Supplementation with Docosahexaenoic Acid Ameliorates Paediatric AD/HD

by

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Abstract

Attention Deficit/Hyperactivity Disorder (AD/HD) is the most prevalent paediatric neurodevelopmental disorder. Research implicates a deficiency of docosahexaenoic acid (DHA) in the aetiology of AD/HD. Supplementation with DHA may improve symptoms of AD/HD in children diagnosed with the disorder. Study One examined plasma phospholipid levels of AA and DHA in 103 children between the ages of 5 and 12 years diagnosed with AD/HD. Blood and buccal swab samples were collected and fatty acid profiles were compared to those of typically functioning children. Medical symptom questionnaires were completed to identify physical symptoms of omega-3 fatty acid deficiency and four-day diet records were completed to measure dietary intakes of DHA. Study Two examined effects of supplementation with DHA on symptoms of AD/HD in 39 children. Children were between the ages of 5 and 13 years. Half the children received a supplement containing either 700 mg or 1050 mg of omega-3 fatty acids per day and the control group received a placebo for 4 months. **Results**: Children with AD/HD had mean plasma phospholipid levels of AA and DHA about half those of normal children despite both groups consuming similar intakes of DHA. This finding suggests children with AD/HD are deficient in AA and DHA and this may be due to metabolic differences rather than dietary intake. Children in the supplement group whose plasma phospholipid DHA levels increased, experienced significant improvements in inattention when assessed with the Conners 3, compared to children whose DHA levels remained constant or decreased. No improvements were observed in the control group. This study suggests that alternative or adjunct treatments to medication may be developed for children diagnosed with AD/HD.

Preface

This thesis is an original work by Ellen Marie Ivity. Research Project One entitled "The Relationship Between Dietary Intake, Plasma Phospholipid Levels of DHA, and Physical Symptoms of EFA Deficiency in Children Diagnosed with AD/HD" and Research Project Two entitled "Supplementation with Omega 3 Fatty Acids Ameliorates Symptoms of Inattention in Children Diagnosed with AD/HD" received research ethics approval from the University of Alberta Health Research Ethics Board, May 2005, No. 5324 and May 2007, No. 6625 respectively.

Dedication

To my mother Bev Ivity, my late father Dennis Ivity, and my brothers Peter, Matthew, and Richard. This achievement is a testimony to the depth of your love and encouragement.

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Contents

Chapter II The Relationship Between Dietary Intake, Plasma Phospholipid Levels of DHA, and Physical Symptoms of EFA Deficiency in Children Diagnosed with AD/HD

Chapter III Supplementation with Omega 3 Fatty Acids Ameliorates Symptoms of Inattention in Children Diagnosed with AD/HD

Chapter IV Significance of Research and Future Directions

Appendixes

List of Tables

List of Figures

Abbreviations

AA	Arachidonic Acid
AACAP	American Academy of Child and Adolescent Psychiatry
AAP	American Academy of Pediatrics
AD/HD	Attention Deficit/Hyperactivity Disorder
ALA	Alpha-Linolenic Acid
AI	Adequate Intake
BASC-2	Behavioral Assessment System for Children – 2nd Edition
CAM	Complimentary and Alternative Treatment Method
CD	Conduct Disorder
CGI	Clinical Global Impression Scale
CRS-3	Conners Rating Scales 3
DHA	Docosahexaenoic Acid
DSM-V	Diagnostic and Statistical Manual of Mental Disorders -5th Edition
EFA	Essential Fatty Acids
EPA	Eicosapentanoic Acid
FA	Fatty Acids
FADS	Fatty Acid Desaturase Genes
FCA	Food Colourings and Additives
FFQ	Food Frequency Questionnaire
LA	Linoleic Acid
MSQ	Medical Symptom Questionnaire

MTA	Multimodal Treatment Study of AD/HD
N-3	Omega-3 Fatty Acids
N-6	Omega-6 Fatty Acids
ODD	Oppositional Defiant Disorder
PUFA	Polyunsaturated Fatty Acids
RCT	Randomized Controlled Trial
RDA	Recommended Dietary Allowances
SFA	Saturated Fatty Acids
SNPs	Single Nucleotide Polymorphisms
WISC-V	Wechsler Intelligence Scale for Children – 5th Edition

Chapter I Introduction

1.1 General Introduction

Attention Deficit/Hyperactivity Disorder (AD/HD) is the most common neurodevelopmental disorder in paediatric populations (AAP 2011; Barkley 2006, 2013; Milte et al. 2009; Rader et al. 2009). Between two and sixteen percent of children are diagnosed with AD/HD (Rader et al. 2009). The core symptoms of the disorder are hyperactivity, impulsivity, and inattention.

AD/HD is well recognized as a chronic condition requiring a multi-modal treatment approach (AAP 2001, 2011; Barkley 2013; CME 2006; DuPaul et al. 2013; NASP 2010). Pharmacotherapy is the most common treatment for children with AD/HD (Biederman et al. 2004; DuPaul et al. 2013, Jitendra et al. 2008; Rader et al. 2009). However, medications do not address aetiological factors of the disorder and up to 40 percent of children do not respond favourably to pharmacological treatment (AAP 2001, 2011; Berne 2002; Davis & Williams 2011).

