and also in the development of the human intellect (Simopoulos, 1999, 2002). More modern diet, on the other hand, has largely increased the amount of vegetable oils rich in omega-6, and at the same time omega-3 fats from fatty fish have decreased (Innis & Jacobson, 2007).

Furthermore, our intake of fatty fish is not only decreasing, the fish we consume also contains different amounts of omega-3. Alasalvar, Taylor, Zubcov, Shahidi, and Alexis, (2002) found that the percentages of two important fatty acids from the omega-3 family, Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) were significantly lower in farm raised sea bass lipids than in wild caught fish, which is in agreement with earlier findings (van Vliet & Katan, 1990) and also that the ratio of omega-3 to omega-6 fatty acids was higher in wild than in cultured sea bass (Alasalvar et al., 2002). The result is that our modern diet, despite being high in calories, is often low in omega-3 fatty acids (Heinrichs, 2010; Innis, 2008; Simopoulos, 1999, 2009). The longest fatty acids in each series, the long-chain polyunsaturated fatty acids (LCPUFAs) are produced from the short “parent” essential fatty acids, linoleic acid (LA) from the omega-6 series, and alpha-linolenic acid (LNA) from the omega-3 series. Humans are not able to synthesize the parent omega-6 and omega-3 PUFAs (LA and LNA), which must be supplied by food or supplements (Bourre, 2005, 2006a).

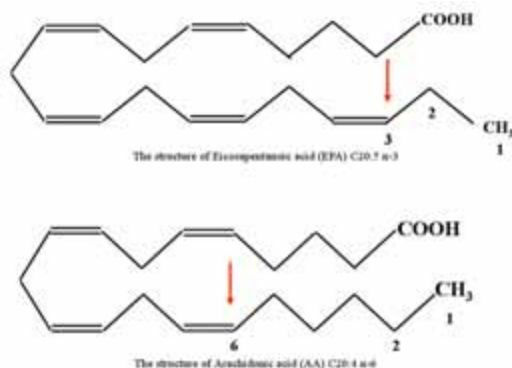


Figure 2. Upper, the omega-6 polyunsaturated fatty acid, AA, the array shows the location of the first double bond. Lower, the omega-3 polyunsaturated fatty acid, EPA, the array shows the location of the first double bond.

The two PUFA families compete for the same metabolic enzymes (Hornstra, 2000), that is, the same series of enzymes (delta-desaturases and elongates) are involved in the incorporation of double bonds and elongation of both the n-6 and n-3 fatty acids. In Figure 2 the location of the first double bonds for AA and EPA, respectively, are shown. Figure 3, shows the different steps in the metabolic chain and the desaturate- and elongate-systems. High activity in the later steps in these metabolic chains is indicating higher levels of the longest PUFAs, and has been used as a marker for a beneficial PUFA status (Mahadik et al., 1996). Due to the competition for the same elongated enzymes, the ratio between omega-6 and omega-3 is of particular interest. A deficient intake of omega-3 or excessive intake of omega-6 would therefore affect the metabolism to favour omega-6 fatty acids and thus further diminish the availability of both DHA and EPA in the brain (Novak, Dyer, & Innis, 2008).

	Omega-6 series (n-6) Vegetable oils		Omega-3-series (n-3) Fish oils
Steps in the fatty acid metabolic chain	LA C18:2 n-6 Linoleic acid	Essential fatty acids	ALA/LNA C18:3 n-3 Alfa-linolenic acid
<i>Step 1</i>		<i>d-6-Desaturation</i>	
	GLA C18:3 n-6 Gamma-linoleic acid	Omega-6 and omega-3 fatty acids compete for the same series of enzymes	SDA C18:4 n-3 Stearidonic acid
<i>Step 2</i>		<i>Elongation</i>	
	DHGLA C20:3 n-6 Dihomo-GLA		ETA C20:4 n-3 Eicosatetraenoic acid Present in breast-milk
<i>Step 3</i>		<i>d-5-Desaturation</i>	
	AA C20:4 n-6 Arachidonic acid Membrane structure	Anti-inflammatory Eicosanoids	EPA , C20:5 n-3 Eicosapentaenoic acid
<i>Step 4</i>		<i>Elongation</i>	
	C22:4 n-6 Adrenic acid		DPA , C22:5 n-3 Docosapentaenoic acid
<i>Step 5</i>		<i>Elongation + d-6-Desaturation + β-oxidation</i>	
	C22:5 n-6 Osbond acid		DHA , C22:6 n-3 Docosahexaenoic acid Membrane structure

Figure 3. Showing pathways for conversion of omega-3 and omega-6 fatty acids and the biosynthesis of these fatty acids.

There is a link between socio-economic back-ground and measured PUFAs, in the sense that poorer socio-economic groups eat low quality food to a larger extent than those with higher socio-economic status and have lower levels of LCPUFAs. Not only do they eat less healthy food, for instance, more refined grains, and added sugar, but they also eat less fish and vegetables (Darmon & Drwonowski, 2008; Kirby, Woodward, Jackson, Wang, & Crawford, 2010). The same pattern seems to be true in all of Europe (Irala-Estevez, Groth, Johansson, Oltersdorf, Prättälä, & Martinez-González, 2000), and there are no reasons to believe Sweden would be an exception.

Fatty acids and the brain

LCPUFAs are important structural elements of cell membranes and, therefore, essential in the formation of new tissue, including neurons and glia cells (Hornstra, 2000, Martinez, 1992). LCPUFAs are also involved in axonal myelination, and they are key components in synaptic functions where they serve as second messengers (Durand, Antoine, & Couet, 1999; Uauy, Mena, & Rojas, 2000). They are associated with neurotransmitter functioning and facilitate release of neurotransmitters, such as dopamine, serotonin, and nor-epinephrine. They also regulate gene transcription and are precursors of pro-inflammatory and anti-inflammatory molecular families (Chalon et al., 2001; Marszalek & Lodish, 2005; Stahl, 2000). Thus, fatty acids can profoundly influence key aspects of cell signalling in brain and body (Bourre et al., 1989; Kurlak & Stephenson, 1999; Yehuda, Rabinowitz, & Mostofsky, 1999).

The most prevalent LCPUFA in the brain is Docosahexaenoic acid (DHA) from the omega-3 group, which is concentrated to nerve cell synapses and important for neural cell signalling and neurotransmitter processes (Bourre, 2004; Yehuda et al., 1999; Stahl, 2000). Another important omega-3 fatty acid is EPA, which is the precursor for DHA; it plays an important role in the brain and is involved in the daily function of the brain. Further, EPA helps making substances, such as prostaglandins, which are crucial for proper signalling between cells (Innis, 2007b). The most important LCPUFA in the omega-6 series is arachidonic acid (AA), which also works as a signal transmitter. It is as the omega-3 fatty acids mentioned also of importance for neural cell signalling (Heinrich, 2010). Recently, also the importance for these fatty acids of the glia cells has been demonstrated (Joardar, Sen, & Das, 2006).

It is generally assumed that the shorter omega-6, Linoleic acid (LA), and the shorter omega-3, Alfa-linoleic acid (ALA), will be converted by humans into the longer DHA and AA, respectively. However, there is reason to doubt whether conversion of dietary EFA is sufficient to reach optimal DHA and AA requirements. Some studies have demonstrated that conversion of ALA to EPA and DHA synthesis is marginal in adults, probably below 5% (Brenna, 2002; Pawlosky, Hibbeln, Novotny, & Salem, 2001; Salem, Pawlosky, Wegher, & Hibbeln, 1999). Regarding infants it is important to be able to properly convert LA and ALA to LCPUFAs in order to cover the needs for brain development, but there seems to be a wide variability among both term and preterm infants to make this conversion (Brenna, 2002). Furthermore, the conversion has been suggested to be even

more inefficient for males where sex hormones are probably related to this effect (Childs, Romeau-Nadal, Burdge, & Calder, 2008).

It is suggested by researchers that the ratio between the omega-6 and omega-3 fatty acids, that is, the relative proportion of the fatty acids, is more important than the concentrations alone (Hulbert, 2003; Joardar et al., 2006; Stevens et al. 2003; Yehuda, 2003). One ratio of particular interest is the AA/DHA ratio, which is important at a cellular level. Hulbert (2003) points at its importance for membrane fluidity, and Joardar et al. (2006) points at the importance of the same ratio for glia cells. The reason why the ratio between omega-6 and omega-3 may be of particular interest is due to their competition for the same desaturase and elongase enzyme systems. Therefore, it is important to note that deficient intake of omega-3 or excessive intake of omega-6 fatty acids can affect the metabolism to prioritizing omega-6 fatty acids and, thus, further decrease the availability of both DHA and EPA in the brain (Hulbert, 2003; Simopoulos, 2009).

Fatty acids in development

In early life, plenty of DHA is needed for the growing brain. DHA is important for construction and structure of brain is especially important during the third trimester of pregnancy and the first two years of life, when brain growth is rapid (Bourre, 2006; Heinrich, 2010, Innis, 2008). The developing foetus and new born children are not able to metabolize important structural fatty acids as DHA, but depends completely on the availability of maternal essential fatty acids from maternal stores via the placenta during pregnancy and breast-milk during infancy (Guesnet et al., 1999). It is therefore a strong positive correlation between PUFA intake in the mother and PUFA status of the new-born child (Uauy et al., 2000).

In understanding the influence of fatty acids on brain and cognitive development, it is important to consider the timing of their delivery in relation to critical or sensitive periods during brain development (Georgieff, 2007; Lucas, 1998). Plasticity of the brain provides protection from external influences as under-nutrition, and allows the brain to adapt to such influences. The plasticity thus, seems to depend on the timing, duration and severity of the incident experienced. Effects from nutrient deficiencies are worse if they occur during rapid growth or sensitive periods. Early mal-nourishment in humans could result in delayed development and may have long term effects on cognitive function (Lucas, 1998; Santos de Souza, Spreafico Fernandes, & das Graças Tavares do Carmo, 2011).

The effect of a diet severely restricted in omega-3 fatty acids is well studied in animals. Research has found altered metabolism of several neurotransmitters, including dopamine and serotonin, and also changes in receptor activities (Innis, 2007b). In non-human primates raised with omega-3 fatty acid deficient diet, several researchers found deficits in behavioural tasks of learning (Bourre et al., 1989, Innis, 2007b; Wainwright, 2002). This indicates the importance of omega-3 fatty acids for development of motor and cognitive functions in human infants. When it comes to studying omega-3 deficiency in humans it is more difficult, not least due to ethical considerations. In the thesis I have a few references to animal studies, but even if this kind of research can give insights into the mechanisms

by which fatty acids affect brain development and function, there are also difficulties. For example, inferences on biochemical aspects and LCPUFA levels, and their extrapolation to humans, are not always evident since the animal species develop and mature different and at varying rates from human development.

Another perspective investigating LCPUFA deficiency is studies from developing countries that suggest that under-nutrition is associated with problems of cognition and behaviour, and with long-term consequences (Grantham-McGregor & Baker-Henningham, 2005). A review concluded that attentional deficits, higher rates of aggressive behaviour and global cognitive deficits were more common among malnourished children when long-term consequences were investigated. However, a dilemma in research in malnutrition is the large number of possible confounders, both social and biological (Eilander, Hundscheid, Osendarp, Transler, & Zock, 2007). Supplementation studies for under-nourished children in the same population have shown significant beneficial effects (Grantham-McGregor & Baker-Henningham, 2005). Lack of omega-3 fatty acids has furthermore been seen in a range of neurodevelopmental disorders. The suggestion is that at least some features of ADHD may reflect an underlying abnormality of fatty acid metabolism (Richardson, 2004, Richardson & Puri, 2002).

LCPUFA supply thus, seems to be essential for normal growth neurological development, and related to functional maturation, including learning and behaviour (Hornstra, 2000; Innis, 2008). Throughout life, adequate supplies of LCPUFA remain essential for optimal brain function. They increase the fluidity of neuronal membranes, which are essential for efficient signal transduction, and certain LCPUFAs - notably AA and EPA - act as second messengers in chemical neurotransmitter systems, as well as contributing to numerous other aspects of signal transduction and neuronal function (Mirnikjoo, Brown et al., 2001; Yehuda et al., 1999). DHA has been implicated in both learning and memory, however underlying cellular mechanisms is not clearly understood. DHA is involved in myelination (Durand et al., 1999) and is important for cell signalling, and theoretically, improvements in synaptic efficiency (Uauy, Mena, & Rojas, 2000) and speed of transmission (Yehuda et al., 1999), could increase the efficiency of processing information.

The impact on PUFAs on specific cognitive abilities is not elucidated. To start, it has been suggested that the effects of PUFAs on visual development, visual perception, and even dyslectic problems may depend on several mechanisms. LCPUFAs, and especially DHA have been found to improve maturation of rod photoreceptor function and visual acuity as well as normal retinal development in humans (Birch, Birch, Hoffman, & Uauy, 1992; Birch, Hoffman, Uauy, Birch, & Prestidge, 1998). Furthermore, visual evoked potentials in infants may be improved by supplementation of fatty acids (Birch, Garfield, Hoffman, Uauy, & Birch, 2000)

Regarding DHA and its importance for memory, one suggestion based on animal research, is that DHA significantly affects hippocampal neuronal development and synaptic function in developing hippocampi (Cao et al., 2009). In neurons supplemented with DHA the spontaneous synaptic activity was significantly larger, this seemed to depend on

enhanced glutamatergic synaptic activity. Further, rat foetuses deprived from DHA showed inhibited neurite growth and synaptogenesis in hippocampal neurons. These findings may represent particular cellular aspects associated to the hippocampus related cognitive processes that is enhanced by DHA. These aspects may be important mechanism explaining why dietary deficiencies of omega-3 fatty acids are associated with learning disabilities and memory deficits (Cao et al., 2009). A conclusion suggested (Chetham et al., 2006) is that speed of which information is acquired and perceived, at least to some extent, depends on the presence of DHA. DHA is also important for the development of mature synapses (Willatts & Forsythe, 2000) processes that underlie cognitive development, which may have impact on problem-solving and visual attention.

There are few studies examining PUFA among typically developed school children. One study (Kirby et al., 2010), investigating 400 children with typical development with cheek cell samples of PUFA levels, found weak but significant relationships between PUFA levels and parent and teacher ratings of behaviour with SNAP and . They found no differences in a cognitive test battery (Kirby et al., 2010). Several investigations of correlations between fatty acids and working memory have been made both in children with typical development (Kirby et al., 2010), but no evidence for associations with omega-3 levels were found.

This brief review of the field of fatty acids and supplementation in children with typical development leads to the conclusion that it is currently unclear whether LCPUFA supplementation affects neurodevelopmental outcome at school age and beyond, but interestingly there are indications that when all intervention studies are taken together, most positive effects of the LCPUFA supplementation on cognitive development were detected by general tests of intelligence. The supply of fatty acids is as previous mentioned depending on availability via placenta during pregnancy and breast-milk during infancy. Breast-feeding and breast-milk is an important issue in this thesis and a section on this topic follows.

Breast-feeding, breast-milk, fatty acids, and cognition

Breast feeding has been related to infant health and nutrition and associated with enhancement of later cognitive ability in childhood (Anderson et al., 1999; Oddy et al., 2004) as well as educational achievement into adulthood (Horwood & Ferguson, 1998; Victora et al., 2005). Some researchers have focused on attachment and bonding between mother and infant as the important factor explaining this relation (Britton, Britton, & Gronwaldt, 2006; Klaus, 1998; Renfrew, Lang, & Woolridge, 2000). Britton et al. (2006) investigated the relationship between breast-feeding, sensitivity in mothers, and attachment security. They found a relation between breast-feeding and maternal sensitivity, which is interpreted by the authors as establishing a relation between attachment theory and infant feeding behaviours; however, they could not show any direct link between attachment security and breast-feeding.

This relation between breast feeding and attachment may affect the performance on tests of intelligence in several ways. Performance on intelligence tests is positively related to the stimulation the child receives during childhood, and mothers who choose to breast-

feed their child may be more prone to stimulate it. In addition, breast-feeding is more common in well-educated groups, and parental education and parents' IQ may be confounding factors (Jacobson & Jacobson, 2002; Rey, 2003; Uauy & Peirano, 1999). One classic study demonstrated that in a sample where children had increased cognitive outcomes on a range of factors (e.g., reading, mathematics, teacher ratings) the advantage of breast-fed children in part was explained by socio-economic factors. Children with higher results on the cognitive outcome measures were more often born into socially advantaged families characterized by mothers who were older, had higher levels of education, did not smoke, had higher socio-economic status, and higher income. But still after adjustment of these factors, increasing duration of breast-feeding was associated with significant better results on the cognitive outcome factors (Horwood & Fergusson, 1998).

Although often promoted as a benefit of breast-feeding, there is little support for the assertion that the bonding itself enhances performance on cognitive tests, instead interest has turned to nutrients, in particular bioactive ingredients, like immune-regulatory proteins and fatty acids, as contributors to this relation (Petherick, 2010). An important step towards the findings of the importance of nutrition was a randomized trial by Lucas, Morley, Cole, Lister, and Leeson-Payne, (1992). They randomized children to receive either breast-milk or formula, and found significantly better results on the Wechsler scales for the children who received breast-milk. The randomized design makes the results unlikely to be affected by confounding factors as bonding, and it was concluded that it was the nutritional composition that had effect. Studies at the same time period demonstrated that infants fed with formulas had lower levels of LCPUFAs in the cerebral cortex compared to children who had been breast-fed (Farquharson, Jamieson, Abbasi, Patrick, Logan, & Cockburn, 1995).

Another important contribution was a meta-analysis, which showed that breast-feeding was associated with significantly higher intelligence quotients than was formula feeding (Anderson et al., 1999). These results remained even after adjustment for confounding variables known to affect intelligence, such as parental education, socio-economic status, birth weight, gestation age and parental smoking. This strengthened the explanation of breast-milk as a provider of important nutrients required for brain development. Long-chain polyunsaturated fatty acids have been pointed out as especially important (Gibson et al., 1996; Innis, 2008).

A cluster randomized trial in Belarus, the PROBIT (Promotion of Breast-feeding Intervention Trial), showed that children whose mothers took part in a WHO programme, with the intention to support breast-feeding, had significantly higher IQ scores at the age of 6.5 years than children whose mothers did not participate (Kramer et al., 2008). The former children had an average of 5.2 IQ scores higher than the latter. By its large sample size, 13889 children, this study contributes uniquely to the support for breast-feeding as positively related to cognitive performance.

Standard formulas used for children not being breast-fed, have classically only been containing the precursors for essential fatty acids (EFA). The precursor for omega-3 is alpha-linolenic acid (ALA) and for omega-6 linoleic acid (LA), as mentioned above. This

means that the infants have to be able to synthesize the necessary LCPUFAs DHA and AA, respectively, on their own. Some studies have indicated that both preterm and term infants fed with this formula have had significantly less DHA and AA in their erythrocytes than breast-fed children. Women with a diet rich in omega-3 LCPUFAs have higher milk DHA levels compared with those with diet low in these fatty acids (Yuhas, Pramuk, & Lien, 2006). In many countries, as in Sweden, most of the formulas are now supplemented with LCPUFAs. Studies on supplemented children often compare formulas with different mixes and dosages of PUFAs; this leads us to the next chapter on supplementation studies.

PUFA supplementation and cognition

More information about the functional importance of omega-3 fatty acids in human brains and for infant development relies mostly on studies of feeding formulas with or without DHA (Innis, 2008). A Cochrane report summarizes 17 studies of LCPUFA supplementation of premature infants and authors conclude that there is not enough evidence to show long-term consequences on cognitive development (Schulzke, Patole, & Simmer, 2011). It is suggested that effects of LCPUFA supplementation may be larger if the time period for supplementation were to be extended). It is necessary to mention that most studies addressing long-term outcomes made assessments when children were between 6 and 24 months of age, and another suggestion is that it is too early to detect cognitive effects and that possible effects can be better detected later, when cognitive tests are more sensitive and reliable (Eilander et al., 2007; Hadders-Algra 2005).

For supplementing term infants, with LCPUFAs in high doses (100 mg DHA and 200 mg AA per day), there is consistent evidence for a beneficial effect on visual development during the first year of life (Eilander et al., 2007). However, findings on cognitive outcomes are not as consistent. A meta-analysis by Makrides et al. (2005) of children born full term did not find any cognitive differences in children supplemented with a formula of DHA and AA compared to those supplemented with a formula with only DHA either. These findings were in contrast to findings from an earlier review of the findings concerning formulas supplemented with DHA, which showed better visual acuity in term infants at two months of age (SanGiovanni, Berkey, Dwyer, & Colditz, 2000). The latest Cochrane review on term infants analyzed twenty randomized studies and found no beneficial effects on LCPUFA supplementation of formula milk in either visual, physical or cognitive outcomes either. The researchers conclude that there is no evidence for suggesting routine supplementation with LCPUFA (Simmer, Patole, & Rao, 2008).

The evidence for the potential benefits of DHA and EPA supplementation in healthy children older than 2 years of age is generally promising, but not yet conclusive (Eilander et al., 2007). There were no significant effects from a DHA infant formula on visual-motor function, IQ or language assessments when children were 39 months of age (Auestad, Scott, & Janowsky, 2003). Another study among older children (10-12 years of age), who were provided 400 or 800 mg supplemental DHA per day for 8 weeks did not find any significant beneficial effect on brain function in healthy children (Kennedy et al., 2009). However, by supplementing healthy 4-year-old children with 400 mg DHA (or placebo) per day for four months, the higher blood levels of DHA, which resulted were

significantly and positively associated with improved scores on the Peabody Picture Vocabulary Test (a test of listening comprehension for the spoken word in Standard English) (Ryan & Nelson, 2008).

To conclude this section, there are only a few “gold standard” studies, using double-blind placebo controlled designs. Results have often been inconclusive with reported positive as well as negative effects. Interesting in this field is that review articles in some sub-areas are almost as numerous as the experimental articles they rely on. It is also important to keep in mind that genetic variations in genes encoding fatty acid desaturases also probably influences fatty acid metabolism and may have impact on individual requirements in different children (Innis, 2008).

Fatty acids in neurodevelopmental disorders in clinical populations

The idea that PUFAs may also play an important role in child mental health was raised by, amongst others, Horrobin (1998). He suggested the possibility that early neurodevelopmental defects present before the onset of psychiatric illness may also be related to an inherited abnormality of cell membrane PUFAs. Thus, the membrane phospholipid hypothesis has more recently been extended to children with developmental disorders (e.g., ADHD, dyslexia, and autism spectrum disorders).

Fatty acid deficiency has been proposed as contributing to several disorders, one of them being dyslexia. Phospholipids have shown to be abnormal in dyslectics brains as opposed to normal brains (Richardson, Cox, Sargentoni, & Puri, 1997). There is furthermore some genetic and neurological evidence that there is a difference in the development of magnocellular systems in dyslectics' brains (Stein, 2000). There also seems to be some possible evidence that supplementation can help alleviate aspects of dyslexia (Richardson et al., 2000; Richardson & Puri, 2002; Stordy, 2000; Taylor et al. 2000). Others have found no support for EPA treatment (Kairaluoma, Närhi, Ahonen, Westerholm, & Aro, 2008).

The conclusion that omega-3 fatty acids may be an effective and well-tolerated treatment, in particular of hyperactive behaviours including disobedience, distractibility, and impulsivity, in children with autism was made in a small pilot study (Amninger et al., 2007). The efficacy of omega-3 fatty acids is supported by an analysis of phospholipids in the plasma of autistic children compared with that of mentally retarded children, showing DHA and total omega-3 levels significantly reduced in children with autistic disorders (Vancassel, Durand, & Barthélémy, 2001).

Clinical trials with fatty acid supplementation for children with ADHD

In this section, a review of supplementation studies for children with ADHD is presented. Clinical trials using omega-3 or a mix of omega-3 and omega-6 supplements during the last decade are included. The aim with this review is to get a picture of the field and to be able to discuss the complexity and diversity of the results, there is no attempt to be extensive, comprehensive or covering all trials conducted.

An early, randomised placebo-controlled double-blind trial of omega-3 fatty acid treatment in children with ADHD involved a supplement of fish oil, supplying mainly omega-3 fatty acids (80 mg EPA, 480 mg DHA, 96 mg GLA, 40 mg AA, and 24 mg alpha-tocopherol acetate) were first published 1995 (Stevens et al., 1995). Fifty children with clinical signs of fatty acid deficiency were included. They did not have a formal ADHD diagnosis, but were according to their parents under the care of a clinician for ADHD. Preliminary data were reported (Burgess, 2000) with the findings that active treatment led to changes in blood fatty acid status. An increase was obtained in the proportion of EPA and DHA in both plasma and red cell membranes. AA increased in plasma, but decreased in membranes, also the omega-6 fatty acids decreased in proportion in the membranes. Active treatment, but not placebo treatment led to a reduction in the ratio of omega-6 to omega-3 fatty acids in both red cells and plasma. The whole study was published in 2003, (Stevens et al., 2003) showing that treatment for four months resulted in significant improvement on two measures, i.e., conduct problems rated by parents and attention symptoms rated by teachers. However, concerning the 14 other outcome measures the differences were not significant. One interesting finding was that oppositional defiant behaviour dropped from clinical to sub-clinical rates in significantly more children receiving active treatment.

Another RCT found supplementation with DHA to be ineffective in children with ADHD (Voigt et al., 2001). This study involved sixty-three school-aged children (6-12 years old) with diagnosed ADHD. The children were treated for four months with DHA (345 mg/day) or placebo. Several of these children were on psycho-stimulant medication, but had a wash-out period of 24 hours before assessment. Outcome measures were parent-rated symptoms and computerized measures of impulsivity and inattention. There were no significant differences between the groups in any of the rating scales or on the computerized test. In fact, the effect of the treatment with DHA even seemed to be lower scores on several outcome measures than for the control group. Voigt et al. (2001) also showed that the children with ADHD had lower levels of plasma phospholipids DHA at baseline than the control group. The treatment with DHA increased the plasma phospholipids DHA content significantly.

Furthermore, yet another DHA supplementation study of children with ADHD was not able to demonstrate any significant effects (Hirayama, Hamazaki, & Terasawa, 2004). This placebo-controlled double-blind study included 40 children with ADHD (including eight ADHD-suspected) children 6-12 years of age. A few of them had simultaneous psycho-stimulant medication. The children received active foods containing omega-3 (3600 mg DHA/week and 700 mg EPA/week) during two months. The control group had control foods without omega-3, but with olive oil (about 9g/week). Assessment included parents and teacher ratings of ADHD-related symptoms according to DSM-IV. Other outcome measures were visual perception, visual and auditory short-term memory, visual-motor integration, CPT tasks, and impatience test. For the visual short-term memory and errors of commission (CPT) the control group showed significantly better results than the active foods group, no other differences were found.

The negative findings by Voigt et al. (2001) and Hirayama et al. (2004) are consistent with other evidence that DHA may not be an important omega-3 fatty acid for the treatment of cognition or behaviour (Peet, Brind, Ramchand, Shah, & Vankar, 2001). DHA alone has been suggested to be ineffective and other fatty acids, especially EPA, may account for the positive findings by Stevens et al. (2003) as suggested by Richardson and Puri (2002). Besides, the study by Hirayama et al. (2004) did not include biochemical measures and had short duration, which limits conclusions.

There are further differences in subject selection; Stevens et al. (2003) used criteria based on fatty acid deficiency while Voigt et al. (2001) excluded children with any other disorders to ensure that the sample comprised only pure ADHD diagnoses. Some studies also included children with on-going psycho-stimulant medication.

One study with a different approach was a study with 41 school-aged children (8-12 years old), all diagnosed with specific learning difficulties. The group consisted mainly of dyslectics with above-average scores on all three global ADHD scales from the CPRS-L. Children were randomized to either treatment during 12 weeks of supplementation with omega-3 fatty acids (186 mg EPA, 480 mg DHA) and omega-6 fatty acids (96 mg GLA, 864 mg LA and 42 mg AA) or to a placebo-group given olive oil (Richardson & Puri, 2002). At 12 weeks there was a one-way cross-over, (the placebo group received active treatment), and both groups received active treatment for a further 12 weeks. Assessment was performed with Conners' rating scale scores, measured at baseline, after 12 and after 24 weeks. No biochemical measures were included. After 12 weeks the children with EPA supplementation had significantly lower scores on inattention, anxiety, and general behaviour problems in CPRS than the control group. After 24 weeks, the cross-over group showed significant improvement in 9 of 14 scales. The group continuing with LCPUFA treatment maintained the improvement or improved further.

In the Oxford-Durham study, (Richardson & Montgomery, 2005), 117 schoolchildren with developmental coordination disorder (DCD) were supplemented with the same combination of omega-3/omega-6 supplements as in the Richardson and Puri study (2002). They found significant reductions in teacher ratings of ADHD symptoms on almost all of the subscales in Conners' teacher rating scales. Moreover, the results showed improvements in both reading and spelling in children receiving EPA treatment, but not in motor function.

Harding, Judah, and Gant (2003) performed a small outcome-based comparison of Ritalin versus food-supplement treated children with ADHD. The supplements were Salmon oil 1000 mg (180 mg EPA and 120 mg DHA) and Borage oil 200 mg (45 mg GLA) with added vitamins, minerals and amino acids. Twenty children diagnosed with ADHD, 7-12 years of age, were included. The children were divided into two groups either receiving four weeks of treatment with fatty acid supplements or Ritalin. Unfortunately, the design was not randomized; instead parents chose which group their child would be placed in. Outcome measures were CPRS:L and a CPT test. Children in both groups showed significant gains on the CPT's full scale, but not on rating scales. This small non-randomized non-controlled trial must be interpreted with caution.

A larger RCT study involved 132 children with high scores on Conners' ADHD index, but lacking formal ADHD diagnosis (Sinn & Bryan, 2007). The children were treated with a combination of omega-6/omega-3 supplement and significant positive treatment outcomes were found on CPRS of core ADHD symptoms, but not on CTRS. Cognitive outcomes were investigated with measures of IQ, verbal learning, working memory (i.e., digit span backwards) and speed of processing from WISC, learning and memory from Rey's auditory verbal learning test (RAVLT), attention, measured with Creature counting from a test of everyday attention for children, ability to inhibit responses with the knock- and tap-test from NEPSY, and distractibility with the Stroop task (Sinn et al., 2008).

According to these outcomes the ability to switch and control attention, as measured on the Creature counting test was significantly improved in the PUFA treated group compared to the placebo group (effect size with Cohen's $d = 0.43$). To summarize the findings from the whole study there were significant positive improvements on the reduction of ADHD symptoms in parents' ratings and limited evidence of positive treatment effects on cognition, with significant positive effect on an attention task.

Fatty acid trials in adolescents are rare, but one study involved adolescents with formally diagnosed ADHD and found that abnormalities in the adolescents were similar to those in younger children. They had lower levels of DHA and total omega-3 fatty acids and higher levels of omega-6 level. The omega-3 status correlated with the scores on some of the Conners scales, implying that lower levels of omega-3 were associated with more severe behavioural problems as measured with Conners (Colter, Cutler & Meckling, 2008).

An RCT study from Sweden with the same combination of omega-6/omega-3 mix as several earlier trials (Richardson & Puri, 2002; Richardson & Montgomery, 2005; Sinn & Bryan, 2008) included 75 children and adolescents (8-18 years old) with formally diagnosed ADHD (almost 50% inattentive subtype). For the whole group of children the results were negative, but a subgroup of boys with ADHD inattentive subtype tended to respond to the treatment (Johnson et al., 2009). About one in eight of the whole group showed considerable clinical improvements (more than 50% reduction in ADHD symptoms). Children with co-morbidity with DCD, reading and writing difficulties, and autistic symptoms were more likely to improve from the PUFA treatment (Johnson et al., 2009).

A trial in school-aged children (7-13 years of age) with formal ADHD-diagnosis used another omega-6/omega-3 formula, (a mix with AA and ALA) or placebo (vitamin C) and a shorter treatment period (7 weeks). The DSM-IV questionnaire for ADHD symptoms (inattention score and hyperactivity/impulsivity score) was filled in by parents, while teachers completed Conners Abbreviated Questionnaire (ADHD score and mood score). Children performed the CPT test, Test of Variables of Attention (TOVA, registering errors of omission, errors of commission, response time, and response-time variability). This study did not find any positive or negative effects of the intervention compared to the placebo (Raz, Carasso, & Yehuda, 2009).

School-aged children were also included in a trial with omega-3 doses higher than in previous studies and with consideration taken of children's weights (20 mg/kg/day to 25 mg/kg/day of EPA and 8.5 mg/kg/day to 10.5 mg/kg/day of D). The 26 included children had formal ADHD diagnoses and were randomized to the EPA and DHA supplement or placebo for treatment during an eight week period (Belanger, 2009). A one-way cross-over design was used and both groups were treated for another eight weeks. CPRS showed a statistically significant improvement in some subscales at eight weeks in the EPA and DHA group relative to the placebo group. Other measured parameters did not show differences between the omega-3 and placebo groups.

The last study reviewed in this section, is an RCT-study among formally diagnosed children with ADHD and with inattentive problems. These children were school aged (8-13 years old) and were randomized to three months of treatment with either phospholipids (156 mg EPA, 95 mg DHA in phospholipid form, 300 mg phosphatidylserine), fish oils (153 mg EPA, 96 mg DHA in triglyceride form, that is, similar to previous studies), or placebo (150 mg LA). CPRS short version and CBCL were scored by parents, and performance on TOVA was evaluated for children before and after treatment. Comparing the fish oil treatment to the placebo treatment shows that the number of children reaching the normal range on TOVA increased significantly. Intervention diminished errors of commission and improved response-times, and response-time variability, compared to the placebo group. There were no effects found regarding the rating scales. Children supplemented with fatty acids increased their EPA, DPA, and DHA concentrations, while AA and adrenic acid concentrations in plasma phospholipids decreased (Vaisman et al., 2008).

To summarize all these fatty acid trials in children, most of the reviewed studies used supplements with a combination of omega-3 and omega-6 fatty acids for treating children with ADHD-related symptoms (Hirayama et al., 2004; Johnson et al., 2009; Richardson & Puri, 2002; Richardson & Montgomery, 2005; Sinn & Bryan, 2007; Stevens et al., 2003). Focus has also moved to higher rates of EPA in relation to DHA after a suggestion that EPA possibly is more efficacious than DHA in treating ADHD symptoms (Richardson & Puri, 2002). The results have been varying, and it is not clear what type of intervention formula might be most effective on what diagnoses (or symptoms). There have also been trials to increase the dosage (Vaisman et al., 2008), which gave effect for the supplemented group on a CPT test, but not on behavioural scales.

There are mixed results regarding the behavioural scales. The absence of results regarding behavioural rating scales in some of the reviewed studies (Johnson et al., 2009; Raz et al., 2008; Vaisman et al., 2008) differed from the positive impact on behaviours observed by Sinn and Bryan (2007), Richardson and Puri (2002) and, to some extent, Stevens et al. (2003), as well as Richardson and Montgomery (2005, thus, supplementing for DCD), which all demonstrated behaviour improvement in behaviour rating scales. In some studies results are found in teacher ratings, in others in parents, this discrepancy is discussed in the discussion section at the end of the thesis.

There is limited support for effects by PUFA supplementation on the cognitive outcome measures. There are only two studies demonstrating significant effects, both have medium-size effects (Sinn et al., 2008; Vaisman et al., 2008), but they only obtained some single significant outcomes out of a large number of cognitive outcome variables. Some studies have shown that DHA concentrations were significantly lower in the ADHD participants compared to controls (Colter et al., 2008; Mitchell et al., 1987; Stevens et al., 1995). Regarding the omega-6 fatty acids, AA was also sometimes found to be significantly lower in children with Chen et al., 2004; Mitchell, Aman, Turbott, & Manku, 1987; Stevens et al., 1995; Vaisman et al., 2008).

Some conclusions from a review will serve as a final word in this section of supplementation studies. The absence of positive findings in some studies may depend on the type of intervention, short treatment duration, or the presence of AA in the active treatment drug. AA can have negative influence on the conversion of ALA into omega-3 LCPUFA (Transler, Eilander, Mitchell, & van de Meer, 2010).

AIMS OF THE CURRENT THESIS

The current thesis had the overall purpose of furthering our understanding of fatty acids in relation to cognition in children. I wanted to investigate two different aspects of this issue. The first type of examination had a longitudinal design and the potential effects of levels of fatty acids in mothers' breast-milk and the infants' blood were analyzed. These data were related to later cognitive development (e.g., intelligence and theory of mind) in the children when they reached the age of six to seven years. The second aspect involved intervention investigated with randomized placebo-controlled studies (RCT). In these studies, the potential effects of treatment with fatty acid supplementation in children with cognitive and behavioural problems (e.g., children with combined ADHD and children identified as being at risk of developing ADHD) were investigated. In order to investigate these aims, some a priori research questions were formulated:

1. What are the effects of breast-feeding on children's subsequent cognitive performance? (*Studies I and II*)
2. What are the effects on LCPUFAs in mother's breast-milk, and in infants' blood, on children's subsequent cognitive performance? (*Studies I and II*)?
3. What are the effects of supplementation with EPA, an omega-3 LCPUFA, on cognitive performance and behaviour in children with ADHD or at risk of developing ADHD? (*Studies III and IV*)
4. How does supplementation with EPA, an omega-3 LCPUFA, influence the levels of the fatty acids in serum and red blood cells of children with ADHD and children at risk of developing ADHD? (*Studies III and IV*)

In addition, another research question was raised during the work with the studies;

5. Do LCPUFAs have differential effects on cognitive functions, such as working memory, inhibition, problem solving, language, or Theory of Mind? (*Studies, I, III and IV*)

EMPIRICAL STUDIES

Methods

The Studies in this thesis have two different kinds of designs. Studies I and II are based on data from the same population-based sample in a longitudinal research project. Studies III and IV are intervention studies with randomized placebo controlled designs, thus using different methods and participants. The methods of all papers are presented in this section.

Participants and procedures

Longitudinal studies (Studies I and II)

The first two studies were derived from the same research project that started in 1993. In this population-based longitudinal study 131 children born in Linköping, Sweden, between January 1994 and July 1997, were included consecutively. The parents had given their consent to participate in this study when they attended Antenatal Health Care Centres during pregnancy between August 1993 and March 1996. The purpose was to investigate relations between mothers' allergies, mothers' breast-milk and their children's allergies. All children were delivered at term and the pregnancy had been uncomplicated. The mothers answered questionnaires about breastfeeding, environmental and socioeconomic factors, prospectively, 2-4 days after delivery, and after 3, 6, 12 and 18 months. Breast-milk samples were taken at birth, at 1 month and 3 months of lactation. Blood samples were taken from cord blood, at 3 and 18 months of age (Duchén et al., 2000). When the children were 6.5 years old they were invited to a follow-up; 73 children (29 girls and 44 boys) participated.

The follow-up consisted of two visits; at the first visit a child psychologist administered approximately half of the subtests of WISC-III (Wechsler, 1991). During this visit, the parent filled out questionnaires (psycho-social back-ground data). At the second visit, the child psychologist completed the WISC-III. The 27 first included children were also assessed with Theory of Mind tasks (Study I) during visit two.

Intervention studies (Study III and Study IV)

Studies III and IV are placebo controlled randomized and double-blinded (RCT) studies. The children were randomized to receive either the test supplement, EPA, or the placebo capsules containing rapeseed oil, during 15 weeks. Both studies involved 15 weeks of treatment and before and after measures. We analysed omega-3 and omega-6 LCPUFA in the blood of the participants in Studies III and IV. Blood data were collected at baseline and endpoint. All children received all other "treatments as usual", with the exception of medical treatment, which implies receiving extra support in school, etcetera, as appropriate.

Participants in Study III were 128 children, having a combined type of ADHD according to criteria in DSM-IV. The children were recruited from eight psychiatric and paediatric clinics in Sweden. Evaluation was made according to the practice of each participating unit, including standard physical examination; however, the following items were to be provided; Cognitive level established (WISC-III, or similar IQ-test) no more than 2 years old, DSM-IV diagnosis of any co-morbidity.

The children were 7–12 years of age and had been evaluated for pharmacological treatment when asked to participate in this study. Participants were included even if they had any neuropsychiatric co-morbidity, but were excluded if they had other medical conditions, such as mental retardation, autism and epileptic seizures during the preceding two years.

There were several drop-outs, 17 children wanted psycho-stimulants instead, leaving 92 children for Intention To Treat (ITT) analysis. Ten subjects had deficient follow-up data, which resulted in 82 Correctly Treated (CT) subjects with data sufficient for analysis according to the protocol (Figure 4).

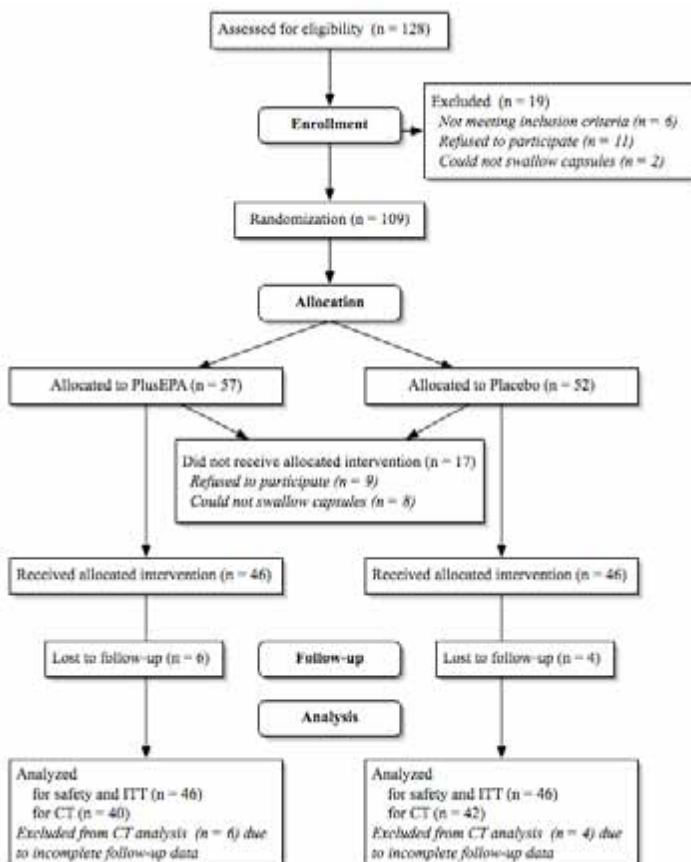


Figure 4. Flowchart of Study III.