Research suggests many families are not comfortable with pharmacotherapy as a treatment option for their children with AD/HD (Davis & Williams 2011; DuPaul et al. 2013; Vitiello & Sherrill 2007). Side-effects of stimulant use can be difficult to manage and safety concerns associated with chronic use of medication by children can be disconcerting (Davis & Williams 2011; Vitiello & Sherrill 2007).

Alternative or adjunct treatments to medication are needed to address symptoms of AD/HD in children (AAP 2001, 2011, DuPaul et al. 2013; Hechtmann et al. 2004).

Research suggests supplementation with docosahexaenoic acid (DHA) may ameliorate symptoms of AD/HD in children (Milte et al. 2009; Richardson 2006, 2008; Sinn & Bryan 2007). DHA is an omega-3 fatty acid and is the most abundant in retina and brain (Kirby et al. 2010; Lim & Suzuki 2001; Mischoulan & Rosenbaum 2002). DHA is an essential fatty acid important for healthy visual and cognitive development (Drover et al. 2009; Richardson 2008; Uauy et al. 2001).

This dissertation is comprised of four parts; a literature review, two papers, and a conclusion. Chapter one reviews recent literature examining the relationship between AD/HD and DHA. Chapter two describes the first study examining dietary intake of DHA and its relationship to plasma levels of DHA and physical symptoms of EFA deficiency in children diagnosed with AD/HD. Chapter three describes the second study examining the effects of supplementation with DHA on plasma levels of the fatty acid, physical symptoms of EFA deficiency, and behavioral symptoms of AD/HD in children diagnosed with the disorder. The final chapter reviews the main findings discussed in the body of the dissertation.

1.2 Attention Deficit/Hyperactivity Disorder

1.2.1 Symptomatology

AD/HD is the most common paediatric neurodevelopmental disorder (AAP 2011; Barkley 2006, 2013; Milte et al. 2009; Rader et al. 2009). Children with AD/HD present with chronic neuropsychological and cognitive deficits that severely impact social and academic functioning (AAP 2011; Barkley 2006, 2013; DuPaul et al. 2011; Hale et al. 2011; Milte et al. 2009; Rader et al. 2009). Several studies suggest that less than 60 percent of children who have AD/HD are properly diagnosed or treated (Barkley 2013). Prevalence rates vary from two to twenty percent depending on diagnostic criteria used (Rader et al. 2009; Smith & Handler 2007). Conservative estimates are between three and eight percent (Barkley 2006, 2013; Kern et al. 2007; Nigg 2006). The DSM-V reports a prevalence rate of five percent (APA 2013).

Follow-up studies have found 60 to 85 percent of children with AD/HD continue to meet diagnostic criteria for the disorder in adolescence (AACAP 2007) and over half continue to meet criteria in adulthood (Barkley 2006, 2013).

The Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DSM-V) is the manual employed by mental health professionals to diagnose AD/HD in North America (APA 2013). The DSM-V is published by the American Psychiatric Association and lists diagnostic criteria for AD/HD under three main categories:

hyperactivity, impulsivity, and inattention. To be diagnosed with the disorder, a child must present with six or more symptoms of hyperactivity/impulsivity and/or inattention at a developmentally inappropriate level. Symptoms must be present for a minimum of six months and must cause significant impairment in social and academic functioning. Symptoms must be present before the age of twelve and not be better attributed to another disorder.

AD/HD is a heterogeneous disorder as no two children present with the same symptomatology (AAP 2011; APA 2000). A variety of symptoms characterize AD/HD. Children with AD/HD may have difficulties sitting still, delaying gratification, inhibiting inappropriate responses, and planning for the future (Barkley 2006, 2013; Milte et al. 2009). Children with AD/HD may make careless mistakes, lose materials necessary for tasks, and have significant difficulties organizing and completing tasks.

One area of significant impairment is the ability to self-regulate thoughts, emotions, and actions (Antshel & Barkley 2008; Barkley 2006, 2013). Some children with AD/HD become more easily frustrated and angry with a task than their typical peers (Antshel & Barkley 2008; Barkley 2006, 2013; Garber et al. 1996). Children with AD/HD often experience extreme mood swings resulting from an inability to stay calm and focused on the situation (Antshel & Barkley 2008; Barkley 2006, 2013; DuPaul et al. 2002, 2011; Hoff et al. 2002).

Garber and colleagues suggest that AD/HD is characterized by difficulties in controlling physiological arousal (Garber et al. 1996). The disorder causes children to be hyperreactive rather than hyperactive. Children with AD/HD find it difficult to control the speed or direction of their thoughts, leading to impulsive decisions and actions. Evidence suggests that hyperreactivity may have a neurochemical aetiology as many children with AD/HD differ from their typical peers on various measures of tension and physiological arousal (Barkley 2006, 2013; Garber et al. 1996; Smith & Handler 2007).

Recently, researchers have proposed that AD/HD is fundamentally an inability to inhibit irrelevant stimuli (Barkley 2006, 2013; Smith & Handler 2007). Contrary to the traditional view that children with AD/HD have difficulties attending, the more recent theory proposed by Dr. Russell Barkley, a leading expert in the field of AD/HD, suggests children with AD/HD attend to all stimuli and must learn to separate the relevant information from all stimuli being presented (Barkley 2006, 2013; Smith & Handler 2007).

According to Barkley, deficits in cognitive inhibition processes lead to the significant impairments in executive functions observed in children with AD/HD (Barkley 2006, 2013). Executive functions are the higher-order cognitive functions of working memory, attentional control, and behavioural inhibition needed to self-regulate responses for goal-directed behaviours (Barkley 2006, 2013; Chang et al. 2012; Mash & Barkley 2006; van der Donk et al. 2013). Stronger internal control

of behaviour allows for increased future-driven action (Mash & Barkley 2006). Impairments in ability to plan, organise, and self-direct behaviour significantly reduce the long-term opportunities available to an individual (Barkley 2006, 2013; Mash & Barkley 2006; van der Donk et al. 2013).

A meta-analysis of 83 studies examining over 6000 participants found impairments in executive functions were common in individuals with AD/HD (AACAP 2007). Executive functions play an important role in academic performance (Barkley 2006, 2013; Mash & Barkley 2006; van der Donk et al. 2013). Deficits in executive functions lead to difficulties organising and completing tasks as attention is significantly influenced by immediate external events rather than focused on delaying gratification for more important goals (Barkley 2006, 2013; Mash & Barkley 2006; van der Donk et al. 2013). Impairments in working memory are associated with learning difficulties and unproductive classroom behaviour (Mash & Barkley 2006; van der Donk et al. 2013). Interactions with others are difficult because children with AD/HD struggle to self-regulate their behaviour and affect as well as properly decipher others' body language and social cues (Barkley 2006, 2013; DuPaul et al. 2002, 2011; Hoff et al. 2002). The Self in a child with AD/HD does not regulate, organize, and execute behaviour as efficiently as in typically developing peers (Barkley 2013).

Neuroimaging studies offer support for Barkley's theory (Arnsten 2006 as cited in Punja et al. 2013; Brown et al. 2010; Nigg 2006). Executive functions may be

mediated by the frontal cortex, especially the prefrontal lobes. These areas of the brain have been found to be smaller and less active in children with AD/HD, compared to typically functioning children (Brown et al. 2010; Hallowell & Ratey 1994; Nigg 2006; Wolosin & Richardson 2009).

Subsets of children with AD/HD exhibit physical symptoms of EFA deficiency (Antalis et al. 2006; Carlson & Neuringer 1999; Richardson 2008; Stevens et al. 2003). Halitosis, rhinitis, headaches, sleep latency, and night awakenings have been reported significantly more often in children with AD/HD compared to controls (Conte et al. 1987; Kaplan et al. 1987; Kaplan et al. 1989). Stevens and colleagues found many children with AD/HD urinated more often, had greater thirst, and exhibited more dry skin than children without AD/HD (Stevens et al. 1995; Stevens et al. 2003). The pervasive nature of AD/HD highlights the need for effective treatments to address the varied symptomatology of the disorder.

1.2.2 Burden of Disorder

The significant academic, personal, and financial burden associated with AD/HD stresses the importance of early and effective management of symptoms of the disorder (Barkley 2006, 2013; Biederman et al. 2004; Chang et al. 2012; D'Amico et al. 2014; Jitendra et al. 2008; Kern et al. 2007). Up to 80 percent of children with AD/HD struggle academically (Barkley 2006, 2013; DuPaul et al. 2002; Jitendra et al. 2008). Grade retention, academic failure, and dropout rates are more prevalent in children with AD/HD (Barkley 2006, 2013; D'Amico et al. 2014;

Hoff et al. 2002; Jitendra et al. 2008; Milte et al. 2009; Schab et al. 2004; Weyandt et al. 2013). Individuals with AD/HD are less likely to attend post-secondary education, have lower grade point averages, and have lower socio-economic status than their typical peers (Barkley 2006, 2013; DuPaul et al. 2002; Milte et al. 2009). Relationships with parents, teachers, and peers are often strained, leading to social rejection, depression, and poor self-esteem (Barkley 2006, 2013; Hoff et al. 2002; Jitendra et al. 2008; Milte et al. 2009; Schab et al. 2004; Sinn 2008).

Comorbidity rates are high in children with AD/HD, increasing the challenges associated with the disorder (Barkley 2007; Hale et al. 2011; Jitendra et al. 2008; Sinn 2008; Smith & Handler 2007). Among children four to nine years of age, comorbidity rates are as high as 79 percent (Smith & Handler 2007). Two of the most common disorders associated with AD/HD are Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD), two severe diagnoses that can lead to total disregard for other living beings' rights to life and property (AACAP 2007; Reddy & Thomas 2007). Rates of ODD and CD vary between 40 and 90 percent in children with AD/HD in contrast to two to sixteen percent in the general population (Reddy and Thomas 2007). Children with AD/HD and comorbid ODD are extremely stubborn, non-compliant, and hostile (APA 2000; Mash & Barkley 2006). They violate rules imposed by caregivers, steal, lie, and may commit aggressive acts. Over 50 percent of children with AD/HD and comorbid ODD experience significant peer rejection (DuPaul et al. 2002; Hoff et al. 2002; Jitendra et al. 2008). A diagnosis of CD is characterized by serious violations of the basic rights

of others and/or societal norms (Jitendra et al. 2008; Reddy and Thomas 2007). CD is strongly associated with the development of Anti-Social Personality disorder in adulthood (Smith & Handler 2007).