Intention to Treat (ITT) “is a strategy for the analysis of randomized placebo controlled trials that compares patients in the group to which they were originally assigned” (Hollis & Campbell, 1999, p. 670). This study was a clinical trial recorded with Case Report Form (CRF) and the study followed the CONSORT statements.

Participants in Study IV were recruited from School Care units in Linköping and Örebro. Children in these units were about to start school in the autumn of 2004 (in Sweden school starts at the age of 7). They were screened for participation if there were considerations about their capability to start school due to poor preschool achievement or problem behaviours, or both in combination. The screening procedure involved using the rating scale “Strengths and Difficulties” (SDQ), filled-in by the children’s pre-school teachers or by school medical doctors during the period May-October 2004. If the total score turned out to be over a cut off (≥ 14) the pre-school teacher or medical doctor also filled in SNAP-IV (see below). The criterion for participation in the study was to exceed cut-off in six items (≥ 6 items rated ≥ 2).

After screening for eligibility 36 children were selected for treatment, of these children 28 remained after drop-outs and were randomized to receive 0.5g EPA or placebo capsules for a 15 weeks treatment period (Figure 5).

Measures

The studies in this thesis examine several cognitive and behavioural issues and involve different measurement methods. These methods are designed to suit children at different ages and children with typical development as well as children with cognitive and behavioural problems; therefore the thesis covers a large number of different tasks and rating scales (Table 1). In the following section the measurements used are described.

Rational of choice of tests

Given the fundamental role of LCPUFAs in supporting most structural and functional aspects in brain, reviewed in the introduction, effects on general cognitive development might be expected. At the same time, fatty acids deficiencies at various time points during development may have quite specific effects on cognitive development. It is therefore important to look at broad outcomes and at the same time search for specific underlying abilities as well. To identify and measure specific aspects of cognitive ability, is a somewhat more sensitive approach to assessing cognitive development.

In the longitudinal studies included in this thesis, fatty acid status at birth is related to subsequent cognitive development, as in most of the previous studies, we choose to examine general cognitive abilities of the children in a standardized well known test battery (WISC-III). We also looked closer to one specific ability, namely, ToM, this test were chosen after a fatty acid literature review finding almost no studies on social cognition. Since ToM is a fundamental ability, I decided to add an age-appropriate ToM task into this research project.

In the supplementation studies, biochemical measures were added to be able to investigate if there were initial deficiencies of some LCPUFAs. Ravens progressive matrices and abbreviated scores from WISC-III for estimation of IQ accounted for the broad perspective, while the subtests from WISC-III and QB-test accounted for specific effects in Study III. The

test battery including, working memory, problem solving and planning, inhibition, reading and spelling and ToM was used in Study IV. Aspects of cognition can be measured in different contexts by using several kinds of outcome variables. Behaviour ratings were included to evaluate overt behaviour with raters from two different settings, parents and teachers. Conner's teacher and parent rating scales have been extensively used in various

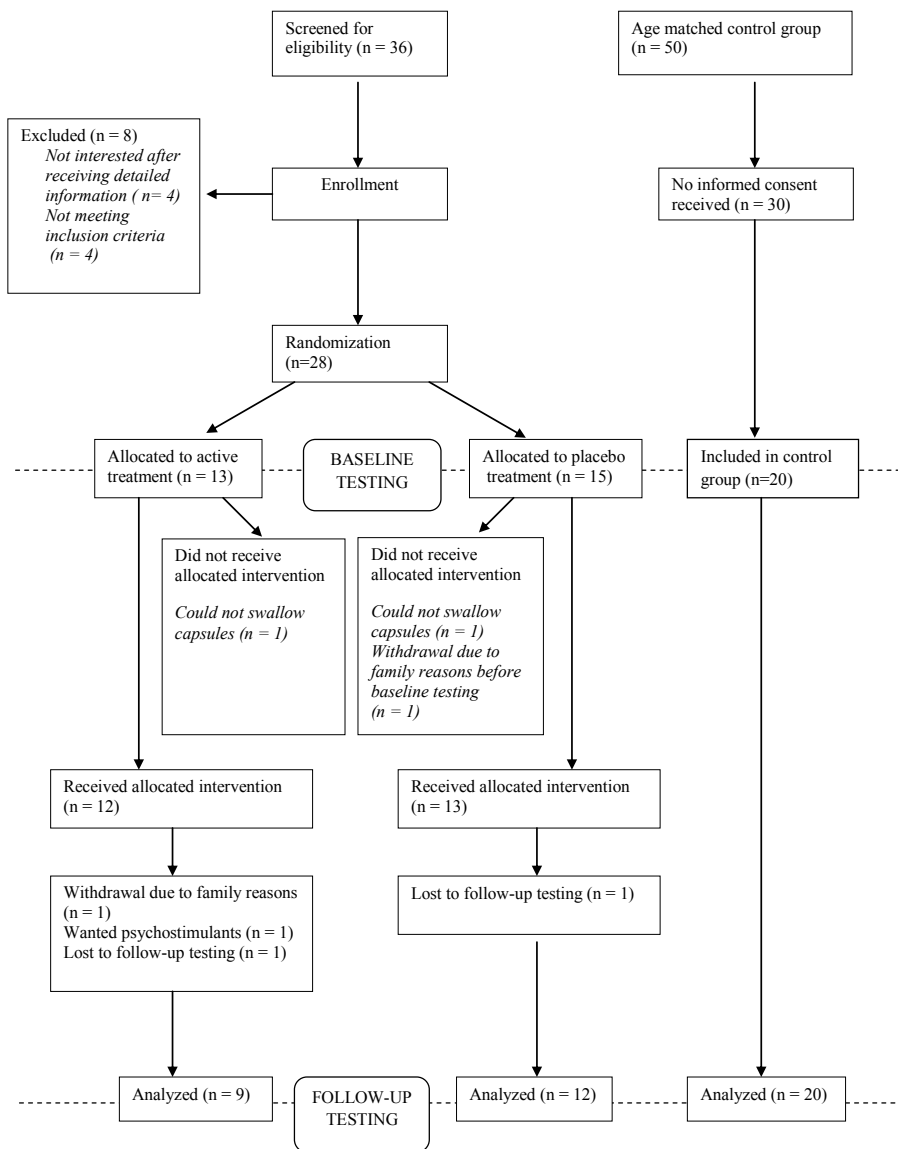


Figure 5. Flowchart of Study IV.

investigations, and are frequently used in clinical trials of LCPUFA supplementation and represents a suitable tool for behaviour evaluation in children.

The specific tasks mentioned above were selected for examining effects of LCPUFA at different levels. The effects that LCPUFA may have on brain structure and function, which is reviewed in earlier sections, were also the rationale for choosing the mentioned tests. Adequate support of LCPUFAs on neurotransmission and sufficient amounts of LCPUFAs have positive effects on cell signalling and impact on second messenger functioning (Yehuda et al., 1999). Synaptic functioning and speed of transmission may theoretically improve efficiency of information processing and influence cognitive function. The importance of LCPUFAs for neurotransmitters as serotonin, norepinephrine and dopamine is important (Heinrich, 2010). Dopamine in the frontal lobes seem to increase working memory capacity (Frank et al., 2007) and may be an important modulator of executive functions and is furthermore integral in studies on ADHD and working memory as well as inhibition. Frontal and hippocampal areas have been demonstrated to be involved in memory and higher-order cognition, such as problem-solving.

Intelligence test battery and tasks

In Studies I and II, the children were administered the full WISC-III (Wechsler, 1991). All children were shown to perform within the upper normal range ($m = 109,5$).

The analyses in Studies I and II used the total score, as well as Verbal IQ and Performance IQ separately. Verbal IQ depicts verbal comprehension, verbal expression, vocabulary, semantic memory and knowledge of language. Performance IQ reflects perceptual and visuo-spatial skills, motor speed, and executive skills. In the total score, measures of attention, memory and speed were partly included. The reliability is high for the full scale, and the overall internal-consistency coefficients are above .90. Test-retest stability coefficients of the Wechsler scales are between .86 and .95 for IQ. However, test-retest reliability for single subtests is not as consistent (Zhu & Weiss, 2005).

In Studies III and IV, four subtests from WISC-III were used, namely Block design, Vocabulary, Coding and Digit Span. These were used to obtain an estimate of the child's full-scale IQ. We chose these four subtests because they have high loadings on both verbal IQ and Performance factors, and are often used as short forms for estimating IQ. The estimated correlation with full scale IQ is .80 (Sattler, 2001).

Raven's standard progressive matrices (SPM). Raven's Progressive Matrices are traditionally used to assess nonverbal reasoning ability. The test consists of a series of complex non-verbal reasoning tasks presented on paper and they are answered by checking in appropriate boxes (Lezak et al., 2004). We used SPM for the baseline assessment and the SPM parallel version at follow-up to avoid test-retest effect. The parallel version is developed to create a discriminative effect in cases where people have been exposed to SPM before. The two versions are similar regarding number of items (60) and degree of difficulty (Raven, Raven & Court, 2000). Raw scores were recorded, with a total of 60 scores.

Table 1. Summary of all measures included in the studies.

	Cognitive assessment	Behavioural rating scales	Psychosocial measures	Biological data
Study I	ToM, Cognitive Cueing WISC-III (the full IQ scale was administered)		Socio-economic conditions Mothers and fathers education and occupation Family size Smoking	Breast-milk samples; colostrum, and at 1 month and 3 months of lactation Blood samples; cord blood, at ages 3 and 18 months
Study II	WISC-III (the full IQ scale was administered)		Breast-feeding Socio-economic conditions Mothers and fathers education and occupation Family size Smoking	Breast-milk samples; colostrum, and at 1 month and 3 months of lactation Blood samples; cord blood, and at ages 3 and 18 months
Study III	Block design, Vocabulary, Digit span and Coding from WISC-III Raven's Progressive Matrices Qb-test	Conner's parent rating scales Conner's teacher rating scales	Food habit questionnaire – Dietist XP	Blood samples; serum phospholipids and RBC, before and after treatment
Study IV	Block design, Vocabulary, Digit span and Coding from WISC-III Wechsler memory scales, story recall Statue and The Tower from Nepsy-R ToM; Cognitive Cueing TOWRE (Test of Word Reading Efficiency) Spelling Test for Preschoolers The Clown	Conner's parent rating scales Conner's teacher rating scales SNAP parents SNAP teachers		Blood samples; serum phospholipids and RBC before and after treatment

Theory of Mind tasks

The Theory of Mind test used in Studies I and IV was developed by Lagattuta et al. (1997) and consists of four illustrated stories in which the characters experience different sad events. This particular ToM test is designed to fit children at ages 5–7 years and for examining children's understanding of the link between emotions and thinking. In Study I, the whole procedure developed by Lagattuta et al. (1997) was applied. Study IV contained a shortened and modified version only involving Personal Cue stories and children's responses were written down instead of recorded.

In the full version, children are presented with four different picture stories intended to assess cognitive cuing and emotional change. Two of the stories use a personal cue, which means that child characters (Maria and Björn) experience sad events in their respective story. Later in the procedure each story character encounters a visual cue that is related to a previous sad experience. The children are told that the character feel sad, and they are asked to explain why. In the shortened version, (Study IV), we only use this personal cues part. There are two parallel stories for Maria and two for Björn, used for baseline and follow-up, see Figure 6 for an example of the pictures used.



Figure 6. Pictures from the Maria story, with Personal Cues (P Cue), in the ToM task (Lagattuta, 1997).

The stories with semantic cues, the Cat and Fish stories, had a different format with a semantic cue section to further probe children's understanding of emotional change in relation to cognitive cuing. In these stories, children were asked why a character felt sad after seeing a cue semantically associated with the past sad episode. Children's explanations were coded into the same categories as were used for the personal cue stories. In the Cat and Fish stories,

the children were also asked to predict how the character would feel and if the emotion would change when thinking of the previous sad incident or thinking of a distracting event. The answers were categorized into one of four categories. The most complete answers were again cognitive-cueing responses. The inter-rater reliability measured with Spearman's correlation of rank was .97 for the semantic stories. Scores varied between 0–4 points depending on number of cognitive cueing answers.

Working memory tasks

The Clown. The Clown is an adaptation of a working memory test, Mr Peanut Man, designed by Kemps, De Rammelaere, and Desmet (2000). It is a visuo-spatial working memory task where the children are presented with a drawn clown on a magnetic board with a varying number of dots stuck onto different locations (Figure 7). All dots are in the same colour. The children were allowed to view the Clown for 10 seconds and were then asked to remove the magnetic dots. After 10 seconds, the child was asked to put the dots back at the right locations. The experimenter arranged the dots in accordance with a designed manual in a fixed order for each trial with the board turned against herself (away from the children's sight). There was different fixed orders for baseline and follow-up trials.



Figure 7. The Clown, here shown with four items.

The task starts with one dot (item) in three trials and maximum number of dots (items) is 7. At every level of difficulty there is a stop-rule, if the child has only one correct answer the trial ends, if two or more correct answers are given the experimenter moves on to the next level. There were two conditions, with and without distraction. The trials with distraction contained an interference task. After the child had removed the dots they were asked to point with one of the magnetic dots at a sheet of paper with four painted dots, in the same colour as the magnetic dots, as fast as they could during 10 s. After the distraction task, they were asked to put the dots back at the same location. This was repeated for every trial.

Digit Span. Digit span is a subtest from WISC-III, in Study IV it was used as measurement of working memory. Children were orally given sequences of numbers and asked to repeat them,

first forward (e.g., 4 1 7 9) and later in reverse (backward) order. Series begins with two digits and keeps increasing in length, with two trials for each length (Wechsler, 1991). The forward task is thought to be a measure of simple memory storage and the backward task is classically considered to be more working memory demanding (Lezak et al., 2004, but see Unsworth & Engle, 2007). Forward and backward tasks were used and scores were calculated for each task separately and also for span length.

Episodic memory tasks

Story recall, Wechsler memory scales. This test was originally developed by Wechsler (1945) and consists of two short stories. The stories were adapted for Swedish children and designed to measure episodic long-term memory and free recall. The experimenter told the child to listen carefully to a story and the child was informed that after listening to the story he or she would be asked to tell it back. The experimenter noted all the answers.

Thereafter the experimenter told the child to listen carefully to the same story again and that they would be asked to remember as much as possible one more time, but this time after a intermission (in experiment IV after some other tasks; i.e. Vocabulary, Digit span and Block design). The experimenter then asked the child to remember all he or she could from the story earlier told (free recall). Two different versions were used for baseline and follow-up, respectively, in Study IV.

Inhibition tasks

Statue from Nepsy-R. To assess inhibition and motor persistence, in Study IV a subtest called “Statue” from the neuropsychological battery Nepsy-R was used. The child was asked to stand still until the experimenter told him or her “stop”. Instructions were given to maintain a still body position (feet slightly apart, left arm at the side, and right arm bent at the elbow perpendicular to the body) and keep eyes closed until hearing the word “stop”. The task lasted for 75 seconds. During the task several distracting stimuli were presented, such as dropping a pen on the floor, and, coughing, (presented at 10, 20, 30 and at 50 seconds as described in the Nepsy-R manual). The investigator marked body movements, opening of the eyes and possible sounds, every 5th second in a test form. The number of errors, in terms of body movements ranging from turning the head to voicing, constituted the data that were collected from this subtest (Korkman, 2000). A score of 2 was recorded for each interval if there were no errors, a score of 1 if there were one error and 0 if there were 2 or more errors.

Problem-solving and planning tasks

Block Design from WISC-III. Block design is considered a good g-factor indicator and was used in Studies I and II together with all other subtests to obtain full IQ score and in Study IV as estimate of IQ level together with three other subtests from WISC-III.

In Studies II and IV it was furthermore used as a measure on problem-solving, perceptual and visuo-spatial ability. Children copied a series of geometric designs by putting together red-and-white blocks in a pattern according to a displayed model. The completion was timed and a bonus for speed was given for the more difficult designs. A total of 69 scores can be achieved.

The Tower from Nepsy-R. In Study IV, the subtest Tower from Nepsy-R was used (Figure 8). It is an adaptation of the Tower of London (Shallice, 1982) and is a highly recognized global measure of executive functioning. It has been considered primarily a measure of response planning, but it also includes visuo-spatial abilities. Furthermore, it requires complex executive functioning abilities, mainly response inhibition and working memory (Asato, Sweeney, & Luna, 2006). The Tower also demands ability to comprehend instructions, attention, and motor skills (Stinnett, Oehler-Stinnett, Fuqua, & Palmer, 2002). In this complex task, the child is viewing a target picture and is told to move three balls of different colour into the target positions. The child is only allowed to use a prescribed number of moves (1–7 moves) and therefore needs to plan a sequence of moves. There is furthermore a time limit (30–60 s) and several complex rules to which the child must adhere (Korkman, 2000). The score is the number of correctly achieved target positions managed within the time limit (maximum 20 points).

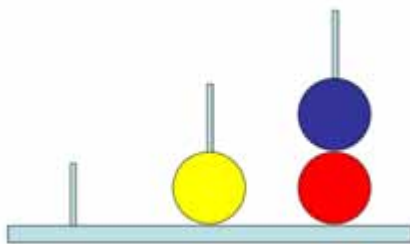


Figure 8. The Tower from Nepsy-III.

Coding from WISC-III. Visual-motor ability and speed are tapped by Coding in Study IV. The Coding version for children under 8 years was used. Children copy figures with different shapes according to a code as quickly as possible during two minutes. Bonuses are given for speed if answers are complete and correct.

Verbal, reading and spelling tasks

Vocabulary from WISC-III. This is one of the strongest g-measures of verbal ability in the scales. In Study IV it was used as a verbal ability measure and together with three other subtests as an estimate of IQ level, (see the section on Intelligence tasks above). Participants were asked to provide oral definitions of words of increasing difficulty. Scoring is 2–1–0, according to the quality of the responses with a total of 30 items (60 scores).

Test of Word Reading Efficiency (TOWRE). Reading ability is assessed with TOWRE (Torgesen, Wagner & Rashotte, 1999), in the intervention studies (III and IV) with a Swedish translation. The test consists of two subtests measuring the two kinds of reading skills that are considered critical in the development of overall reading ability. The first subtest concerns the ability to accurately recognize familiar words as whole units or "sight word efficiency" (SWE) and consists of a list with printed high frequency words. The second subtest is the

ability to sound out words quickly, “phonemic decoding efficiency” (PDE) and consists of a list of pseudo-words. The child’s task is to read aloud as many sight words or pseudo-words as possible in 45 seconds. Each subtest has two forms, A and B, of equivalent difficulty here used as parallel forms for base-line and follow-up. The TOWRE is a standardized test.

Spelling test for preschoolers. Spelling test for preschoolers (Lieberman, Rubin, Duques, & Carlisle, 1985) were administered in Study IV, chosen to be appropriate for this age group. It consists of a list of 14 words (man, train, come etc.) that the examiner reads out loud, one at a time, first separately and then included in a sentence. The last four words on the list are non-words (ig, sut, frof, yilt) read three times in a row. Children are told to write the words read on a sheet of paper. The test was adapted from Lieberman et al., (1985) and further developed by Byrne and Fielding-Barnsley (1993). A scoring system values correct representation of the individual phonemes and gives a maximum of 6 points per word (maximum 84 points).

Behavioural assessment

QB-test. QB-test is a type of Continuous Performance Test (CPT), which is a computer-based test designed to measure hyperactivity, inattention and impulsivity. The QB-test uses an infrared motion analysis system with a reflective marker attached by an elastic band to the front of the child’s head. The child is performing a continuous performance task during 15 minutes, in this task the child is told to press a button every time he or she sees a star on the computer screen, during this task head movements are recorded with the infrared motion system. Both number of head movements and performance on the continuous performance task are recorded as described by Teicher, Ito, Glod, and Barber, (1996).