A diagnosis of AD/HD leads to significant financial burden (Barkley 2006, 2013; Mash & Barkley 2006; Schab et al. 2004). According to the National Institutes of Health, an additional three billion dollars is incurred annually by school systems to manage symptoms of AD/HD and health care costs are more than double in this population (Barkley 2006, 2013; Schab et al. 2004).

Because the psychological and financial burden associated with AD/HD is severe, an early diagnosis and commencement of effective treatment is indicated to reduce the negative impact of the disorder (Berne 2002; DuPaul et al. 2011; Hallowell & Ratey 1994; Kern et al. 2007).

1.2.3 Aetiology

The aetiology of AD/HD is multifactorial. Genetics, neuropsychological deficits, and nutritional deficiencies are associated with a diagnosis of AD/HD (Berne 2002; Russell 2014; Smith & Handler 2007). Genetic studies suggest AD/HD may be heritable (AACAP 2007; Berne 2002; Hallowell & Ratey 1994; Russell 2014; Smith & Handler 2007). Large twin studies have found significantly high heritability for AD/HD, ranging from .75 to .97 (Barkley 2006, 2013; Mash & Barkley 2006). Faraone and colleagues reviewed 20 independent twin studies

and found heritability for AD/HD to be 76 percent (AACAP 2007). Twins reared apart have higher concordance rates for the disorder than children living in the same household and AD/HD is more prevalent in monozygotic rather than dizygotic twins (Hallowell & Ratey 1994; Mash & Barkley 2006). Higher rates of AD/HD are found in biological parents of children with AD/HD compared to adoptive relatives (Mash & Barkley 2006). Research suggests parents who have AD/HD have a 57 percent risk of having children with the disorder, which adds support to the genetic theory of AD/HD (Barkley 2006, 2013; Mash & Barkley 2006).

Neuroanatomical and neurochemical deficits have been implicated in the aetiology of AD/HD (Brown et al. 2010; Gow et al. 2009; Hinshaw et al. 2002; Nigg 2006; Weyandt et al. 2013; Wolosin & Richardson 2009). Neuroimaging studies of children with AD/HD have found differences in regions of the brain responsible for executive functions compared to control children (Chang et al. 2012; Nigg 2006; Weyandt et al. 2013). Four main regions of the brain are implicated; the prefrontal cortex, basal ganglia, cerebellum, and corpus callosum (Nigg 2006).

The prefrontal cortex is a large area at the front of the brain responsible for working memory and inhibition of inappropriate responses (Nigg 2006). The area is heavily interconnected with other areas of the brain and is an important endpoint for dopamine neural projections (Nigg 2006). Research suggests the prefrontal cortex is smaller in size (Nigg 2006; Wolosin & Richardson 2009) and

has decreased blood flow (Hallowell & Ratey 1994; Weyandt et al. 2013; Wolosin & Richardson 2009) in children with AD/HD compared to controls. Total cerebral volume is reduced in children with AD/HD by over five percent (Nigg 2006; Wolosin & Richardson 2009), suggesting understimulation in critical areas necessary for proper cognitive and behavioural functioning. The basal ganglia, cerebellum, and corpus callosum are interconnected with the prefrontal cortex and are necessary for complex motor and cognitive functions (Nigg 2006). These areas have been found to be smaller in many children with AD/HD compared to controls (Nigg 2006; Weyandt et al. 2013).

Neurochemical studies implicate the dopaminergic system as a key factor in the aetiology of AD/HD (Banaschewski et al. 2005; Brown et al. 2010; Hinshaw et al. 2002; Nigg 2006; Rader et al. 2009; Weyandt et al. 2013). Dopamine is a monoamine neurotransmitter, synthesized from the amino acid tyrosine (Nigg 2006). Dopamine modulates cognition, attention, and executive functions in regions of the brain (Nigg 2006). Neural activation studies have found deficits in cerebral stimulation during cognitive tasks measuring response inhibition in children with AD/HD compared to controls (Gow et al. 2009; Nigg 2006; Weyandt et al. 2013). These findings suggest the dopaminergic system may be impaired in the former population, possibly due to deficits in synthesis, release, and/or reuptake of dopamine in cerebral regions.

Nutritional factors have been implicated in the aetiology of AD/HD (Gaitens et al. 1998; Kaplan et al. 2007; Milte et al. 2009; Richardson 2008; Stevenson 2010; Walsh 2012). Food intolerances, allergies, and excesses or deficiencies of nutrients have been shown to lead to symptoms of AD/HD (Boris & Mandel 1994; Dorfman 2011; Jacobson & Schardt 1999; Kaplan et al. 2007; Milte et al. 2009; Richardson 2008; Stevenson 2010; Walsh 2012). Research suggests sensitivities to sugar, artificial sweeteners, milk, eggs, or wheat lead to symptoms of hyperactivity and inattention in subsets of children (Boris & Mandel 1994; Dorfman 2011; Rojas & Chan 2005; Stevenson 2010; Walsh 2012).