Conner’s rating scales. Standardized questionnaires were used to estimate behavioural symptoms in Studies III and IV. Short forms of Conner’s parent rating scale (CPRS) and the Conner’s teacher rating scale (CTRS), which are norm-based behaviour rating scales used to assess children at 4–16 years of age, were applied. They include items of attention, cognition, hyperactivity, social problems, and emotion (Conners, 2000). CTRS is designed to assess children’s classroom behaviours, and CPRS is adapted to fit for parents. The Swedish versions were translated by Birgegård and Jones. The short versions consist of 28 brief statements and each statement is rated in a Likert-type scale as follows; 0 – not true at all, 1 – just a little true, 2 – pretty much true and 3 – very much true. The extent to which different problems have been present during the last week is estimated. Responses are divided into four subscales, oppositional, cognitive problems including inattention, hyperactivity, and ADHD index. This index is based on the best set of items for identifying children at risk of developing ADHD.

SNAP-IV. A Swedish translation of SNAP-IV was used (Johnson & Gustafsson, 2002) in Study IV. The 26 items are based on DSM-IV criteria for ADHD and include 9 items for inattentive symptoms, 9 for hyperactivity/impulsiveness and 8 for ODD symptoms. Besides we added three questions about emotional problems, and questions about sleep, motor difficulties and tics. Items are ranged on a 4 point scale from 0 – not at all to 3– very much. The same items are used for both parents and teachers. Subscale scores are obtained for inattention, hyperactivity/impulsiveness and opposition/defiance areas (Swanson et al., 2004).

Strengths and Difficulties Questionnaire (SDQ). In Study IV, SDQ (Goodman, 1997), was used for screening preschool children before school start. It is an instrument for rating mental health in children aged 3–16 years. There are two Swedish publications on the Swedish version of SDQ (Smedje, Broman, Hetta, & von Knorring, 1999; and Malmberg, Rydell, & Smedje, 2003). The questionnaire contains 25 questions concerning emotional symptoms, conduct problems, hyperactivity including attentional problems, peer and relational problems and prosocial behaviour, each with scores ranging from 0 to 10.

Dietist XP. Dietist XP (kost och näringsdata, Bromma, Sweden) is a food frequency questionnaire. Parents were in Study III asked to fill in a questionnaire with a 24-h recall of the diet to assess the child's food habits. Total energy intake, distribution of different fats and the amount of fish in the diet were asked for. The questionnaire was repeated at the 15 weeks follow-up to evaluate the stability of food habits.

Breast-milk and blood samples measuring fatty acids

In all studies blood samples were taken and analyzed. The method used for analyzing the fatty acids in Red Blood Cells and in serum is described in detail in Folch, Lees, Sloane, and Stanley, (1957) and in Gronowitz, Mellström, and Strandvik (2006).

In blood samples measures of levels in serum, red blood cells were taken as well as in membranes. Single PUFAs of interest were; the omega-3 PUFAs; LNA, EPA, DPA, DHA and the omega-6 PUFAs; LA, GLA, DHGLA, AA, C22:4n-6 and C22:5n-6. Total amount of LCPUFA omega-3 and omega-6 was examined as well as quotients between the long chain polyunsaturated acids; AA/DHA, total LCPUFA n-6/total LCPUFA n-3. Finally, ratios between AA plus EPA over DHGLA plus eicosatetraenoic acid (ETA; 20:4n-3), the C22:4n-6/C22:5n-6 and the DPA/DHA ratios were calculated to identify rate limiting metabolic steps important for cognitive development.

In Studies I and II, breast-milk samples were taken. The child was allowed to suckle for 2 minutes before the sample was taken. The same PUFAs were analyzed in breast-milk as in blood, see above.

Ethical considerations

The studies were conducted in accordance with the Declaration of Helsinki 2004. In all studies in the present thesis, the information requirement was fulfilled with information letters and oral information to the presumptive participants before they were included in the studies. Participant teachers received information letters with information about whom to contact for questions. In Studies III and IV letters of consent were filled-in by both parents before study start, which is in accordance with the Declaration of Helsinki code (2004), paragraph 25. Before the assessment, children were informed of their right of withdrawal at any time during the assessment. Despite this procedure there may be more subtle dilemmas, such as when to push the child to continue in a difficult test and when to let go. Here the principles of the ethical code paragraph 22 were used (Declaration of Helsinki, 2004) and the test leaders were trained in ethical and assessment considerations.

Research journals were established and they are kept secure at the respective clinic. Studies III and IV were conducted in accordance with GCP (Good Clinical Practice) and a case report form (CRF) was filled-in for every person, marked only with initials of the child. Data are only used for research, thus this requirement of confidentiality is considered to be met.

Safety is another issue in clinical trials like Studies III and IV. No adverse events were expected from the omega-3 supplementation, however, all subjects starting the treatment were asked for adverse events during each visit. Each clinic was responsible for reporting any abnormal sensation or unwanted event. All answers were recorded in the CRF.

Moreover, due to fairness reasons, all subjects were offered treatment with omega-3 for 15 weeks after their 15 weeks study treatment (one-way cross-over), since the randomization code could not be broken. Also, subjects who wanted to discontinue the study prematurely were able to return to regular clinical treatment, including psycho-stimulants, if appropriate.

SUMMARY OF THE STUDIES

Study I

Background and purpose

Omega-3 and omega-6 poly unsaturated fatty acids have been found to play a central role in development and in brain functioning. Long chain poly-unsaturated fatty acids are involved in numerous brain processes, from membrane fluidity to affecting neurotransmitter uptake. Findings from research of very long chain polyunsaturated fatty acids on early brain development, during pregnancy and infancy, indicate that they benefit later development (Innis, 2008). DHA, for example, is a major structural component of the brain, and it is important for normal development and maintenance of brain functions (Bourre, 2006; Heinrich, 2010; Innis, 2008).

Nutrition during pregnancy and infancy may programme later neurodevelopment (Innis, 2008; Lucas, 1998, 2007), and deficiency of important micronutrients and omega-3 fatty acids has severe consequences for animals' behaviour and learning. Therefore, deficiencies are also thought to affect children's cognitive development (Innis, 2008). There have been conflicting results in longitudinal studies measuring effects of LCPUFA. One Cochrane report showed no effects in children born full term (Simmer et al., 2008), whereas another Cochrane report showed that children born preterm benefited from supplementation (Schulzke, Patole, & Simmer, 2011). Some supplementation studies have also shown that supplementation of omega-3 fatty acids to mothers during pregnancy and lactation influences IQ measures later on (Eilander et al., 2007; Helland et al., 2003).

Based on this body of evidence regarding nutrition, cognitive growth, and neuropsychiatric disorders in children we hypothesize that there is a relationship between early intake of n-6 and n-3 polyunsaturated fatty acids and cognition. We specifically wanted to examine the relation between mothers' levels of fatty acids in breast milk and their children's development of ToM. In addition, we examined the relation between children's levels of omega-3 PUFAs in serum at 3 months and development of ToM at 6.5 years of age.

We also hypothesized that the PUFA composition in the first breast-milk (colostrum), particularly of omega-3 LCPUFA and the DHA/AA ratio as a reflection of maternal PUFA biosynthesis could be related to development of ToM as measured by the tasks developed by Lagattuta et al. (1997).

Results and discussion

Correlations between the ToM tasks and the results in WISC-III revealed that the results regarding the stories with personal cues, but not the semantic cues, were significantly related to total IQ ($r = .42, p < .05$) and verbal IQ ($r = .52, p < .01$) in this group with 27 children.

Regarding separate PUFAs in colostrums, particularly the levels of omega-6 LCPUFA, were all negatively correlated to results in ToM in children at 6.5 years of age, meaning that children performed better on the tasks if the first breast-milk contained lower amounts of omega-6 PUFA. However, for results in WISC-III the omega-3 fatty acids correlated positively (Table 2).

There are several factors known to have impact on cognitive abilities. Therefore, partial correlations in order to investigate the influence of these abilities were made. The results showed that parental education and duration of breast-feeding were important determinants of results in ToM and WISC-III, although the results still largely remained after controlling for these factors.

Turning to the findings of different quotients between fatty acids in breast milk we found that some of them were related to cognitive measures. In ToM tasks there were significant positive correlations between personal cues and DHA/AA ($r = .62, p < .05$) and between personal cues and DHA/DPA ($r = .74, p < .01$) after accounting for effects of parental educational level and length of breast-feeding. Turning to results regarding WISC-III, the correlation between DHA/AA and total WISC scores was $r = .76, (p < .01)$ verbal IQ $r = .59, (p < .01)$ performance IQ $r = .81, (p < .01)$ after accounting for parental education and length of breast-feeding. WISC-III and DHA/DPA also showed positive correlations with total IQ ($r = .55, p < .01$), verbal IQ ($r = .54, p < .01$) and performance IQ ($r = .48, p < .05$) with control for the same demographic variables as above.

One apparent interpretation of these findings is that it is the balance between the different kinds of fatty acids that is important, and specifically a quota with high levels of omega-3 in relation to lower amounts of omega-6. This pattern is also shown in other studies. The relations with parental education and length of breast-feeding in the partial correlations are expected, these factors are well known for influencing results in intelligence tests and ToM tasks. More interesting is the fact that the relations with the PUFAs largely remained after this adjustment. This may be an indication of the fatty acids subserving important functions during the structural building of the child's brain. For example, the relative deficiency of DHA may make neurons more vulnerable to lesion, and a relatively high amount of AA may affect the neurotransmission. Together they show the importance of the balance between omega-3 and omega-6 fatty acids and may be a possible explanation to these findings.

Correlations were found between the first breast-milk and cognition, and there were no correlations with the blood samples taken from the children, it is therefore also of interest to discuss the process to synthesize omega-3 and omega-6 during the first years of life. Our findings may reflect the PUFA status of the mother during pregnancy and thereby stress the significance of the mother's omega-3 status during pregnancy for the child's later development. Since the levels of fatty acids in colostrum seem to be mirroring the levels during pregnancy, this may simply reflect the availability during pregnancy (Houwelingen & Hornstra, 2000).

To summarize, this study found relations between breast-milk and theory of mind, as well as intelligence, in a group of 6-year old children with typical development. There were no relations with fatty acids in children's blood samples. Correlations between cognitive tasks and single omega-6 PUFAs as well as with ratios between omega-3 and omega-6 fatty acids in breast milk remained significant even when accounting for other factors known to influence cognition, such as parental education and length of breastfeeding. Due to the small number of participants the results must, however, be interpreted with caution.

Table 2. Relations between PUFAs in colostrum and psychological measures when effects of demographic variables were accounted for i.e., # parental educational level (WISC-III n = 19, ToM n = 10), □ length of breast-feeding (WISC-III n = 15, ToM n = 10), or § parental educational level in combination with length of breast-feeding, (WISC-III n = 14, ToM n = 9). Separate PUFAs and ratios in the n-6 and n-3 series are presented. Results denote correlations in terms of Pearson's product-moment correlation, r. Non-significant results are not shown. Personal Cue = P Cue

	WISC-III (n = 22)	WISC-III adjusted	ToM (n = 13)	ToM adjusted
n-6 series				
LA				
GLA				
DHGLA			P Cue, r = -.60 *	
AA	<i>IQ</i> , r = -.44*	<i>IQ</i> , r = -.45*	P Cue, r = -.64*	P Cue, § = -.71 *
	PIQ, r = -.45*	PIQ, □, r = -.44*		
DTA			P Cue, r = -.61 *	P Cue, □, r = -.58*; #r = -.64*; §r = -.70*
C22:5 n-6				
<i>Total LCPUFA n-6</i>			P Cue, r = -.71 **	P Cue, □, r = -.70*; #r = -.72**; §r = -.77**
n-3 series				
LNA				
<i>C18:4 n-3</i>	Not studied	Not studied	Not studied	Not studied

ETA	$IQ, r = .42^*$	$IQ, r^2 = .50^*$	
EPA	$PIQ, r = .54^{**}$	$PIQ, r^2 = .50^{**}, \xi r = .59^*$	
DPA			$P\ Cue, r = -.57^*$
DHA			$P\ Cue, r = -.58^*$
Total LCPUFA n-3			
Ratios			
DHA/AA	$IQ, r = .59^{**}$ $VIQ, r = .48^*$ $PIQ, r = .65^{**}$	$IQ, r^2 = .77^{**}, \xi r = .76^{**}$ $VIQ, r^2 = .62^{**}, \xi r = .59^*$ $PIQ, r^2 = .82^{**}, \xi r = .81^{**}$	$P\ Cue, \xi r = .62^*$
DHA/DPA	$IQ, r = .51^*$ $VIQ, r = .51^*$ $PIQ, r = .46^*$	$IQ, \xi r = .55^{**}, r = .66^{**}$ $VIQ, \xi r = .54^{**}, r = .51^*$ $PIQ, \xi r = .48^{**}, r = .71^{**}$	$P\ Cue, r = .64^*$ $P\ Cue, r^2 = .60^{**}, \xi r = .74^{**}$
C22:5N-6/DTA			
Total LCPUFA n-6/	$PIQ, r = -.48^*$	$IQ, r^2 = -.54^{**}$	
LCPUFA n-3		$PIQ, r^2 = -.61^{**}, \xi r = -.56^*$	

* $p < .05$, ** $p < .01$

Study II

Background and purpose

There is increased awareness of the nutritional and medical benefits of breastfeeding, such as reduced incidence of infection and allergic reaction. Interest has also turned to effects of breastfeeding on cognitive development and several studies have published data about effects on preterm infants fed with either breast milk or formula by tube where results indicate that breastfed children score slightly higher than those fed on formula in children's IQ tests (Anderson et al., 1999; Kramer et al., 2008). A more recent meta-analysis from WHO also concludes that breast-feeding "is associated with increased cognitive development in childhood" (Horta, Bahl, Martines, & Victora, 2007. p.38) where it is also concluded that breast-feeding is positively related to academic achievement. Formulas have traditionally only been supplemented with the precursors of the essential PUFAs and some investigations have demonstrated that infants fed with formulas have lower levels of LCPUFAs in the cerebral cortex compared to children who have been breast-fed (Faquharson et al., 1995; Makrides, Neumann, Byard, Simmer, & Gibson, 1994).

These findings, among others, have highlighted the role of early exposure to the omega-3 LCPUFAs such as DHA, and the possible consequence of benefitting subsequent cognitive development. The purpose of this study was therefore to examine this relation with a longitudinal design and to take into consideration the potential influence of variables known to influence cognitive performance.

At the date of planning of our study, there were few, if any, studies that had examined whether the pregnant mother's prenatal omega-3 fatty acid status was associated with the child's later cognitive development. But lately some other studies have been conducted (e.g., Hadders-Algra, Bouwstra, van Goor, Dijk-Brouwer, & Muskiet, 2007).

The aim of Study II was to examine if breastfeeding could affect later general cognitive ability in children with typical development as measured by WISC-III, and also to investigate the composition and biosynthesis of omega-3 and omega-6 fatty acids in mothers' breast milk and in children's serum phospholipids in relation to the same children's later general cognitive performance.

Results and discussion

The results showed that there was an association between length of breastfeeding and children's later IQ results in WISC-III. The correlation between the full scale IQ and length of breastfeeding was $r = .30$, ($p < .01$). There were also correlations between socio-economic class ($r = .56$) and mothers' educational level ($r = .45$). Children who had been breastfed for five months, or more, had significantly higher IQ results at 6,5 years of age than those who had shorter duration of breastfeeding.

To investigate the influence of breast-feeding, a multiple regression analysis was used. Relevant cofactors, such as socioeconomic status and gestation week were introduced in the models, and in the best models, 46% of the variation in total IQ was explained. Regarding verbal IQ and performance IQ, 44% and 32%, respectively, were explained by this model. The length of breast feeding contributed significantly to children's total IQ ($\beta = .23$, $p = .02$)

and verbal IQ ($\beta = .20, p = .04$), while performance IQ was not significant ($\beta = .21, p = .06$). Every week of added breastfeeding predicted 0.27 units of total IQ of the child (Figure 9).

There were no relations between breast-feeding and specific subtests in the WISC-III battery, but as reported in Study I positive correlations with a ToM task were demonstrated. This indicates a need for further examinations of other abilities such as different aspects of executive functions.

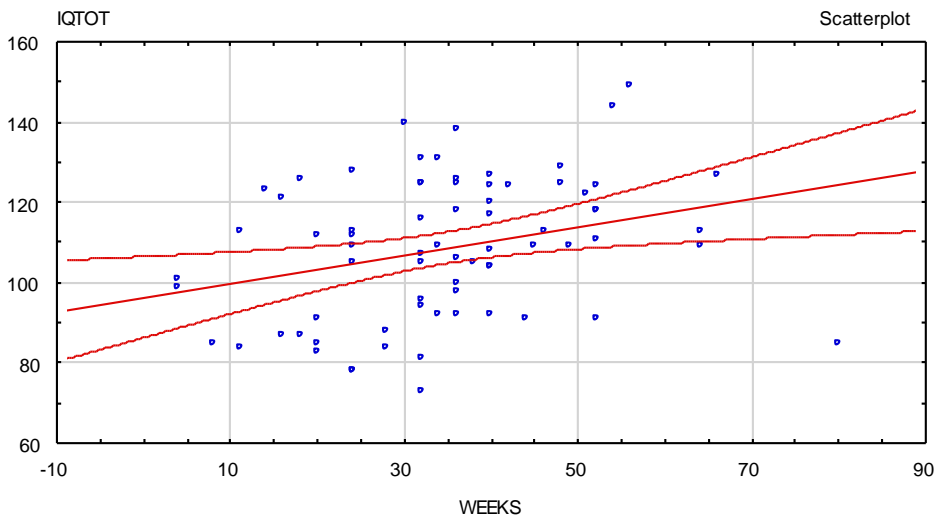


Figure 9. Correlation of length of breast-feeding and total IQ ($r = .298$, (straight line) 95% confidence interval (curved lines), $n = 73$)

Turning to findings concerning PUFAs and cognition there were no significant single correlations between PUFA and measures of cognitive development. In a multiple regression analysis of colostrum, however, we found significant relations between the quotient DHA/AA and total IQ ($r = .63$) and also in desaturases (steps 4+5) between the fatty acid metabolic chain and total IQ ($r = .56$), see Figure 10.

Different steps in the metabolic biosynthesis in colostrum were introduced as independent variables in a multiple regression analysis with a step-wise forward method introducing one confounding factor at a time. The steps were categorized as; 1+2, 3, and 4+5 and these three were considered the relevant steps to include. Step 4+5 correlated significantly with IQ and if length of breastfeeding and gestation week were entered, the model could explain 67% of the variance of total IQ. The other cofactors did not contribute significantly to the model (Table 3).

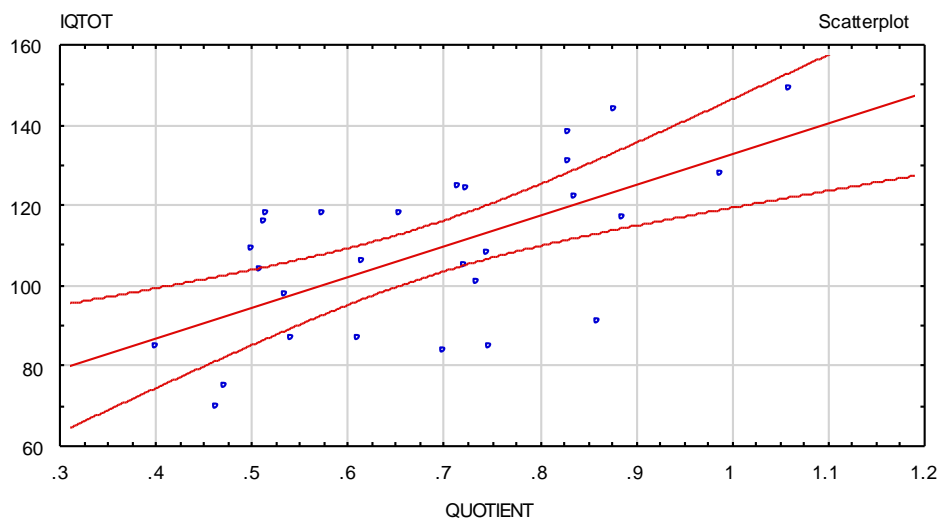


Figure 10. Correlation between the quotient DHA/AA in colostrum and total IQ ($r = .629$ (straight line), 95% confidence interval (curved lines), $n = 28$)

The quotient DHA/AA was furthermore examined in a multiple regression model. Together with length of breastfeeding and gestation week 76% of the variation in total IQ was explained. DHA/AA remained significant together with length of breastfeeding in all the models. The findings for both verbal and performance IQ were similar to the results for total IQ. No other cofactor gave significant results.

In this study, a higher amount of DHA compared to AA was related to IQ and we found that this pattern remained even when the impact of other factors known to influence IQ, such as socioeconomic status, mother's education, and family size, for example, were examined. Since we did not find any correlation with the quotient EPA/AA, which is often found in treatment research, we hypothesized that DHA may be more important for brain structure and EPA more important for brain function, as others have suggested before (Das, 2003; Makrides et al., 1994; Uauy, Mena, Rojas, 2000).

Furthermore, these findings can be interpreted as a contribution to the explanation of why breast feeding is important for cognitive development; i.e., breast-milk seems to be an important source of PUFA in the newborn. In other words, the longer duration of breastfeeding the higher amounts of PUFA in the child.

The interpretations of the findings in this study need to be made in the light of some limitations. To start with, the sample size was relatively small. The correlations found between PUFA and cognition were exclusively found in colostrum, and not in any other breast milk samples, nor in blood samples.

Table 3. Steps of biosynthesis of PUFA in colostrum and cognitive development. Multiple regressions with steps 4+5 in the biosynthesis process, weeks of breast-feeding; and gestation week, birth weight, mother's education level and socio-economic class as the third independent variable, respectively.

	Model 1 <i>p</i>		Model 2 <i>p</i>		Model 3 <i>p</i>		Model 4 <i>p</i>	
	Total IQ		Total IQ		Total IQ		Total IQ	
% explained variance	67%	< .001	58%	< .001	57%	< .001	56%	< .001
Variable	Beta		Beta		Beta		Beta	
Steps 4+5	.444	< .001	.486	< .001	.476	.001	.477	.004
Weeks of breast-feeding	.562	< .001	.537	< .001	.490	.002	.488	.001
Gestation week	-.315	.010						
Birth weight			-.131	ns				
Mother's education level					-.122	ns		
Socio-economic class							.098	ns

Study III

Background and purpose

Long-chain omega-3 polyunsaturated fatty acids (omega-3 LCPUFAs) have been associated with neurodevelopmental disorders, such as ADHD, and an increasing number of studies of supplementation of omega-3 fatty acid as treatment for ADHD have been carried out with varying results. There is reason to believe that fatty acid supplementation can be effective in at least some subgroups of children with ADHD. Studies by Johnson et al. (2009) and Sinn et al. (2008) give evidence that omega-3 fatty acids, and specifically EPA, can be an effective treatment for ADHD of the inattentive type.

A number of supplementation studies have been criticized for lacking measures of pre- and post-existing fatty acid status. Evidence is furthermore suggesting that children with ADHD often have a pre-existing lack of fatty acids, thus we decided to examine fatty acid status before and after treatment with PUFA.

The aim of Study III was to investigate possible treatment effects of supplementation with EPA, an omega-3 LCPUFA, on cognition and behaviour in children with ADHD of the combined subtype, or to any subgroup of these children.

Results and discussion

There were no initial differences between the placebo and treatment groups. Regarding the Conner's Teacher and Parents rating scales there were no significant treatment effects in the ITT group of children with ADHD ($n = 92$). Thus there were no differences between the EPA and placebo groups, neither in the combined parents' and teachers' rating scales nor in their individual ratings at the follow-up. However, in the Conner's teacher rating subscale

for inattention, including cognitive difficulties, children who received the EPA supplement exhibited significant improvement ($ES = 0.28, p = .04$). Effect size obtained with Cohen's d .

Concerning analyses of the children who were classified as having coexisting oppositional behaviours at baseline ($n = 43$), those children improved from EPA treatment (Table 4). This effect of EPA treatment on the combined scores of parents' and teachers' ratings was statistically significant ($ES = 0.51, p = .026$). The result depended almost exclusively on improvement in Conner's teacher rating scales for the EPA-treated group. Among these children, more than fifty percent of those who consumed the EPA supplement exhibited improvement of at least one standard deviation in teachers' ratings for oppositional behaviour and inattention or cognitive problems. Those who responded to the EPA supplement also had lower serum phospholipid EPA and higher arachidonic acid concentrations at baseline compared with non-responders.

Table 4. Children with ADHD and coexisting oppositional behaviour ($n = 43$) improved significantly with EPA treatment.

Treatment	n	M	SD	p (t -test)	p (M-W test)	ES
Placebo	20	3.30	9.58	.015	.021	0.80
PlusEPA	23	12.65	13.73			

Another subgroup was a group of children who ranked below median in hyperactivity/impulsivity on the QB-test ($n = 44$), here called the group with *less hyperactivity/impulsivity*. Twenty-two children were classified as being included both in the group with less hyperactivity/impulsivity and, at the same time, in the group with coexisting oppositional defiant symptoms. Of these, thirteen children were supplemented with EPA, and nine received placebo. Eight of the EPA children showed a 25% or greater improvement in hyperactive and oppositional behaviour, while only one in the placebo group improved. These responses were significant ($p = .03$).

Turning to the analyses on fatty acids, children who had been treated with EPA during 15 weeks showed a 159% rise in their EPA concentration in serum and a 160% rise in RBC membranes ($p < .001$). Furthermore, almost all omega-6 PUFA were decreased in both serum and RBC membrane phospholipids. We also showed that children with oppositional behaviours who responded to EPA had significantly lower levels of EPA in their serum phospholipids compared with non-responders at base-line.

To summarize the results, no treatment effects were found when analysing the whole group of children with ADHD as one general group. But, in two subgroups with ADHD, i.e., those who had high levels of oppositional behaviours and those with less hyperactive-impulsive behaviours, the children responded to the supplementation of EPA with significant reductions in their symptoms, as assessed by their teachers. These findings support earlier findings by Richardson and Puri (2002), Sinn & Bryan (2007), and Johnson et al. (2009). All of these studies showed that subgroups with more inattention or oppositional behaviour or both in combination were more likely to respond to omega-3 fatty acids. In this present

study, relations were found in teacher ratings and not in parent ratings. One explanation to this may be that teacher ratings are more sensitive for detecting behavioural changes than are parent's ratings. Parents and teachers observe and relate to their children in different settings where the class room situation may demand more from children's behaviour.

To our knowledge there is no other study that used a pure EPA supplement, included both teacher and parents rating scales, and at the same time used biochemical measures. This makes the results in the present study an important contribution to the understanding of possibilities of treating children with ADHD with fatty acids.

Study IV

Background and purpose

There has been large interest in the changed life style and food intake with excessive intake of omega-6 in relation to omega-3 (Bourre, 2006; Simopoulos, 1999, 2009), and, at the same time, a discussion of the coincidence with an increasing number of children being diagnosed with neurodevelopmental disorders (Atladdottir et al., 2007; Mandell, Thompson, Weintraub, Destefano, & Blank, 2005; Kelleher, McInerney, Gardner, Childs, & Wasserman, 2000). A link between these facts have been suggested (Richardson, 2006; Schuchardt, Huss, Stauss-Grabo, & Hahn, 2010; Young & Conquer, 2005).

Neurodevelopmental disorders, consist of several heterogeneous conditions, characterized by a delay or deviance in development (Andrew, Pine, Hobbs, Anderson & Sunderland, 2009). Examples of common neurodevelopmental disorders are ADHD, Autism, Dyslexia, and Tourette syndrome. They share several factors, (1) onset is in childhood, (2) the disorders are diagnostically closely aligned, (3) the disorders are overlapping and often co-morbid (Gillberg, 2010, Rasmussen & Gillberg, 2000), (4) the disorders include cognitive abilities such as language, communication, emotional regulation, memory, and learning (Rutter, Kim-Cohen & Maughan, 2006; Shevell, 2010).

The suggested link between fatty acids and neurodevelopmental disorders has led to numerous supplementation trials with omega-3 fatty acids, but most of them focused on separate diagnoses. About the suggestion of an underlying common ground, the frequent overlap, co-morbidity and heterogeneity of these disorders, this present study has an inclusive approach and includes children with several neurodevelopmental symptoms.

The purpose of Study IV was to investigate a group of 7-year old children with unspecific neurodevelopmental symptoms. Children for whom there were considerations about their capability to start school, depending on behavioural problems and poor preschool achievement, were screened for eligibility. Children reached the criterion for ADHD on the rating scales, but have not been formally diagnosed by a clinician.

Several PUFA trials have pointed at the need for not only standardized general test batteries for evaluating treatment effects, but also for experimental cognitive tasks designed to suite the included age group (see for example Cheatham et al., 2006). In this study, I therefore choose to use an extended test battery with tasks selected for measuring cognitive skills, as well as parents' and teachers' rating scales (SNAP and Conners). The same RCT design as in

Study III was applied to examine if this group with neurodevelopmental immaturity, at risk developing ADHD, would benefit from treatment with (0.5g) EPA supplementation. An age-matched control group was added to investigate whether the screened children had cognitive deficits and behavioural problems in comparison to children with typical development, and to make it possible to compare normal development during a school semester.

Results and discussion

Children with neurodevelopmental problems, at risk of developing ADHD, did not benefit from treatment with (0,5 g) EPA supplementation during 15 weeks, on any of the cognitive tests or the behaviour rating scales. These findings are similar to the findings in the larger multicentre trial in this thesis, i.e., Study III. The sample, however, was in this study too small to be able to make any subgroups and it is therefore not possible to compare the studies regarding findings in subgroups. Although not being the principal aim of this study, unfortunately, also the biochemical data is missing, as several of the children were injection phobic and some samples were not possible to analyze.

To examine treatment effects split plot factorial repeated measures, 3x2 ANOVAs, were used. Main effects were found for two working memory tasks, Digit span backwards ($p = .07$) and the Clown ($p = .08$), showing significant differences between the groups over time. Planned contrasts show that differences are between the control group and the two neurodevelopmentally immature groups, in the sense that the control group improved less over time than the children with neurodevelopmental immaturity. Main effect was also obtained for the reading test, TOWRE ($p = .08$), and planned contrasts gave that the control group also here differed from the neurodevelopmentally immature groups.

Non-parametric tests were made to examine if the children's behaviour ratings changed after the treatment period. Regarding teacher ratings, in SNAP there were differences on several subscales; hyperactivity/impulsivity ($p = .02$), inattention ($p = .01$) as well as the combined ADHD scale ($p = .01$). Significant effects were also found for parents' ratings CPRS hyperactivity index ($p = .00$). The results indicate that children with neurodevelopmental immaturity improved their behaviour according to the ratings (lowered their scores), while children in the control group having low scores at baseline increased slightly. No other significant differences were found in either cognitive tasks or rating scales.

Studies with assessment of cognitive tasks are not as common as rating scales in supplementation studies and results are mixed, often with only a few significant correlations found out of a whole test battery. Sinn et al. (2008) used a cognitive test battery in a fatty acid treatment trial with children with ADHD, their tests assessed speed of processing, memory, attention, inhibition, working memory, and distractibility. They found no treatment effects other than on a test of the ability to learn to switch and control attention, (the Creature Counting test) their PUFA group compared to placebo ($n = 129$, $p = .002$). Zhang, Hebert and Muldoon, (2005) demonstrated better performance for the PUFA group on the Digit Span subtest of the WISC-III and better reading after controlling for potential confounders. Better reading and spelling was also demonstrated in a study among children with dyspraxia (Richardson & Montgomery, 2005). In a supplementation study of healthy 5-year old children, results demonstrate that the supplemented group have significantly higher scores on Peabody Picture Vocabulary Test (PPVT) than the placebo group (Ryan & Nelson, 2008).

PPVT is a test of listening comprehension for the spoken word in English and is a measure of vocabulary (Dunn & Dunn, 1997).

One-way ANOVA shows significant differences between the groups regarding performance on the cognitive tests at baseline. Planned contrasts revealed that the control group performed better at baseline than the two groups with neuropsychological immaturity on all tests except the immediate recall on the Wechsler memory task, Block Design, Tower, Coding, and the ToM tasks. The rating scales differed significantly on both parents and teacher ratings in Conner's and in all teacher ratings in SNAP, showing that the control group was rated as having less behaviour problems at baseline than the group with neurodevelopmental immaturity.

To summarize the findings in the group of children with neurodevelopmental immaturity, they showed significantly poorer cognitive functioning as well as significantly lower scores on teacher and parents rating scales than the children with typical development in the age-matched control group, at baseline. This indicates that the selected group with neurodevelopmental immaturity had significant difficulties regarding both cognition and behaviour and therefore were eligible for treatment.

Children with neurodevelopmental immaturity, with several behavioural symptoms similar to children diagnosed with ADHD, differed from an age-matched control group with typical development in several cognitive tests assessed in Study IV. Foremost, they performed worse regarding working memory, both visuo-spatial and verbal working memory, episodic memory, inhibition and in verbal tasks including vocabulary, reading and spelling. They did not differ in visuo-spatial or planning tests such as Tower and Block Design or on Coding. Parents and teacher rating scales differed in almost all subscales, children with neurodevelopmental immaturity were rated worse. The exceptions were in scales tapping sleep and emotionality.

DISCUSSION

The aim of this thesis was to investigate the role of omega-3 fatty acids on cognition in children. The overall research questions presented in the Introduction will be addressed and attempts to answer them are summarized below. Thereafter, strengths and limitations of this thesis are discussed followed by methodological issues. Possible directions for future research finalize the discussion.

MAIN FINDINGS OF THE EMPIRICAL STUDIES

What are the effects of breast-feeding on children's subsequent cognitive performance?

There were significant effects on breast-feeding and children's subsequent cognitive performance as measured by a standardized cognitive test battery (WISC-III) in Study II. The duration of breast-feeding was significantly and positively correlated with verbal IQ and the full IQ scale. Children who had been breast-fed for 5 months or more had significantly better results than those breast-fed for a shorter period than 5 months. This relation remained even after controlling for factors well-known to influence cognition. An interesting finding was that breast-feeding improved mean IQ score with 0.27 units per week. Note, however, that the largest proportion of mothers in our study stopped breastfeeding before children reached 1 year of age, and only a few mothers continued after that time (the longest duration was 80 weeks).

These findings support previous studies indicating that breast-feeding is correlated with higher intelligence quotients later in childhood (Anderson et al., 1999; Kramer et al., 2008; Oddy et al., 2004). The size of the IQ advantage for children breast-fed longer is in line with findings in other trials. In for example the PROBIT study children exclusively breast-fed for 6 months had an average of IQ score 5.2 points higher than those breast-fed shorter, at the age of 6.5 years.

Other studies have obtained a larger improvement regarding verbal IQ than regarding performance IQ (Horwood, Darlow, & Mogridge, 2001; Kramer et al., 2008). Results being more pronounced for verbal IQ have been reported in other studies as well. Longer duration of breast-feeding was positively correlated with language ability in PPVT-R (Peabody Picture Vocabulary Test – Revised), in children at 10 years of age. This effect remained after adjustment for socio-environmental conditions, such as mother's education (Whitehouse, Robinson, Li, & Oddy, 2010). Positive correlations between duration of breast-feeding and PPVT-R scores, with i.e., increasing scores with increased duration of breast-feeding were

found in a study of healthy 5-year old children (Quinn, O'Callaghan, Williams, Najman, Andersen, & Bor, 2001). Results after adjusting for psychosocial and biological confounders demonstrated an adjusted mean of 8.2 points higher for girls and 5.8 points for boys, regarding children who were breast-fed for more than 6 months, in comparison to children never breastfed.

One possible explanation to the effect on language may be that breast-feeding influences the mother-child interaction, enhancing the relationship and possibly also the way they relate through language. There is evidence that close physical interaction, such as breast-feeding increases levels of oxytocine (Grewen, Davenport & Light, 2010). Breast feeding is known to be involved in altering levels of different hormones (see Horta et al., 2007 for a review of health benefits from breast-feeding). This interaction may induce a positive biological response in the child and indirectly promote cognitive growth in the child.

A substantial effect of psychosocial confounders on the relationship with cognitive performance was demonstrated in our data. Argument exists as to whether the entire effect that breast-feeding appears to have on cognition can be explained by these factors (Der et al., 2006; Jacobson et al., 1999). However, as mentioned before, the results largely remained after adjustment for these confounders. Even though the results suggest that associations between duration of breast-feeding and subsequent cognitive growth persisted, the possibility remains that the relation found in this study is non-causal and arises from the effects of confounding factors that have not been controlled adequately in the analysis. One important factor we have not included in our studies is the mother's intelligence quotient. Mothers' IQ is a variable that has proved to be an important confounder for children's results (Jacobson & Jacobson, 2002; Rey, 2003; Uauy & Peirano, 1999). Including maternal IQ in models explaining relations between breast-feeding and cognitive performance in children has indicated that effects have disappeared (Der et al., 2006; Jacobson et al., 1999). Nonetheless, there are studies with positive results even after adjustment for maternal IQ (Rao, Hediger, Levine, Naficy, & Vik, 2002), and when taken in conjunction with the existing literature on this topic, (Anderson et al., 1999; Horwood & Ferguson, 1998; Kramer et al., 2008; Lucas et al., 1992; Oddy et al., 2004; Victora et al., 2005) the weight of the evidence supports the view that breast-feeding in fact is associated with increases in childhood cognitive development and cognitive performance.

Our data in Study II do not clarify whether effects of breast-feeding on cognitive performance are confined to effects upon IQ, or whether these influences affect other domains as well, in Study I, however, we investigated the relation between ToM ability and duration of breast-feeding. We could not demonstrate any direct correlations between ToM and breast-feeding, but the results indicate that duration of breast-feeding is an important mediator as to ToM. The relations demonstrated between breast-feeding duration and cognitive performance on Wechsler IQ test in this thesis is likely to be multi-factorial. One straightforward proposal is that the association found, reflects the effects that LCPUFA levels and, particularly DHA, levels have on early neurodevelopment.

What are the effects of LCPUFAs in mother's breast-milk, and in infants' blood, on children's subsequent cognitive performance?

Breast-milk provides essential nutrients to the child, and in particular PUFAs, are thought to explain the often found relation with cognitive performance (Anderson et al., 1999; Horwood & Ferguson, 1998; Oddy et al., 2004; Victoria et al., 2005). In Studies I and II the fatty acids present in the first breast-milk, colostrum, were correlated with children's cognitive performance at 6,5 years of age, associations were found for both intelligence measured by WISC-III and for ToM assessed with a ToM task (Lagattuta et al., 1997). Children with high scores on WISC-III and ToM had significantly lower levels of omega-6. The strongest correlations with cognitive test scores were yielded by some of the quotients of omega-3 and omega-6 fatty acids in colostrum, especially DHA/AA and DHA/DPA. The findings indicate that a larger amount of the omega-3 fatty acid DHA in relation to the omega-6 fatty acids AA and DPA is favourable for higher levels of intelligence as well as for ToM ability at six to seven years of age. These results remained after accounting for variables known to influence cognition, as parental education and gestational week. There were no relations with fatty acids in breast-milk samples taken at three months and succeeding cognitive performance.

In Studies I and II, blood samples taken from children at the age of three months were not found to be significantly related to subsequent cognitive performance at six to seven years of age, in either the full intelligence test (WISC-III), or any of the included subtests in Study II. Nor did the ToM test, used in Study I, yield any significant correlations with serum samples.

There are two types of experimental designs investigating PUFA and early cognition, observational breast-feeding studies, where children breast-fed for different duration are compared and randomized controlled trials involving supplementing pregnant or lactating women and formula feeding women (McCann & Ames, 2005). Our observational breast-feeding study contains samples from both mothers' milk and children's blood. Results involving breast-milk samples are rare in investigations of fatty acids and associations with cognitive performance, which makes our study a unique contribution to this field. However, fatty acids in mother's breast-milk and blood samples in the new born baby are highly correlated (Peng, Zhang, Wang, Zetterström, & Strandvik, 2007; Warstedt, personal communication, 2011). In this discussion, studies including breast-milk samples and blood samples are discussed in the same section.

The surprisingly high explained variance in the relation between DHA/AA in mothers' milk in relation to the children's total IQ scores, when combined in a model with duration of breast-feeding and gestational age, deserves to be discussed. First, it is the longest and most unsaturated omega-3 fatty acid in relation to AA that is involved. DHA is thought to be of special importance for construction of the brain during the last weeks of pregnancy, by its involvement in neurotransmission and neuroprotection (Bourre, 2006a; Hornstra, 2000; Innis, 2007a, 2008). The ratio is important since omega-3 and omega-6 families share the same enzyme- and elongase-system; an excessive intake of omega-6 can decrease the availability of both DHA and EPA in the brain (Hulbert, 2003; Simopoulos, 2009).

Relations between breast-feeding and intelligence scores have shown to be significant, but after adjustment for confounding factors it turned out weak. We entered, instead, the co-variables into a model with gestational age and duration of breast-feeding, together with DHA/AA. The importance of gestational age for IQ has been reviewed regarding children born premature (Johnson, 2007) as well as healthy children born at term (Yang et al., 2010). These studies showed that depending on gestational age, cognitive performance assessed by WISC abbreviated scores at age 6.5 years were lower in children born at 37 or 38 weeks, and in children born late, in week 43. Cognitive ability was demonstrated to be increasing with each additional week of gestation between 37 and 41 weeks (Yang et al., 2010). Duration of breast-feeding has been discussed previously, but to recall, it is found to be of significance for children's performance on intelligence tests. One interpretation of these findings is that the quotient of DHA/AA, together with the co-variables of duration of breast-feeding and gestational week, are unique contributors to IQ in children.