Over the past two decades, deficiencies in DHA have been implicated in the aetiology of AD/HD (Gow et al. 2009; Kaplan et al. 2007; Milte et al. 2009; Mitchell et al. 1987; Richardson 2008; Stevens et al. 2005). Compared to controls, many children with AD/HD have lower concentrations of DHA in plasma phospholipids and exhibit physical symptoms of EFA deficiency such as increased thirst, frequent urination, skin problems, and sleep deficits (Kaplan et al. 1989; Mitchell et al. 1987; Richardson 2006; Stevens et al. 2005). Supplementation with n-3 fatty acids has been shown to ameliorate EFA deficiency symptoms and core symptoms of AD/HD including inattention and hyperactivity (Richardson 2006; Sinn & Bryan 2007; Young & Conquer 2005). Research suggests supplementation with DHA is a promising and safe alternative or adjunct treatment to medication for children with AD/HD (Richardson 2008; Sinn & Bryan 2007; Young & Conquer 2005).

AD/HD is the most prevalent neurodevelopmental disorder in children with varying aetiology and symptomatology. Early assessment and commencement of effective treatment are important to minimize the burden of the disorder.

1.2.4 Assessment

Assessment of AD/HD can be challenging due to the heterogeneity of the disorder (Levy & Hay 2001). Best practice guidelines recommend a multifaceted approach employing a variety of assessments, informants, and settings (AACAP 2007; AAP 2011; McConaughy & Ritter 2002; NASP 2010; Rader et al. 2009; Smith & Handler 2007). Use of multiple informants and settings allows for assessment of behaviour in multiple contexts. For example, children may display hyperactivity in the structured setting of the classroom but not in the home environment. Obtaining information from parents, teachers, other caregivers, and the child aids the clinician in conducting a thorough assessment and arriving at an accurate diagnosis (AACAP 2007; Rader et al. 2009; Smith & Handler 2007).

The American Academy of Child and Adolescent Psychiatry suggests conducting thorough clinical interviews with parents, teachers, and children (AACAP 2007). Obtaining detailed medical histories of children is recommended to assess for disorders or life events that may be causing symptoms of AD/HD (AAP 2011; Garber et al. 1996; Hallowell & Ratey 1994). Conditions such as allergies, deficiencies or excesses of nutrients, hearing or visual impairments, and head injuries can lead to symptoms of hyperactivity, inattention, and aggression (Smith

& Handler 2007). Recent life events such as parental divorce, death of a loved one, or birth of a new sibling can be important contributing factors to development of behaviours observed in AD/HD (AACAP 2007).

Psychiatric and physical health of primary caregivers are important to assess as poor health of caregivers can exacerbate symptoms of AD/HD in children (AACAP 2007). Research suggests the clinician meet with the child and teacher to obtain information about the child's mental and physical health (NASP 2010; Rader et al. 2009; Smith & Handler 2007). The child and teacher may have important insights to offer about the child's symptoms and effective treatment options.

Best practice guidelines suggest obtaining detailed information about symptoms and their functional significance to aid in development of effective interventions (McConaughy & Ritter 2002). Detailed lists of symptoms can be obtained from caregivers and the child (AACAP 2007). Research strongly encourages clinicians to conduct clinical observations to observe the child's interactions with caregivers and peers (AACAP 2007; McConaughy & Ritter 2002; Smith & Handler 2007). Conducting functional behaviour analyses of the various symptoms aids in designing an effective treatment plan for the child (Smith & Handler 2007).

Use of standardized assessments to measure cognitive and behavioural functioning is recommended as part of a comprehensive assessment (AACAP 2007; McConaughy & Ritter 2002; Rader et al. 2009; Smith & Handler 2007).

Cognitive tests like the *Wechsler Intelligence Scale for Children-5 edition* (WISC-V) (Wechsler 2014) assess areas such as working memory, verbal comprehension, and processing speed (Smith & Handler 2007). The WISC-V is the most popular intelligence scale used with children (Hinshaw 2002; Smith & Handler 2007).

Broadband assessments such as the *Behavioral Assessment System for Children-2 Edition* (BASC-2) (Reynolds & Kamphaus 2006) and the *Conners 3 Rating Scales* (CRS-3) (Conners 2008) measure socio-behavioural functioning across multiple domains (Smith & Handler 2007). The *Conners 3* is a commonly used assessment with children with AD/HD (AACAP 2007; Hoff et al. 2002). CRS-3 is a broadband rating scale designed to assess severity of behaviour problems in a variety of domains including anxiety, shyness, and perfectionism. In addition, the CRS-3 assesses symptoms of AD/HD and improvements in symptoms observed during treatment (Conners 2008).

Best practice guidelines recommend incorporating a variety of standardized measures, along with clinical interviews and observation when conducting psychiatric assessments with children (AACAP 2007; Rader et al. 2009). Thorough assessments are required to devise effective multimodal treatment plans.

1.2.5 Treatment

Multimodal treatment plans are the most effective in treating symptoms of AD/HD in paediatric populations (AAP 2001, 2011; Garber et al. 1996; Konikowska et al. 2012; Rader et al. 2009; Vitiello & Sherrill 2007). Various treatment options, including pharmacotherapy (Biederman et al. 2004; DuPaul et al. 2002, 2011; Rader et al. 2009; Weyandt et al. 2013), psychoeducation (AAP 2001, 2011; Antshel & Barkley 2008; DuPaul et al. 2002, 2011; Rader et al. 2009; Weyandt et al. 2013), and nutritherapy (Milte et al. 2009; Richardson 2008; Rojas & Chan 2005) have been found to ameliorate symptoms of AD/HD in children diagnosed with the disorder.