Theory of mind ability was in Study I, associated with both omega-3 and omega-6 fatty acids, but co-variables were important mediators to these results. However, the results regarding the relations to ToM remained after controlling for these confounding variables. A main finding was that the ratios DHA/AA and DHA/EPA in the final step of omega-3 fatty acid metabolism were related to ToM. This result is thus extending the previous cognitive findings in WISC-III with DHA/AA to also involve a later step in the metabolic chain, namely DHA/EPA. This latter finding also strengthens the importance of the balance between the omega-3 and omega-6 ratios. In Study II, we also calculated on steps of desaturases and found relations with step 4+5 in desaturation. Relative deficiency of LCPUFA may be the result of too little substrate for the process of biosynthesis or restricted factors in this desaturating process itself (Mahadik et al., 1996). In support of a deficit in the enzyme and elongase-system itself, we could not demonstrate a link between intake of animal or fish fat and the cognitive results. Nor was there any correspondence between IQ and the precursors LA and ALA. We therefore suggest that factors affecting the biosynthesis of PUFA other than the dietary intake of omega-3 may be of importance. Factors possibly affecting this influence on cognition can include genetic mechanisms of the biosynthesis (Innis, 2007a) or hormonal modulation of desaturases (e.g., through direct effects of insulin (Das, 2003).

Positive results regarding general cognitive ability and intelligence quotients in standardized test batteries have been found before, but in supplementation studies (Helland, 2003; Willatts, Forsyth, DiModugno, Varma, & Colvin, 1998). Supplementation of pregnant and lactating women with DHA demonstrated correlations between IQ scores in healthy children at 4 years of age and concentrations of the omega-3 fatty acids DPA and DHA. These findings, however, were in plasma phospholipids at 4 weeks of age, and no differences in breast-milk samples related to cognition were obtained (Helland et al., 2003). In another study, infants at 10 months of age, fed with formula containing AA and DHA, had significantly higher problem-solving scores than infants who received no LCPUFA (Willatts, et al., 1998). Mothers supplemented with DHA during lactation had children with significantly better scores on Bayley's Psychomotor Development index compared to the a placebo group, at 30 months of age, but there were no differences in Mental Development

index at the same age. Investigation in the same groups, with the same indexes at 12 months of age, did not reveal any differences (Jensen et al., 2005).

A review concludes that supplementation with DHA to pregnant or lactating mothers is positively related to mental functioning and cognitive abilities of the child (Eilander et al., 2007). The effects were small, inconsistent and tended to occur when measured at some ages but not others. One study of supplemented mothers during lactation even found negative correlations between levels of DHA at four months and word comprehension at 1 year of age, but findings disappeared at the age of 2 years (Lauritzen, Jorgensen, Olsen, Straarup, & Michaelsen, 2005). Other studies investigating prenatal fatty acid availability in healthy children in the same age category as ours, 6-7 years of age, found no associations between DHA or AA in plasma phospholipids from birth and cognitive performance as measured by the KABC. They did not find any relations in plasma samples taken at 7 years of age either (Bakker et al., 2003). A DHA + AA supplementation study in healthy four year-old children found no statistically significant relations regarding assessment with WPPSI (Birch et al., 2007).

Relations between PUFA levels and childhood development are examined more frequently at younger ages and assessment of visual development and visual acuity has often been made. DHA supplementation during infancy has shown inconsistent findings regarding effects on visual acuity (Birch et al., 2000; Malcolm et al., 2003; SanGiovanni et al., 2000). For example, there were significant relations at the age of 2 months but results were no longer significant when children became older (6-12 months) (SanGiovanni et al., 2000). In children born preterm, the latest Cochrane study concludes that there were no clear long-term effects on visual development (Schulzke, Patole, & Simmer, 2011). The sensitivity of tests and of the composition of fatty acids used in supplements, as well as the dosage has been discussed. It has been suggested that using electrophysiological methods, such as EEG, is a more sensitive way of finding possible effects of fatty acid supplementation (Cheatham et al., 2006; SanGiovanni et al., 2000).

To elaborate on the breast-milk findings we only found relations in colostrum, the first breast-milk, and not in mature milk at 3 months. This finding may simply reflect the levels of PUFA that the child achieves during pregnancy (Innis, 2007b). The findings could thereby simply point at the importance of omega-3 fatty acids during pregnancy. But we also showed that the longer the duration of breast-feeding the better cognitive function, and even if mature milk tends to have lower amounts of PUFAs, the child will still receive larger total amounts of fats the longer they have been breast-fed. Since breast-milk is an important source of PUFA in the infant, these findings substantiate the link shown between breast-feeding and cognitive development. Moreover, Dunstan et al. (2007), showed that DHA supplementation to pregnant mothers modified the breast-milk composition in itself, it did not only give a rise in DHA and EPA levels in the new-born baby, but also modified children's fatty acid composition at 1 year of age in the same way. This finding supports the view that it is not only the pregnancy fatty acid levels that are of importance but also the breast-milk composition.

Children develop rapidly and certain skills are not fully developed until school age, or even later. To be able to detect possible positive effects on cognition and behaviour from PUFAs upon the brain during its intense period of construction, two aspects is of special importance. First, comparisons must be made in the same age categories, and, second, the age group must have reached an age in which these abilities are developed (in our case at 7 years of age). At this age ToM ability is thought to be fully developed, children have further more advanced working memory, whereas inhibitory control and reading skills are often at a developing stage.

To summarize this discussion, we have demonstrated a relation between PUFA levels at birth and cognition in terms of full IQ scores on WISC-III as well as better ToM ability at 6.5 years of age. The quotient of DHA/AA in colostrum seems to be of particular importance and is highly related to IQ together with duration of breast-feeding and gestational week. There were no relations indicated in mature milk or in blood samples. Most studies investigating PUFA and cognition have used blood samples from the child; our findings suggest that further studies of breast-milk and long term outcome are necessary.

What are the effects of supplementation with EPA, an omega-3 LCPUFA, on cognitive performance and behaviour in children with ADHD or at risk of developing ADHD?

Regarding supplementation with EPA the results in this thesis showed no overall effect in children with ADHD or in children with neurodevelopmental immaturity, (at risk of developing ADHD) after 15 weeks of treatment, compared to placebo groups. The groups did not differ regarding any of the cognitive tests or regarding the teacher and parent behavioural rating scales, except for a significant result in a subscale in teacher ratings of inattention and cognitive difficulties in CTRS, where the EPA group improved significantly after treatment. Moreover, there were some findings regarding two subtypes of children with ADHD in Study III; one with additional oppositional symptoms and another with less hyperactivity/impulsivity. Descriptively, both groups improved in the teacher rating scales, but results were only significant in the first group.

To start with, the negative findings, as regards not being able to decrease core symptoms of hyperactivity in children with ADHD with a supplementation of fatty acids, our findings corroborate earlier findings (Hirayama et al., 2004, Johnson et al., 2009; Raz et al., 2009; Vaisman et al., 2008; Voigt et al., 2001). General findings of behaviour symptom reduction in the whole group of children with ADHD are rare (Transler et al., 2010), but some other studies apart from ours, also found reductions of Conners rating index of attentional and cognitive problems seen in the whole ADHD group (Belanger et al., 2009; Johnson et al., 2009; Richardson & Puri, 2002; Stevens et al., 2003; Sinn et al., 2007). In the present study, a high rate of co-morbidities was accepted; this design is similar to one of the previous studies (Johnson et al., 2009), and differs from the others. Johnson et al. (2009) also had a high degree of inattentive children, about 50%.

Turning to the results of the subtypes we demonstrated that a subgroup of children with less hyperactivity and impulsivity at baseline had greater treatment effects than children with hyperactivity/impulsivity. This finding differs from an RCT study showing that children

with ADHD were improved regarding Conners parent ratings of hyperactivity and impulsivity (Belanger et al., 2009).

Results seem mostly to be gathered around inattentive symptoms (Johnson et al., 2009; Richardson & Puri, 2002; Sinn & Bryan, 2007). This is interesting since it has been suggested that the attentive subtype of ADHD is an entirely different kind of disorder (Lahey & Applegate, 2001; Milich et al., 2001). Children with more inattentive behaviour have been discussed as being qualitatively different from the ADHD hyperactive/impulsive subtype. This subtype is more common than the combined or the hyperactive/impulsive subtype and the attention problems may be different (Barkley, 1997a). The attention problems are more general and related to deficits in selective attention and a lower IQ. This subtype may also have a different development and different characteristics, as more socially withdrawal and lower levels of academic achievement. Moreover, they tend to have different co-morbidity patterns than the hyperactive/impulsive subtype, including more mood disorders and less externalizing problems (Barkley, 1997a).

We found no significant effect of treatment according to the parents' ratings. This was probably due to a large placebo response in parents' ratings. It might be argued that the parents, since they agreed to participate in the study, had a positive interest in omega-3 and therefore had a bias towards rating improvement in their children. The low number of girls makes it difficult to draw any conclusions for females. The clinical effect was moderate, i.e., about half of what can be seen with stimulant treatment. Results between studies differ regarding parent and teacher ratings; is unusual with significant effects in both, but some studies have effects in parents' ratings (Sinn & Bryan, 2007) other in teachers' ratings (Richardson & Montgomery, 2005). This difference is discussed further in the Rating scale section below.

In Study IV we widened the focus on treatment to include children with unspecific symptoms of neurodevelopmental immaturity or delay, all at risk of developing ADHD. The cognitive test battery was extensive and covered a large number of tests including executive tasks. There were no significant differences between the groups after the EPA supplementation in any of these measures. Earlier studies have shown limited significant improvements in some cognitive outcome measures, for example reading and, spelling, in children with DCD (Richardson & Montgomery, 2005),

Regarding cognitive outcome measures there is limited support for effects by PUFA supplementation. Several studies have obtained significant, though medium-sized, effects (Sinn et al., 2008; Vaisman et al., 2008), but only in few, out of a large number, of cognitive outcome variables. These findings involve improvements in ability to control and switch attention (Sinn & Bryan, 2008). Multiple neuropsychological phenotypes of ADHD may also account for variability in omega-3 fatty acids supplementation studies. According to the different subtypes, children with the combined subtype may be more likely to have deficits in inhibition relative to the inattentive subtype (Barkley, 1997b).

We used a pure EPA supplement, while most of the reviewed studies used supplements with a combination of omega-3 and omega-6 fatty acids (Hirayama et al., 2004; Johnson et al., 2009; Richardson & Puri, 2002; Richardson & Montgomery, 2005; Sinn & Bryan, 2007;

Stevens et al., 2003). The results from the mixed formulas have been varying, and it seems that a pure EPA at least is as good as the mixed formulas in treating behaviour symptoms of ADHD.

A review concludes that absence of positive findings in some studies may depend on the type of intervention, short treatment duration or the presence of AA in the active treatment drug. AA can have negative influence on the conversion of ALA into omega-3 LCPUFA (Transler et al., 2010).

How does supplementation with EPA, an omega-3 LCPUFA, influence the levels of the fatty acids in serum and red blood cells of children with ADHD and children at risk of developing ADHD?

Blood samples taken before and after treatment with EPA shows that children treated with EPA actually have an increased EPA concentration in both serum and red blood cell membranes in Study III. This increase was about 160%, which is significant. Omega-6 fatty acids decreased at the same time in serum as well as in RBC-membranes. Children with additional oppositional symptoms, and who responded to the treatment, had significantly lower levels of EPA at baseline assessment compared to the children in this group who did not respond to the treatment. The increase of EPA in serum and membranes is not surprising, as this is shown in several studies (for a review, see Kremmyda, Vlachava, Noakes, Diaper, Miles, & Calder, *in press*).

Some studies have shown that DHA concentrations were significantly lower in the ADHD participants compared to controls (Colter et al., 2008; Mitchell et al., 1987; Stevens et al., 1995). We found that children with co-morbid ODD had significantly lower concentrations of total omega-3 PUFA and EPA as well as higher AA/EPA and AADHA ratios in serum phospholipids. Similar findings were observed regarding the omega-3 PUFA levels in RBC membrane phospholipids, as well as significantly lower concentrations of dihomo-gamma-linoleic acid (DHGLA), AA and total omega-3 PUFA. These relationships have also been found in other studies of children with ADHD. Interestingly, clinical improvement in this group of children with ADHD and ODD showed increasing EPA and decreasing omega-6 FA concentrations in serum phospholipids and RBC, indicating that this subtype may have a PUFA deficiency possible to “repair”.

Earlier findings have demonstrated increasing omega-3 PUFAs and decreased omega-6 PUFAs in plasma phospholipids after supplementation (Voigt et al., 2001). These changes may influence the balance of omega-3 and omega-6 and may be related to the mechanism of the desaturase enzyme system, such as delta-6 and delta-5 desaturase, which catalyze the conversion from precursors to DHA and Adrenic acid, respectively (Vaisman et al., 2008).

Unfortunately, there were too few blood samples taken in the neurodevelopmental group to make any analyses, but there is no reason to believe that they should diverge from the results in the larger multi-centre study.

Do LCPUFAs have differential effects on cognitive functions, such as working memory, inhibition, problem-solving, language, or theory of mind?

In this thesis, a range of specific cognitive abilities have been assessed in different studies on both typically, and, atypically developed children. The findings will be reviewed in the light of other fatty acid studies investigating cognitive functions.

In Study I, we found that children at 6.5 years of age had better performance on a ToM task when ratios between omega-3 and omega-6 fatty acids were in favour of omega-3 in mothers' breast-milk (colostrum) at the time of the children's birth. This indicates that a higher proportion omega-3 is better for ToM development. In Study II, we could not demonstrate any relation between fatty acids and any of the subtests in WISC-III (but significant relations with Full scale IQ and verbal IQ, as discussed above). The lack of findings in the subtests from WISC-III, but at the same time considerable effects on full scale IQ and verbal IQ, may indicate that the subtests used were not sensitive enough to find differences.

In Study III the results of cognitive measures included are not reported yet, cognition is, however, rated by teachers and parents in one of the Conners indexes ("cognitive problems/inattention"). Children with ADHD receiving omega-3 supplementation (EPA) improved their results in teacher ratings and lowered their scores in comparison to the placebo group, thus indicating that cognitive difficulties decreased in this group. Study IV examined treatment effects in children with neurodevelopmental immaturity, at risk of developing ADHD, and embraces a large battery of cognitive tests, related to several cognitive domains, ToM, working memory, episodic memory, inhibition, problem-solving and planning, verbal tasks (including reading and spelling). We did not obtain any differences between the groups on any of the tests.

One obvious interpretation from all of these studies is that we do not find any short- or long-term relations between PUFAs and cognitive abilities, except from ToM. Possible explanations for the few findings of relations between specific cognitive functions and fatty acids, involve methodological limitations, such as small sample size, and, regarding the treatment studies, the type of supplements (EPA) used and the dosage (0,5 mg). Methodological issues are discussed further, later in this section.

In the following discussion the cognitive findings will be discussed point by point, starting with working memory

Working Memory

According to the findings in several of the included studies, no single PUFA, or any ratios between omega-6 and omega-3, seem to be able to single out working memory as measured in the studies in the present thesis. This corroborates earlier findings (Hirayama et al., 2004; Sinn et al., 2008; Stevens et al., 2003), although only Sinn et al., (2008), used the same working memory task as one of ours, i.e., digit span from WISC. One study have related signs of fatty acid deficiency (FADS) to significantly poorer working memory performance in a digit recall test in dyslectic children aged 8-12 years. The results applied only to boys (Richardson et al., 2000). Another study demonstrated that children aged 6-16 years with

higher intake of total omega-3 and omega-6 PUFA (10 g/day increase) in their diet showed lower risk for poor performance on the Digit Span test, but not on Block Design, Arithmetic, or a Reading comprehension task. Furthermore, higher levels of dietary cholesterol were associated with lower risk of poor performance on digit span (Zhang et al., 2005). The two positive findings suggest that higher LCPUFA intake could be beneficial for children's working memory, although there were no biochemical data included in any of those studies, which makes the link to fatty acids weak.

According to Baddeley's model (2000) the phonological loop, with its storage of verbal material is separate from the visuo-spatial sketchpad that handles visual and spatial representations. Most studies have used auditory working memory tasks; only Hirayama et al., (2008) included a visual working memory task, thus implying that results regarding auditory working memory are inconsistent, whereas there is a lack of visual working memory tasks. Working memory deficits have also been related to difficulties in learning, e.g., reading, spelling and mathematics in young children (De Jong, 1998; Gathercole & Alloway, 2008). In children with ADHD, working memory deficits are frequently shown, and, two reviews have concluded that visuo-spatial deficits are larger than verbal working memory deficits (Martinussen et al., 2005; Willcutt et al., 2005). The only visuo-spatial task used in trials with LCPUFAs, to my knowledge, is the Clown used in Study IV. Despite the negative findings regarding the Clown task, the clear association between DHA and visual functioning (Birch et al., 1998a; Birch et al., 1998b; Birch et al., 2000) together with the support for DHA involvement in hippocampal activities (Cao et al., 2009) and in spatial learning (Santos de Souza et al., 2011), suggests further exploration of visuo-spatial working memory in relation to LCPUFAs.

Problem-solving and planning

There were no relations between LCPUFA and Block design or Tower, the tasks used for investigating possible effects of omega-3 fatty acid treatment in Study IV. Several other studies have been using the Block design test in PUFA supplementation studies of school-aged children with ADHD without being able to demonstrate any effects, which confirms previous results (Sinn et al., 2008; Zhang, Hebert, & Muldoon, 2005).

Several investigations with positive results have, however, been made in infants 9-10 months of age. One supplementation study (with DHA and AA) demonstrated that infants who received the supplemented formula had significantly higher problem-solving scores than infants who received no LCPUFA (Drover, Hoffman, Casteneda, Morale, & Birch, 2009) and another study in which mothers had DHA-enriched diet during pregnancy had children with significantly better problem-solving (Judge, Harel, & Lammi-Keefe, 2007). However, two more studies showed no such effects on problem-solving (Lauritzen et al., 2005; Willatts et al., 1998). One possible explanation to the negative findings may be that Willatts et al. (1998) used a relatively low concentration of DHA in their formula, and Lauritzen's (2005) study of mothers supplemented during breast-feeding had high levels of DHA in breast-milk in the control group (Drover et al., 2009). DHA is, as discussed in the introduction, implicated in processes that underlie cognitive development. Theoretically, improvements in synaptic efficiency (Uauy et al., 2000) and speed of transmission (Yehuda et al., 1999), and

myelination (Durand et al., 1999), could increase the speed and accuracy of information processing.

Verbal tasks, reading and spelling

In Study II, the verbal IQ scale was positively related to LCPUFAs in our group of healthy children with typical development. This corroborates earlier findings in 4-years old healthy children supplemented with DHA, in that study there were no effects found from the supplementation, however, a statistically significant positive relation was found in the level of DHA in children's blood and better performance on the Peabody Picture Vocabulary Test (PPVT) (Ryan & Nelson, 2008). Another study also found positive correlations between omega-3 RBC membrane lipids with reading in adults with dyslexia, and in fact also in the controls. Further, the ratio omega-6/omega-3 correlated negatively with reading in dyslectics (Cychlarova et al., 2000). Improved vocabulary can alternately result from improved attention and less hyperactivity, which in turn results in longer periods of reading and retention of information, as there seems to be a link between inattentive behaviour and language difficulties for instance in reading skills (Mather & Wendling, 2005). If low omega-3 LCPUFA levels in some way contribute to poor verbal ability and reading performance, then optimizing the balance of omega-6/omega-3 LCPUFAs through omega-3 LCPUFA supplementation may demonstrate beneficial results. Further, it is important to include biochemical blood analysis before and after supplementation.

One study, in which mothers were supplemented (DHA and EPA) during pregnancy, found no differences in vocabulary (PPVT) in healthy children at the 2.5 years follow-up. DHA supplementation has been examined in relation to language production and verbal comprehension in 14-month olds as well. One study of term infants found no improvements (Auestad et al., 2001), and supplementing with DHA led to lower scores on Vocabulary than in the controls. However, a follow up at 39 months did not replicate this finding, and tests of receptive and expressive language showed no differences between the supplemented group and the placebo group (Auestad et al., 2003).

In Study IV we obtained no differences regarding the EPA-supplemented group compared to the placebo group regarding any of the age appropriate verbal tasks, Vocabulary (from WISC-III), reading (a word reading task), or spelling. Previous studies show inconsistent findings, as there are no significant improvements in Vocabulary among the treated children in the ADHD-group in relation to the placebo group (Sinn et al., 2008). Improvements have, however, been found regarding reading and spelling in children with DCD (Richardson & Montgomery, 2005). A study of fatty acid supplementation among children selected for dyslexia (Richardson & Puri, 2002) found improvements but only regarding parents' ratings of cognitive problems, and similar results were found. The lack of studies measuring verbal ability, reading, and spelling in children with ADHD is somewhat surprising. Studies of dyslectics, with a large clinical overlap with ADHD, indicate some cautiously optimistic findings regarding both LCPUFA status and improvements in symptoms after supplementation.

Inhibition

Inhibition, as measured with Statue in Study-IV, showed no treatment effects regarding supplementation in children with neurodevelopmental immaturity at risk of developing ADHD. Statue is primarily a motor inhibition task, which requires the child to stand still with eyes closed despite disturbing events. Previous studies with objective measures of impulsivity or inhibition have not showed any effects either (Sinn et al., 2008; Voigt et al., 2001). Voigt et al. (2001) used Children's colour trail test and Sinn et al. (2008) used knock-and tap-test from Nepsy. LCPUFA have been reported by teachers (Richardson & Montgomery, 2005) and parents (Sinn & Bryan, 2007) to improve impulsivity.

Impulsivity is a symptom of inhibitory control deficiency and impulsivity is one of the core symptoms in ADHD (Castellanos & Tannock, 2002). Impulsiveness is often linked to the prefrontal cortex (Dalley et al., 2008; Fuster, 2002), and is usually understood as a lack of response inhibition. This indicates that difficulties to withhold a previously rewarding response, will eventually lead to impulsive behaviour. In adults impulsivity may manifest as aggression and other disorders characterized by impulsivity are a diverse group of conditions related to aggression; violent behaviour, deliberate self-harm, or the phenomena of suicide, or even homicide (Hallahan & Garland, 2004). Some studies have shown a decrease in impulsive behaviour after supplementation with LCPUFAs. DHA supplementation in a group of university students has for example proved beneficial in stabilizing aggression (Hamazaki, Sawazaki, & Kobayashi, 1996). Another supplementation RCT study involved young adult prisoners, supplements containing DHA, EPA and ALA (Gesch, Hammond, Hampson, Eves, & Crowder, 2002) reduced their offences by 35% when supplemented for 2 weeks or more; interestingly there was a linear relation with the greatest reduction for the most serious, violent behaviours. Some evidence that modification of serotonergic neurotransmission may be a mode of action of LCPUFA supplementation has been suggested (Hallahan & Garland, 2004). To conclude, the association between disordered impulsivity and decreased levels of omega-3 LCPUFAs, in combination with some beneficial results from supplementation of this deficiency, leads to the suggestion to further evaluate inhibition and impulsivity in children.

Theory of Mind

In Study I, a relation between an advanced ToM task and LCPUFAs in 6.5 year old healthy children was demonstrated. A quotient with higher levels of omega-3 in relation to omega-6 in mothers' breast-milk at birth was favourable for having more advanced ToM ability. To my knowledge, ToM ability has not been investigated in relation to fatty acids in healthy children before. However, two previously related findings regarding pro-social behaviour are reported (Kirby et al., 2010a; Hibbeln et al., 2007). The first was a study supplementing healthy school-aged children, which found significant differences in parents' ratings of the children's pro-social behaviour (in Strengths and Difficulties Questionnaire, SDQ) after 16 weeks of supplementation (DHA and EPA). However, these findings showed a decrease in pro-social behaviour in the control group. The researchers suggest that the treatment with LCPUFA may have a protective effect. The other study investigated the effect of mothers' seafood intake during pregnancy and children's subsequent development at 6 years of age. Low intake of seafood in mothers was related to a worse pro-social behaviour in SDQ. These

results are of particular interest since there seems to be a link between ToM ability and pro-social behaviour in children (Eggum et al., 2011; Renouf et al., 2010).

The first study, (Eggum et al., 2011) investigated links between emotional understanding, ToM and pro-social behaviour in children at different time periods during development. The second study examined the relation between ToM ability, indirect aggression in kindergarten, and children's pro-social behaviour. They found a relation between ToM and indirect aggression, but this relationship was moderated by pro-social behaviour and was significant only for children with a low or average level of pro-social behaviour (Renouf et al., 2010). These findings are also interesting in relation to the reports on beneficial effects of omega-3 supplementation in reducing aggression in adults (Gesch et al., 2002; Hamazaki et al., 1996, 2005) and to some extent in children with typical development (Itomura et al., 2005).

In Study IV the same ToM task, but adapted and shortened, was used among children with neurodevelopmental immaturity, at risk of developing ADHD, for evaluating possible treatment effects from EPA supplements during 15 weeks. No treatment effect was shown. One possible explanation is the alteration of the task, instead of recording children's full answers and making categorizations retrospectively, the responses were written down. The changed procedure may have had an impact on the experimenter's relation to the child, and making them less prone to elaborate their answers.

In the present thesis none of the laboratory cognitive tasks, except from ToM, yielded any positive effects from PUFAs. One possible explanation is that there is no effect, but there might be other explanations. First, there seems to be only moderate effect sizes regarding deficits in executive functions in children with ADHD compared to controls. This may indicate that executive dysfunction is not a unifying feature of ADHD (Sergeant, et al., 2002) and individual variability may cause effect in some cases and not in others. Another explanation to the lack of treatment effects on cognitive measures may be multiple phenotypes of ADHD (Sonuga-Barke, 2005), which may be underlying subgroup findings in the results of supplementation studies. Children with inattentive ADHD type may be more sensitive to treatment with omega-3 fatty acids. They may also be more likely to have impairments in working memory (Alloway et al., 2004), and regarding tasks demanding inhibitory control they are slow and show a cautious response style (Adams et al., 2008). The possibly different subtypes of ADHD need to be more clarified as well as the neuropsychological profiles of the different subtypes, before we can evaluate treatment effects more exhaustively. Furthermore, neuro-imaging studies have demonstrated that children with ADHD, compared to children without ADHD, use larger and more diffuse networks of brain regions when performing, not only complex, but also simple, executive tasks (Durston et al., 2003). Tasks of executive functions are demanding and require simultaneous application of multiple cognitive processes. In children with delayed frontal maturation (Biederman & Faraone, 2005), LCPUFAs may not be able to improve these deficits.

To summarize, there are inconsistencies regarding effects on cognitive tasks. One explanation of the inconsistency of the findings is that only children with fatty acid

deficiency respond to treatment. Another explanation might be that other micronutrients are needed as well. A review concluded that out of 13 controlled studies on micronutrients 10 reported positive results (Benton, 2008). This indicates the importance of pre-controlling fatty acid status and also micronutrient status.

Moreover, even though some objective tests of memory, impulsivity, problem-solving, planning, attention, verbal tasks, reading skills, and spelling have been applied, they have been used in different studies and no two studies have used the same battery of cognitive tasks. The lack of consistency regarding cognitive tasks employed, make comparisons of results in different studies difficult. Many of the measures are behaviour rating scales and not cognitive neuropsychological laboratory tasks. Furthermore, the issue remains as to what the different tests of executive abilities are actually tapping. The lack of cognitive neuropsychological tasks included in the trials is unfortunate. Including this kind of tasks in clinical trials demands neuropsychological competence as well as an extensive amount of work, demanding both time and effort. Nevertheless, the theoretical grounds for the assumption that LCPUFA may be of importance for frontal lobe performance, speed of cell signalling, transmitter release and uptake are obvious. Empirically, the effects of LCPUFA suggest possible effects on executive function and thus require further attention.

METHODOLOGICAL CONSIDERATIONS

In this section, the focus is on aspects that are important for the methodology used and for the conclusions drawn from the studies. The attention is first turned to assessment issues, thereafter to the specific issue of measuring cognition in relation to nutrition. Fatty acid issues are the next focus with a discussion about difficulties with evaluating effects in fatty acid studies, problems related to type of supplementation and also dosage. Some words about sample size end the methodological considerations section.

Most studies concerning fatty acids and cognition use behavioural rating scales to either parents or teachers. We included experimental measures for assessing a range of cognitive and executive functions as well as theory of mind. The inclusion of a ToM task is unique for our studies. This kind of measure is seldom used in LC-PUFA supplementation studies despite the known associations between PUFA, brain-behaviour models of PUFA and certain tasks. This is also the case for ADHD, a typical example is working memory, which is central to many brain-behaviour models of ADHD (Castellanos & Tannock, 2002; Pennington & Ozonoff, 2006; Sonuga-Barke, 2005). By using laboratory tasks we are enabled to measure cognitive abilities more directly, and without the relational bias from parents or teachers.

The concurrent use of biochemical measures, is another strength in our studies. These data makes it possible to investigate compliance and deficiency in the clinical trials. We also have breast-milk samples from different points in time, which are of importance since it probably reflects the PUFA levels of the child during a crucial period of brain development, i.e., pregnancy. Moreover, the combination of studies with prospective longitudinal and cross-sectional designs strengthens our results. The inclusion of children with typical development as well as children with disabilities also gives a unique contribution to the field.

Issues of cognitive and behavioural assessment

Rating scales issues

In most studies the main informants regarding the child's behaviour are the parents, who also are part of the family system, and a bias in the description of the child may thus reflect other factors than the child's behaviour. If the parent for example is not well that may influence the ratings (Murray et al., 1999). Furthermore, raters are often coloured by their relation to the child, and children identified positively are more likely to be rated in a positive manner, even in cases with some unfavourable characteristics in the child's behaviour, while a child identified negatively are more likely to be rated in an unfavourable way despite several favourable behaviours (Gupta & Kar, 2010).

Behavioural rating scales are constructed to measure traits or dimensions to capture the problem behaviours of the child, thus the items often are negatively expressed. Some items are also vague and abstract and their meaning can vary from rater to rater, which may affect the consistency in ratings. Besides, the raters have different views of what the rating categories mean, for example the category "seldom" may mean once a week for one rater and once a year for another; this bias lowers the consistency of ratings and, thereby, their reliability (Gupta & Kar, 2010).

In order to get estimations of the children's behaviour in two different settings, we have used both teacher and parent's ratings in Studies III and IV. However, there often are discrepancies among reports from different sources (Sawyer, Clark, & Baghurst, 1993; Verhulst & van der Ende, 1992). In Study III teacher ratings were more discriminative than parents ratings, this is in line with the suggestion that parents ratings are less informative for evaluating ADHD behaviours when considering sensitivity, specificity, and overall classification accuracy, than teacher ratings (Tripp, Schaughency, & Clarke, 2006).

Another factor to take into consideration when comparing rating scales between parents and teachers is the school setting, which requires certain abilities from the child, such as being able to sit still, to complete assignments and to participate in group activities at the same level as the peers, to mention a few (Teeter et al., 2009; Kamphaus, Reynolds, & King Vogel, 2009). In Study III parents ratings seemed to reflect a positive interest in the participation and possibly an expectancy of improvement, both the active group and the placebo group rated improvements in children's behaviour.

Neurocognitive laboratory assessment issues

The rationale for the choice of neuro-cognitive tests is described in the Empirical studies section. We have used both a broad and a specific approach, and there are, unfortunately, some problems associated with both.

Standardized cognitive test batteries, as the Wechsler scales, are used in the thesis to evaluate overall cognitive capacity. In nutritional studies such standardized tests have been frequently used. However, these tests are constructed to identify children who develop atypically, and may have limited possibilities to discriminate effects of nutritional differences (Cheatham et al., 2006; Hughes & Bryan, 2003). Moreover, the global measures may not be sensitive to manipulations that produce specific effects (Kamphaus et al., 2009).

Several researchers stress the importance of using experimental laboratory measures instead of measuring overall functioning, as is often done in standardized scales like Bayley scales of infant development and the Wechsler scales. We have used a battery of laboratory tests within several cognitive domains for increasing the possibility to detect specific aspects of cognitive functioning, which has been suggested for nutritional research (Busch, 2007; Wainwright & Colombo, 2006). In order to tap specific relatively pure aspects of a cognitive or executive function it is also important to find tasks with high specificity (Lezak et al., 2004). Executive tests used by researchers tend to be blunt and underspecified in terms of what cognitive processes they involve (Burgess, 1997). We have also tried to find age-appropriate tasks.

The assessment of ADHD in our studies included both cognitive tests, as with WISC-III, behavioural rating scales, and CPT-tests. It is, however, important to note that there is only a low correspondence between these rating scales and WISC-III (Naglieri et al., 2005).

Repeated measures are a common design in treatment studies, but improvement at end-point may be due to practice effects rather than real changes (Mollica, Maruff, Collie, & Vance, 2005). Our investigations of fatty acid supplementations have used test-retest designs for evaluating possible effects of supplementation. Test-retest reliability is essential to consider in a test that is likely to be used serially. WISC is a stable instrument with average test-retest coefficients of .93 for FSIQ.

Practice effects are in Wechsler tests largest for children 6-7 years, and after one month they have shown to improve about 8.3 points at full scale IQ. These effects become smaller with increased age (Kamphaus et al., 2009). Where possible we therefore have used parallel versions of the tests included, also, the randomized design and the adding of an extra control group (in Study IV) are actions taken to avoid this problem.

Definitions of executive functions vary, so comparison among studies using executive functions is difficult. This applies also to executive tests which are differently defined, for example, one test can at one time fall under the broad term “working memory”, at another time be called “verbal working memory”, and, finally, in a third article it may be specified as “verbal aspects of the central executive in working memory”.

Cognitive tasks designed to tap different specific cognitive abilities often have problems with validity. Tasks are seldom “pure” measures of a single ability, but often tap abilities adjacent to the ability it is intended to assess. Planning and inhibition are typical examples of impure tasks (Towse & Cowan, 2005). There are differences between experimental tasks in test settings and everyday life tasks, often discussed as ecological validity. In experimental and neuropsychological tasks this lack of ecological validity has often been criticized (Burgess et al., 2006).

Assessment of cognition in relation to nutrition

Cognitive measures in nutrition studies have been discussed thoroughly (Chetham et al., 2006; Rosales et al., 2009; Wainwright & Colombo, 2006). Most studies evaluating cognitive effects from omega-3 fatty acids use a high number of outcome variables, and so also in this thesis, thus raising the risk of false-positive findings (type I error). We therefore

corrected for multiple comparisons with an alpha level of .05. However, there is not only a problem with the large number of different tests, there is also a methodological advantage to use different tests, since different tests can help to isolate the relevant variables (Towse & Cowan, 2005).

Validity relies here on two particularly important factors, first, on the necessity to have reliable outcome measures that are sensitive to detect a behavioural change, and, second, on the construct validity, here defined as the possibility to control for alternative explanations also discussed as confounding factors. Wainwright & Colombo (2006, p. 965) underline that "various outcome measures may be necessary to deconstruct the experimental manipulations in light of plausible alternative explanations before concluding that the observed effects are attributable to a specific cognitive process".

In studying cognition and nutrition in children, it is important to consider the developmental aspects. Many important developmental changes occur during the last trimester of pregnancy and during the first years of childhood. Furthermore, knowledge of neuropsychological development is important, for example DHA is accumulated in the brain during different periods of development (Chetham et al., 2006). One other aspect is that LC-PUFA influences can be seen in a long-term perspective; effects can occur during the foetal period and may possibly last for the entire life span. For example, early nutrition has been related to a range of long-term outcomes through various neural mechanisms, such as blood pressure, obesity and intelligence (Lucas, 1998; 2007). Nutritional effects can also occur in different periods of life and may have a relatively short-term effect on cognition or behaviour. Since there is still little knowledge of when periods of sensitivity occur in relation to LC-PUFAs, it is difficult to optimally time supplementation (Rosales et al., 2009).

The cognitive tests used in the longitudinal studies are intended for 6.5-year old children, and the measures in our intervention studies are attempted to be adapted for use in seven year olds, and, school-aged children, respectively. Effects from fatty acids may be subtle, since nutrition is only one of many influential factors on cognitive and brain development during childhood (Hughes & Bryan, 2003). One such important question concerns whether rating scales are more sensitive in tapping change in supplementation studies than are neuro-cognitive laboratory tasks. Most clinical fatty acid studies have found results regarding rating scales and more seldom in a neuropsychological test battery, which is the case also in our studies. In our studies there were only some moderate correlations between the rating scales and the neurocognitive measures. Naglieri et al. (2005) found statistically insignificant and low correlations between WISC-III IQ scores and the severity of parent- and teacher-rated inattention and impulsive-hyperactive behaviours, but the inattentive and impulsive-hyperactive behaviours were not registered during the administration of the Wechsler IQ test.

Assessment in children

Measuring cognition in children render special considerations compared to adults. For example, when assessing working memory in children it is important to consider that children's memory span is shorter, and it may be that also the forward condition of a span-task taps processing (since effortful processing is needed even in relatively simple tasks) and

therefore becomes a measure of working memory rather than short-term memory. This might also be true for other populations with low memory capacity (Towse & Cowan, 2005).

Children are flexible and can adopt different strategies in different test situations (Miyake, Emerson et al., 2000), which may lead to low test-retest reliability. Parallel forms of the tests are, unfortunately, linked with the same difficulty, i.e., even though new contents and items may be used, the task format remains the same (Phillips, 1997).

Assessments in children with ADHD

Some characteristics of children with ADHD may influence the assessment, for example, the cognitive performance of individuals with ADHD is often characterized by variation and fluctuations in cognitive control. It is also often reflected by a highly inconsistent and inaccurate response style (Van Meel, Heslenfeld, Oosterlaan, & Sergeant, 2007). It has, moreover, been suggested that individuals with ADHD differ from normally developing individuals in behavioural adjustment to errors, for example, they get slower after inhibition failures and are slowed down to a greater extent (Van Meel et al., 2007). This is important to consider when deciding or choosing tasks for measurement in studies.

Administration issues

In Study IV the test battery was extensive (2h), which may inflate performance variability by increasing fatigue and attention and even decrease participation compliance. Children with ADHD are also known to have a lengthy administration time. To minimize fatigue and loss of attention or motivation we had breaks when needed and at least one break per trial, during which fruits were served. The child was also encouraged by the experimenter to move around. Our perception is that almost all children enjoyed the assessment session and the full attention they received from the experimenter. However, it is possible that some of the dropouts in Study IV depended on the extensive test battery demanding a large effort from the child.

Issues related to LCPUFA studies

Making comparisons between the controlled trials of fatty acid treatment in ADHD is difficult. The few proper studies that are made differ regarding methods, compositions of fatty acids and also concerning biochemical measurements. *Methods* have varied in several aspects, from identifying the study populations, the study designs to the outcome measures. The *compositions of fatty acids and placebos* used have varied; the earliest studies were focusing on omega-6 fatty acids while more recent studies have been using omega-3 fatty acids. The placebo capsules have had olive oil or castor oil. In addition only a few trials have included *biochemical measures* of fatty acid status, which could be used with different purposes, either in the selection of subjects, to monitor treatment compliance, or to investigate associations between blood fatty acid levels and outcome measures or baseline characteristics. Measures of fatty acid levels have been made in serum or in plasma, as well as in red blood cell membranes (Busch, 2007; Richardson, 2006).

The type of supplements chosen is discussed in the introduction. We used a pure EPA supplement. Choice of placebo supplements is also important since it often has been olive oil or rapeseed oil, which may affect the balance between the fatty acids. Blinding is difficult in randomized placebo controlled studies if the participants are able to taste the flavour of fish

(Damico et al., 2002). In Study III, however, there was the same number of dropouts between the groups indicating that dropping out did not depend on identifying the substances. Some children had difficulties swallowing the capsules even though they were designed for children.

There is a lack of standardization of *dose of fatty acids* administered (Heinrichs, 2010) and optimal doses for different developmental ages need to be established (Ryan et al., 2010). One trial examined if different dosages given to patients with schizophrenia gave different results. The findings could indicate that there is an optimal level of benefit in schizophrenia, where both too low and too high doses showed less results (Peet et al., 2001). However, more trials are needed for testing optimal dosage and different dosages might be required for different diagnoses and different age groups. A number of clinical studies lack biochemical data. There is still no standardized method or practice of analyzing and assessing levels of fatty acids in plasma or tissue (Heinrich, 2010).

Inclusion criteria differ among studies; some included children with co-morbid disorders, and others had strict inclusion criteria. Regarding ADHD as a diagnosis, “pure” ADHD is also uncommon (perhaps 20% of presented cases), and the majority has co-morbid problems of the kind mentioned in the introduction (Rasmussen & Gillberg, 2000). Regarding establishment of a diagnosis, what subtypes that are included, levels of symptoms, onset of symptoms, co-morbidity etc., vary heavily between studies, thus making comparisons difficult. There are, for example, fewer studies regarding the hyperactive/impulsive subtype. This makes it difficult to compare studies and important to clearly define inclusion and exclusion criteria.

In Study III we used Intention To Treat (ITT) which is a method applied for trying to get a pragmatic estimate of the treatment effect from the actual change rather than from only the patients who received treatment exactly following the plan (Hollis & Campbell, 1999). However, full application of ITT is not possible if there are missing outcome data for any of the randomized subjects. The method of “carry forward” to use the last observed responses is a way to adjust for this problem, as applied in Study III. But to fully appreciate the potential influence of missing responses there needs to be a sensitivity analysis.

In some studies, variables such as income, education and maternal IQ are not adequately taken into account. Studies that do take these variables into account often find little or no association between breastfeeding and cognitive outcomes except in the case of premature or low birth weight infants. We lack data on mothers’ IQ in Studies I and II, which is a short-coming; however, we have measures on parents’ education which is known to be linked to parents’ IQ.