Best practice guidelines suggest treatment recommendations be based on data obtained during thorough assessments and be tailored to the needs of the individual child (DuPaul et al. 2002, 2011; Garber et al. 1996; Vitiello & Sherrill 2007). Presenting symptoms and level of impairment, needs of the family, and availability of financial and material resources must be considered when designing treatment plans (AAP 2001, 2011). Research suggests adherence to and effectiveness of treatment are increased when treatment plans have clearly defined target outcomes and methods of monitoring progress (AAP 2001, 2011).

Pharmacotherapy has been employed for over 60 years to treat learning and behavioural problems (Berne 2002; DuPaul et al. 2013). An extensive body of research supports use of medication in managing symptoms of AD/HD (AACAP

2007; Biederman et al. 2004; DuPaul et al. 2013; Mash & Barkley 2006; Rader et al. 2009). Psychostimulants are the first line pharmacological treatment indicated for AD/HD (AAP 2011; Biederman et al. 2004; Davis & Williams 2011; DuPaul et al. 2013; Rader et al. 2009). Sixty to eighty percent of children with the disorder respond favourably to stimulant medication (AAP 2001; Berne 2002; Mash & Barkley 2006). Methylphenidate (Ritalin®) is the most commonly researched and prescribed stimulant for AD/HD (Biederman et al. 2004; Mash & Barkley 2006; Punja et al. 2013). Other prescribed stimulants include dextro-amphetamine (Dexedrine®), mixed amphetamine salts (Adderall®), and magnesium pemoline (Cylert®).

Stimulants are thought to enhance dopaminergic and noradrenergic neurotransmission in the prefrontal cortex, leading to improved executive functioning (Arnsten 2006 as cited in Punja et al. 2013; Biederman et al. 2004). Each stimulant has a different mechanism of action causing some children to respond more favourably to one medication over another (Biederman et al. 2004).

Stimulants improve core symptoms of AD/HD, leading to increased academic performance and improved social interactions (Biederman et al. 2004; Hechtmann et al. 2004; Mash & Barkley 2006). The American Academy of Pediatrics (AAP) discusses three meta-analyses and one review of reviews supporting use of stimulants in reducing core symptoms of AD/HD (AAP 2001, 2011). However, this class of drug has not been found to improve intelligence or normalise behaviour

(AAP 2001, 2011). Therefore, other treatment options are recommended in addition to or in place of pharmacotherapy (AAP 2001, 2011; Hechtmann et al. 2004).

Though stimulants are generally well tolerated, significant concerns have been raised about use of these medications in children, especially preschool children (AAP 2011; Biederman et al. 2004; Davis & Williams 2011; Hoff et al. 2002). A few studies have examined the use of stimulants in preschool populations (AACAP 2007; Biederman et al. 2004). In 2002, Connors reviewed nine studies employing a total of 206 children with AD/HD and found increases in irritability, crying, and social withdrawal due to use of stimulants (AACAP 2007). The National Institute of Mental Health funded the preschool AD/HD Treatment Study employing 183 children with AD/HD between the ages of 3 and 5 years (AACAP 2007). Methylphenidate was effective in managing symptoms of AD/HD but in lower doses (AACAP 2007). Stimulants are approved for short-term use in children over the age of six years (Biederman et al. 2004; Hoff et al. 2002). However, no long-term safety data exists on the neurological effects of stimulant use in preschool children (AAP 2011; Davis & Williams 2011; DuPaul et al. 2013; Hoff et al. 2002).

Stimulant use is associated with a number of side effects; the most common being appetite suppression and sleep disturbances (AAP 2001, 2011; Biederman et al. 2004). Headache, abdominal pain, increased tearfulness, lethargy, fatigue,

increased blood pressure, stunted growth, and acute motor tics are also observed (Biederman et al. 2004; DuPaul et al. 2013; Konikowska et al. 2012).

Rarely, stimulant use may lead to severe side effects such as major depressive-like syndrome and toxic psychosis, usually due to prescribed doses being too high (AAP 2001, 2011; Biederman et al. 2004; Davis & Williams 2011). Dextro-amphetamine has a black box warning printed on the packaging for abuse potential and cardiovascular risks. Methylphenidate carries a warning for abuse potential and possible withdrawal complications. Because these medications are often prescribed to young children (the former being approved for children as young as three years of age), these side-effects and warnings are particularly alarming.

Research suggests many families are not comfortable with pharmacotherapy as a treatment option for their children with AD/HD (DuPaul et al. 2013; Vitiello & Sherrill 2007). Fabiano and colleagues found families prefer avoiding or limiting use of medication by their children (Vitiello & Sherrill 2007). Side-effects of stimulant use can be difficult to manage and safety concerns associated with chronic use of medication by children can be disconcerting (Davis & Williams 2011; Vitiello & Sherrill 2007). The fact stimulants do not increase skills or normalise behaviour also support the use of other options when treating children with AD/HD (AAP 2011; DuPaul et al. 2013; Gresham 2002; Hechtmann et al. 2004).

Three types of psychoeducation have documented success in ameliorating symptoms of AD/HD in children: parent training, classroom management, and cognitive-behavioural therapies (AAP 2001, 2011; Antshel & Barkley 2008; DuPaul et al. 2002, 2011; Mash & Barkley 2006; Rader et al. 2009; Vilardo et al. 2013). These treatment options encompass a variety of interventions aimed at altering the home and school environment and increasing self-awareness to encourage positive behaviours and minimize maladaptive ones (AAP 2001, 2011; DuPaul et al. 2002, 2011; Gresham 2002).

Parent training techniques educate parents about AD/HD and teach them skills to better manage their children's symptoms (AACAP 2007; AAP 2001, 2011; Mash & Barkley 2006). Over the course of a few months with a trained therapist, parents are taught how to instruct their children and reinforce adaptive behaviours while minimizing negative ones (AAP 2001, 2011; Mash & Barkley 2006). Parent training techniques have been found to improve children's functioning but not sufficiently to normalise behaviour (AAP 2001, 2011).

Classroom management techniques increase the structure of the school environment, encouraging more positive academic and interpersonal functioning (AAP 2011; DuPaul et al. 2011; Mash & Barkley 2006; Vilardo et al. 2013). Examples of classroom techniques include active teaching of school and classroom rules and altering the classroom environment to encourage learning (DuPaul et al. 2002, 2011; Vilardo et al. 2013). For example, a child may be

placed at the front of the classroom or paired with a peer to facilitate instruction (DuPaul et al. 2002, 2011; Vilardo et al. 2013). Token economies, brief time-outs, and choice strategies in which the child chooses between two or more activities are also effective strategies for ameliorating symptoms of AD/HD in children (AAP 2011; DuPaul et al. 2002, 2011; Mash & Barkley 2006; Rader et al. 2009).

Cognitive behaviour therapies educate a child with AD/HD about the disorder and teach skills to help manage symptoms of the disorder (AAP 2001, 2011; Barkley 2013; DuPaul et al. 2002, 2011; Mash & Barkley 2006; Tamm et al. 2013). For example, a child with AD/HD may receive instruction on how to monitor, evaluate, and reinforce her own behaviour at regular intervals, using a Likert scale (DuPaul et al. 2011). Self-evaluations would be compared to teacher or parent assessments. Once the child with AD/HD is capable of accurately monitoring her own behaviour, only self-assessments would be used to determine progress. Self-assessment techniques are often used in conjunction with other teacher-mediated behavioural approaches.

According to Tamm and colleagues, attention may be viewed as a set of subprocesses that can be taught individually (2013). Orienting to a stimulus, attending, sustaining attention, and being capable of dividing attention between two or more stimuli are skills that can be learned (Tamm et al. 2013). More efficient attentional processes may enhance executive functions, leading to improved cognitive and behavioural functioning.

Research has found psychoeducation coupled with pharmacotherapy is more effective in treating symptoms of AD/HD in children than monotherapy (AAP 2001, 2011; DuPaul et al. 2013; Hechtmann et al. 2004; Mash & Barkley 2006; MTA 1999). The large 5-year Multimodal Treatment Study of AD/HD (MTA) found that over 14-month treatment periods, children receiving a combination of pharmacotherapy and behavioural therapy experienced increased academic and social functioning compared to either treatment option alone and compared to controls (MTA 1999). Combination therapy lead to reductions in anxiety scores, greater normalization of functioning, and need for lower doses of medication compared to pharmacotherapy alone (CME 2006; MTA 1999). Parents were significantly more satisfied with the combined treatment option, which is important as adherence to treatment leads to greater treatment effects (AAP 2011; MTA 1999).

AD/HD is well recognized as a chronic condition requiring a multi-modal treatment approach (AAP 2001, 2011; Barkley 1998; CME 2006; Rader et al. 2009). Vitiello and Sherill (2007) suggest most research examining treatments for AD/HD is concerned with symptom reduction rather than cure. Research focusing on treatments with curative effects are needed (AAP 2001, 2011).

Nutritherapy is one such type of treatment option for AD/HD. Nutritherapy is considered a complementary and alternative treatment method (CAM) (Rojas & Chan 2005). Use of CAM for AD/HD is widespread worldwide with 12 to 64

percent of families employing them (Bussing et al. 2002 as cited in Rucklidge et al. 2009; Rojas & Chan 2005). Nutritherapy has been found to ameliorate behaviour in 45 to 65 percent of children with AD/HD (Kaplan et al. 1989, 2007). Three types of nutritherapy that have been found to be effective in treating symptoms of AD/HD in children are Feingold-type diets (Kaplan et al. 1989, 2007; Rojas & Chan 2005), multinutrient supplementation (Benton 2007; Kaplan et al. 2007), and omega-3 fatty acid supplementation (Milte et al. 2009; Richardson 2008; Rojas & Chan 2005).

Feingold-type diets are based on Dr. Feingold's work in the 1970's (Kaplan et al. 1989, 2007; Rojas & Chan 2005). Feingold observed positive relationships between increased use of food colourings and additives (FCA) and learning disorders and hyperactivity (Rojas & Chan 2005). Feingold observed that eliminating FCA and salicylate-containing foods from children's diets led to behavioural improvements. His work led to multiple studies and considerable media attention (Rojas & Chan 2005).