Sample size

Measurements in the longitudinal project (Studies I and II) were collected at several points in time, thus, the design is exposed for attrition problems. Breast-milk samples and blood samples were collected at sensitive periods and the researchers used a strict ethical approach, with missing data from some of the occasions as a consequence; for example, there is a lack of colostrum, and since collecting this were first breast-milk may be an intrusion. Particularly in Study I this became a problem, since only the first 27 consecutively recruited

children participated in the ToM follow up, and, unfortunately, there were missing data regarding colostrum samples. Looking at the results, it seems though that the number of children were enough to enlighten the relationship between omega-3 and omega-6 LCPUFA ratio and ToM development in children.

In Studies III, and IV, the outcome measurements were the differences in ratings, or neurocognitive test results, between groups. To reach a statistical power of .80, with a probability level of .05, and with three groups (as used in Study IV), 14 children in each group is needed (e.g., Hinkle & Oliver, 1983, cited in Hinkle, Wiersma & Jurs, 2003). Thus, in Studies III and IV, the number of participants included can be considered satisfied. In biological studies of omega-3 and omega-6 LCPUFA milk (Studies I and II) and blood samples (Studies III and VI), to reach a statistical power of .90 with a probability level of .05, 45 children are needed in each group. Thus, in Study III, there are enough children included to assess differences in LCPUFA levels.

Study IV was designed as a pilot study, and the amount of supplements was limited. Unfortunately, attrition problems were the case also here, which caused dropouts. Reasons for that may have been the time consuming test procedure (2h) with only 15 weeks between the assessments. Moreover, families with children with ADHD-like problems often live under stressful conditions, time demanding contact with school, for example. Some families have several children with diagnoses, and one family withdrew for that reason. The small number of participants in Studies I and IV thus may result in a type II error.

CLINICAL IMPLICATIONS OF THE STUDIES

Consumption of fish is considered important for children, and recommended in several countries, for example in the US, Canada (Kris-Etherton & Innis, 2007) and Sweden. In Sweden, the recommendation from Livsmedelsverket is a consumption of 2–3 portions a week for both children and adults. However, most people eat less than that. Pregnant and nursing women are recommended to limit their consumption of fish that may contain dioxin and PCB. Speculations have arisen that some women may avoid fish consumption due to difficulties in knowing which type of fish to avoid.

Our results indicate that there might be a positive general effect on intellectual development by increasing intakes of omega-3 fatty acids during pregnancy and lactation. However, the effect seen in Study II is slight to moderate and would probably not make any difference at an individual level in the western world. On the other hand, in developing countries, where diets tend to be low in ALA and absent of DHA and where infant malnutrition is a common cause of morbidity and mortality, this could be of importance. A recent thesis showed that no benefits of additional DHA intakes were found on cognitive performance, but the author speculates that higher PUFA intakes could be needed in future studies (Eilander, 2009). For cognitive functioning in later life, children may benefit from higher intakes of the long chain omega-3 fatty acids (Whalley, Fox, Wahle, Starr & Deary, 2004). Further longitudinal evaluations are definitely needed to develop recommendations on fatty acid intake for children's future health.

Supplementation in the whole group of children with ADHD is inconsistent. Our results indicates that monotherapy with EPA is no more likely to give effects on children with ADHD than supplementations containing DHA. We found that children with PUFA deficiencies before supplementation benefited more from supplementation than children without this lack, which supports earlier findings (Burgess, 2000; Stevens et al., 2005). Moreover, we also found certain subgroups responding to the supplementation, which also are in line with previous findings (Sinn & Bryan, 2007; Johnson et al., 2009). These findings together may indicate that some subgroups may be more prone of a PUFA imbalance and therefore would benefit more from supplementation. This however, needs to be clarified from future research. In particular, the time is now ripe for delineation of the biological mechanism behind effects of PUFA (e.g., genetic variations or enzyme-system deficiencies).

Several methodological suggestions have been discussed regarding the relatively few positive findings of supplementation for ADHD. Among them, the type of intervention, and short duration time, are common suggestions (see Schuckhardt, et al., 2009 and Transler et al., 2010). However, the research findings regarding infants and young children indicates that early supplementation give rise to more apparent effects (Eilander et al., 2007), and also, as our results indicate, give rise to effects seen first later in childhood. Thus, supplementing school-children with ADHD may be too late. If the effects from fatty acids on brain development occur early in development or only during specific sensitive periods of development (Lucas, 1998; Rosales et al., 2009), then brain alterations may be difficult to establish at this age.

DIRECTIONS FOR FUTURE RESEARCH

Children develop rapidly and certain skills are not fully developed until school age, or even later. To be able to detect possible positive effects from PUFA during the construction of the brain and on the whole range of cognitive skills and behaviour in childhood, it is of importance to investigate different age categories and not only outcome measures in infancy or early pre-school age. Our results indicates that PUFA biosynthesis during pregnancy and early infancy is important for subsequent cognitive functioning, however, due to the design we are not able to tell if it is a co-variation or a causal relation. A supplementation study with the same assessment methods, at the same ages, would be able to contribute to the clarification of this issue. Our research group has started such a supplementation study with LCPUFA given to pregnant and lactating women. The study has a RCT-design and the follow-up in the children, who now is about to be 8-years old, is planned to start this year.

My conclusion is that future studies would benefit from assessing effects from supplementation during pregnancy, in even older children, than in the present investigations, (6–8 years old), tentatively at later school age when the brain is more differentiated and different neurocognitive tests, which assess more specific aspects of cognition, can be applied.

Turning to the research on ADHD, a reflection is that if supplementation in school-aged children with ADHD is given too late for being able to affect cognition in a coherent way, it is of outmost importance to detect these children earlier than at school start. Our findings in

Study IV shows that children about to start school (7 years of age) already demonstrate clear cognitive deficits in comparison with children with typical development. The relatively simple screening procedure could easily be applied in younger children. Moreover, we are planning to relate biochemical breast-milk samples with subsequent cases of ADHD. If it is possible to detect biochemical differences already in samples from breast-milk, these results would be of great importance for the possibility for early supplementation.

Regarding LC-PUFAs it is important to explore the type of supplement that is best suited for the purpose of the study planned, for example, if a mix between omega-3 and omega-6 or a pure single fatty acid is most appropriate. Dose and duration of treatment needs to be established. New research has shown genetic variations in FADS1 and FADS2, the genes coding for delta-6 and delta-5 desaturase in the metabolic pathway of LCPUFA, influencing the levels of omega-3 and omega-6 in human milk and blood. The importance of these genetic variations needs to be studied to understand their influence in supplementation studies.

The results regarding ToM are intriguing, particularly in the light of links to pro-social behaviour and aggression in children, and findings in pro-social behaviour in studies on fatty acids (Hibbeln et al., 2007; Kirby et al., 2010a). It would be interesting to combine and refine assessment methods to investigate this potential relation. Moreover, application of social cognition tasks in future studies of fatty acids and cognition would be a proposal near at hand. Also, other methodologies, such as brain imaging techniques, could help in future trials to detect subtle effects on brain structure and function (Black & Ackerman, 2008). Some interesting findings have already been made with EEG, for example (Henriksen et al., 2008). Furthermore, I would agree to the claims from several researchers (e.g., Rosales et al., 2009; Wainwright & Colombo, 2006) as regards the importance to move away from the use of global tests for measuring cognitive development in nutrition studies and instead strive to use tests based on a hypothesis that the neural or cognitive system is expected to be improved or altered by a dietary supplement.

GENERAL CONCLUSIONS

Breastfeeding is, according to our results, related to children's subsequent cognition. The duration of breast-feeding was positively correlated to children levels of intelligence as measured with WISC-III. Our data suggests that the LCPUFA composition in the first breast-milk, colostrum, is of importance for this relationship. Together with factors known to have impact on children's cognition, the quotients of omega-3 and omega-6 fatty acids, explain a large part of the variance. In particular, the quotient DHA/AA seems to be involved in performance on the intelligence scale. The ratio omega-3/omega-6 ratio in colostrum is also correlated to children's Theory of Mind development. A higher level of omega-3 in relation to omega-6 seems to be favourable.

Supplementation with omega-3 fatty acids (0.5 g EPA per day), in a group of school aged children diagnosed with ADHD did not remove core symptoms of ADHD. Furthermore, neither did children with unspecific neurodevelopmental immaturity, at risk developing ADHD, benefit from the supplementation. However, two subgroups of children with diagnosed ADHD, those with high levels of oppositional behaviours and those with less hyperactive/impulsive behaviours responded to the supplementation of EPA. These results corroborate with earlier findings. Treatment with EPA, thus, seems to be at least as efficient as the mixed LCPUFA formulas previously used. From a clinical view it is gratifying that there seem to be an alternative treatment for children with oppositional symptoms, since they seem to have less benefit from psycho-stimulants.

The uniqueness in this thesis is the attempt to investigate the effect of fatty acids from two different viewpoints; a longitudinal perspective and a short-term intervention perspective. The application of an extensive number of experimental tasks is also unique in fatty acid research, and together with biochemical measures of fatty acid status the results are important contributors to the field.

SVENSK SAMMANFATTNING

Bakgrund

Barns kognitiva utveckling inbegriper olika förmågor som är betydelsefulla för barnets vardagliga fungerande. Några av dessa förmågor som begåvning, theory of mind (ToM), arbetsminne och inhibering, diskuteras i inledningen av denna avhandling.

Hjärnans utveckling och funktion är beroende på den näring vi tillför den. Långa fleromättade fettsyror är en viktig källa. Det finns två särskilt viktiga fettfamiljer, omega-3 och omega-6 fetter, de är livsnödvändiga och måste tillföras via kosten. Både omega-3 och omega-6 är essentiella för tillväxt, utveckling och funktion av hjärnan. AA och DHA är de viktigaste fettsyorna i cellmembran, speciellt i den grå substansen där de utgör 6% av dess torra vikt (Bourre, 2006; Heinrich, 2010; Innis, 2007b, 2008).

Barn får under fostertiden sina långa fleromättade fetter från modern, via placenta. Moderns intag av dessa fetter samt hennes förråd i fettvävnaden är därför viktiga för fostrets utveckling (Innis, 2007b, 2008). Efter födelsen är det innehållet av långa fleromättade fettsyror (LCPUFA) i bröstmjölken som säkerställer barnets behov av AA och DHA för hjärnans utveckling. Världshälsoorganisationen (WHO) sammanfattade nyligen de viktigaste studierna på detta område med slutsatsen att bröstmjolk har positiv betydelse för barnets kognitiva utveckling (Horta et al., 2007). Det finns konsistenta fynd om positiva effekter på visuell utveckling för barn som fått mjölkersättning med höga doser LCPUFA jämfört med de barn som fått placebo (Eilander et al., 2007). Gällande kognitiv utveckling finns inkonsistenta fynd. Vissa studier visar på att tillskott till gravida och ammande kvinnor har gynnsam effekt för deras barns begåvningsnivå mätt med intelligenstest Helland et al., 2003; Willatts et al., 199), medan en metaanalys inte visade in någon sådan effekt hos barn som fått tillskott med fettsyror (Makrides et al., 2005).

Intresset för dessa LCPUFA har varit stort när det gäller eventuella effekter för barn med specifika svårigheter, inte minst när det gäller barn med neuropsykologiska svårigheter som ADHD, dyslexi och autismspektrumstörningar, men även gllande depressioner (Bourre, 2005, 2006, Freeman et al., 2006; Harbottle & Schonfelder, 2008; Richardson et al., 2006; Soh et al., 2009). Behandlingsstudier som hittills har genomförts visar varierande resultat. Vissa har påvisat effekter på barn med ADHD när det gäller uppmärksamhet, medan andra inte har visat några behandlingseffekter. Metodologiska omständigheter att ta i beaktande vid jämförande av olika studier är att det är olika sammansättning av fetter som har använts i olika studier, vissa har både omega-3 och omega-6, andra har använt någon av de långa fleromättade fettsyorna. Behandlingstiden varierar mellan studier även om den numer vanligtvis är runt 12-15 veckor. Det finns ingen fastställd optimal behandlingslängd i nuläget även om många forskare anser att fetterna bör ha omvandlats i kroppen på ca 8 veckor.

Syftet med denna avhandling har varit att, (1) relatera olika fetter under graviditet och amning till barnens senare kognitiva förmågor i 6–7 års ålder. Dessa longitudinella studier på typiskt utvecklade barn har undersökt barns begåvningsnivå, amning och även deras ToM förmåga, (2), att med två randomiserade dubbelblinda placebokontrollerade studier undersöka om behandling med ett tillskott av omega-3 fettsyran EPA under 15 veckor kunde minska symptom eller förbättra kognitiv förmåga hos barn med allmän neuropsykologisk omognad, (i risk för att utveckla ADHD) eller hos barn med diagnosticerad ADHD, jämfört med de som fått placebo.

Metod

De två första studierna i avhandlingen härstammar från samma forskningsprojekt. Familjer som kom till mödravården i Linköping blev tillfrågade om de ville delta i ett longitudinellt projekt. Fettsyreanalyser gjordes i den allra första bröstmjölken (colostrum) samt i barnets blod vid 1 och 3 månader. När barnen fyllt 6,5 år blev de tillfrågade om att delta i denna uppföljning och 77 st tackade ja. Barnen fick göra hela WISC-III, ett allmänt begåvningstest bestående av ett antal olika deltest (Wechsler, 1991), och även göra ett ToM test, bestående av fyra bildberättelser om barn som upplever olika händelser (Lagattuta et al., 1997). ToM studerades med avseende på förståelse av emotionella konsekvenser av att (genom kognitiva ledtrådar) ha blivit påmind om en tidigare sorglig händelse. Barnen fick höra fyra berättelser med tillhörande bilder och presenterades verbala och visuella kognitiva ledtrådar relaterade till den tidigare händelsen. Barnen ombads förklara den känslomässiga reaktionen hos huvudpersonen i berättelsen. ToM krävs för att förstå reaktionerna hos huvudpersonerna

Studierna III och IV är båda randomiserade dubbelblinda placebo kontrollerade studier. I dessa behandlades barn med svårigheter med omega-3 fettsyran EPA under 15 veckor. Alla barnen testades med tester både före och efter behandlingen. Även föräldrar och lärare fick fylla i skattningsformulär. Studie III är en multicenterstudie gällande barn med diagnosticerad ADHD. De som kom till någon av de åtta barnpsykiatriska mottagningar som ingick i projektet, och som kommit ifråga för behandling med läkemedel (Psykostimulantia) för sin ADHD blev tillfrågade om deltagande i denna studie. Studie IV inkluderade barn med mer allmänna symptom på neuropsykologisk omognad. Detta gällde 7-åriga barn där det fanns oro inför skolstart, på grund av rastlöshet, motoriska problem eller svårigheter med uppmärksamhet. I denna studie ingick ett omfattande neurokognitivt testbatteri avseende; verbalt och visuo-spatialt arbetsminne, inhibering, problemlösnings- och planeringsförmåga, läs- och skrivfärdigheter samt ToM.

Resultat och slutsatser

Ett huvudresultat är att längre amningstid leder till signifikant bättre kognitiv utveckling i 6,5 års ålder, resultaten gäller både för total intelligenskvot (IK) och verbal IK mätt med WISC-III. Resultaten kvarstår även efter att vi kontrollerat för kända kovariabler som föräldrarnas utbildning och graviditetslängd. När det gäller fettsyrorna är kognitiv utveckling relaterat till nivåerna av omega-3 fetter i den tidiga bröstmjölken, colostrum, och framförallt till balansen mellan omega-3 och omega-6. Detta styrker tidigare hypoteser om att fetterna i bröstmjolk kan vara en av de faktorerna som gör att amning leder till bättre kognitiv utveckling hos barn (se bl. a. Horta et al., 2007). Skillnaderna är inte stora, men viktiga på gruppnivå. Dessutom tyder fynden i dessa studier på att barns utveckling av ToM kan

påverkas av denna balans på så sätt att barn som har mer omega-3 i förhållande till omega-6 har en mer avancerad förståelse av känslomässiga reaktioner mätt med ToM test. Detta är ett unikt fynd, då oss veterligen inga andra studier om LCPUFA hittills undersökt denna förmåga.

Ett annat huvudresultat i är att ett kosttillskott med omega-3 fettsyran EPA till barn med ADHD inte visar på några fördelar för hela gruppen, bortsett från att problem med uppmärksamhet och kognition mätt med lärarskattningar signifikant minskar efter behandlingen. Däremot finner vi positiva behandlingseffekter i för en subgrupp barn med avvikande omega-3 PUFA balans. Dessa barn fungerar efter behandlingen bättre än tidigare avseende lärarnas skattningar. Detta är i linje med flera tidigare fynd. Även en subgrupp med barn som har mer trotsbeteenden och en subgrupp som har förhållandevis låg nivå hyperaktivitet från början, förefaller bli hjälpta av detta kosttillskott. När det gäller det kognitiva testbatteriet finner vi inga skillnader i någon av behandlingsstudierna.

När det gäller den sista studien (Studie IV) där gruppen med mer allmän neuropsykologisk omognad (i risk för att utveckla ADHD) fick samma behandling som barnen med ADHD finner vi inte heller några effekter av behandlingen. Däremot finns tydliga kognitiva skillnader mellan barnen med den allmänna omognaden jämfört med en åldersmatchad kontroll grupp. Detta indikerar att det redan tidigt kan finnas en kognitiv nedsättning som relativt enkelt kan screenas fram via föräldra- och lärarskattningar.

Det är svårt att studera omega-3 och omega-6 fetters betydelse för den psykiska och kognitiva utvecklingen hos barn. Kognitiva funktioner har vanligen studerats hos spädbarn genom Bayley Scales of Infant Development (BSID) och hos lite större barn med standardiserade testbatterier som Wechslerkalorna. När det gäller behandlingsstudier av barn med kliniska diagnoser är det vanligast att behandlingseffekt har studerats med skattningsformulär av barnets beteenden. En styrka med avhandlingen är att den undersöker de komplicerade sambanden mellan LCPUFA och kognition hos barn från två olika utgångspunkter. Dels med longitudinella studier avseende barn med typisk utveckling och dels med interventionsstudier för barn med ADHD (eller i risk för att utveckla ADHD).

Ytterligare en styrka i avhandlingen är att förutom de inkluderade skattningskalorna och standardiserade testen har även experimentella tester speciellt designade för barnens ålder använts. Dessutom har vi använt *både* föräldra- och lärarskattningskalor. En annan styrka är att vi har inkluderat biokemiska mått före och efter behandlingen med omega-3 fetter för att kunna säkerställa eventuella skillnader. En av de största begränsningarna i de utförda studierna är urvalsstorleken, där vi i flera av studierna har små studier med få deltagare.

Rekommendationer för framtida forskning om nutrition och kognition innefattar bland annat fler experimentella åldersanpassade tester. Dessutom vore det önskvärt att även inkludera undersökandet av social kognition och heta exekutiva funktioner i framtida LCPUFA studier.

CONTRIBUTION REPORT

All research projects included in this thesis is collaboration between me and my supervisor Thomas Karlsson from the Department of Behavioral Science and Learning, and my co-supervisors Per Gustafsson and Karel Duchén from Hälsouniversitetet, all at Linköping University.

Studies I and II: Per Gustafsson and Karel Duchén planned the original research project. Karel Duchén was responsible for the fatty acid expertise and fatty acid analyses. Thomas Karlsson and I entered the project at follow-up. In Study I, I contributed with the Theory of Mind perspective and was responsible for the test instrument, including adjustment to Swedish conditions. I did the psychological testing, analyzed the data together with Thomas Karlsson, and wrote the paper with contributions from the co-authors. In Study II, I performed the psychological tests together with two psychologist candidates. Per Gustafsson and Thomas Karlsson made the major part of the data analysis, all co-authors discussed the analysis and the results. I contributed equally with co-authors in writing the manuscript.

Study III: Per Gustafsson was principal investigator in this study. I participated in the planning process together with the research group, including writing an application for the Clinical trial to Läkemedelsverket. Together with Thomas Karlsson, I planned the test battery and I was responsible for training and supervising the nurses performing the tests. I wrote information letters to the recruiting medical doctors, as well as for parents, children and teachers. The medical doctors were responsible for enrolling participants into the study. Per Gustafsson analyzed the data. Karel Duchén was responsible for the fatty acid analyses. Per Gustafsson made the largest part of writing the manuscript, with contributions from me and the other authors.

Study IV: I planned the project together with my supervisors and I also planned the test battery and designed the visuo-spatial working memory task: the Clown. I was responsible for all data collection, including monitoring the study; including handling contacts with parents, teachers and medical doctors. I performed a large part of the tests, but supervised and trained 3 psychologist candidates that contributed as well. Karel Duchén was responsible for the fatty acid analyses. I made the data analyses and wrote the manuscript with contributions from the co-authors.

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