Several early meta-analyses found the Feingold diet to be ineffective in treating AD/HD (Rojas & Chan 2005). More recent studies reducing dietary intake of specific FCA and foods found to be common allergens in children have shown treatment effects (Schab & Trinh 2004; Rojas & Chan 2005; Stevenson 2010). Subsets of children experience allergic or food intolerance reactions to certain substances in food, causing symptoms of AD/HD such as hyperactivity and

impulsivity (Benton 2007; Schab & Trinh 2004). Reducing or eliminating these substances from the diet leads to improvements in behaviour (Benton 2007; Boris & Mandel 1994; Kaplan et al. 1989, 2007).

Multinutrient supplementation improves cognitive and behavioural functioning in psychiatric populations (Benton 2007; Dorfman 2011; Kaplan et al. 2004, 2007; Schoenthaler & Bier 2000; Walsh 2012). Supplementation with a variety of nutrients has been found to reduce antisocial behaviour in young offenders and delinquents (Gesch et al 2002). Increased dietary intake of multinutrients ameliorates memory, attention, and eye-hand coordination (Benton 2007; Dorfman 2011; Walsh 2012); improves mood and temper in children with mood and anxiety disorders (Dorfman 2011; Kaplan et al 2004, 2007; Walsh 2012); and improves mood in adults with bipolar disorder (Kaplan et al. 2007). Multinutrient supplementation has been found to increase serum levels of prescription medication, potentially reducing required doses of medications (Kaplan et al. 2007).

The importance of DHA for healthy cognitive and behavioural functioning has received increased attention over the past decade (Colombo 2001; Konikowska et al. 2012; Lien 2005; Richardson 2008). DHA is the most abundant n-3 fatty acid in brain. Adequate intakes of DHA are critical for healthy development and functioning of visual and cognitive systems, including healthy neurotransmission (Carlson et al. 1996; Clandinin et al. 2005; Drover et al. 2009; Richardson 2006).

Because deficiencies of DHA lead to AD/HD-like symptoms in animals (Carrié et al. 2000; Zimmer et al. 1999), research suggested deficiencies in DHA may be associated with symptoms of AD/HD in children (Drover et al. 2009; Colombo 2001). Studies support this hypothesis (Richardson 2007; Stevens et al. 2003; Uauy et al. 2001). Therapeutic intakes of DHA have been found to improve visual acuity (Birch et al. 2000, 2007; Carlson et al. 1999; Drover et al. 2009; Lien 2005), speed of information processing (Werkman et al. 1996), psychomotor and cognitive development (Birch et al. 2000, 2009; Clandinin et al. 2005; Drover et al. 2009, 2011), language and emotional regulation (Drover et al. 2011), and enhance growth (Clandinin et al. 2005) in infant populations.

DHA supplementation has been found to reduce physical symptoms of EFA deficiency such as excessive thirst, frequent urination, and skin problems in children with AD/HD (Richardson 2006; Stevens et al. 2003). Research suggests DHA supplementation improves behaviour, inattention (Sinn & Bryan 2007; Stevens et al. 2003), reading, spelling (Richardson & Montgomery 2005), and reduces hostility (Richardson 2006; Stevens et al. 1995) in children with AD/HD.

A number of treatment options, including pharmacotherapy, educational training, classroom management, and nutritherapy have been found to ameliorate symptoms of AD/HD in children. Pharmacotherapy is the most common treatment, however, research suggests many parents are opposed to the use of medications to manage their children's symptoms. Natural, safe, and effective

alternatives to pharmaceuticals are needed. Supplementation with DHA has been found to ameliorate symptoms of AD/HD in subsets of children. DHA supplementation is a safe, promising alternative or adjunct treatment to medications to manage symptoms of AD/HD in children.

This section has focused on AD/HD in children. AD/HD is the most prevalent neurodevelopmental disorder in paediatric populations with varying aetiology and symptomatology. Multimodal treatment plans based on thorough assessments are recommended to address the various symptoms of AD/HD. Pharmacotherapy, behavioural therapy, and nutritherapy are effective in treating AD/HD in children. Early diagnosis and commencement of effective treatment are important to minimize the burden of AD/HD (Richardson & Ross 2000).

1.3 Polyunsaturated Fatty Acids

1.3.1 Nomenclature

Most dietary fatty acids are linear hydrocarbon chains with a carboxylic acid group at one end and a methyl group at the terminal end (Bradbury 2011). Saturated fatty acids (SFA) contain no double bonds whereas unsaturated FA are characterized by one or more double bonds. Generally these bonds are arranged in the *cis* configuration; the two hydrogen atoms on either side of the double bond are located on the same side of the fatty acid. This configuration causes a bend in the molecule at the site of the double bond (Bradbury 2011).

The n-3 and n-6 fatty acids, known collectively as polyunsaturated fatty acids, contain two or more double bonds (Rolfes, Pinna, & Whitney 2006). Multiple double bonds cause PUFA to be more flexible and more highly folded than SFA. This flexibility leads PUFA oils to be liquid at room temperature unlike SFA which are solid at twenty degrees Celsius. This difference in melting point explains why cell membranes that have higher levels of PUFA than SFA are more fluid at body temperatures. Increased fluidity is important for healthy intra- and intercellular signal transduction (Jumpsen & Clandinin 2005; Salem et al. 2001).

PUFA are classified by the length and degree of unsaturation of the hydrocarbon chain (Lattka et al. 2013), and the distance of the bonds from the methyl group (Rolfes, Pinna, & Whitney 2006). For example, DHA is also written as 22:6n-3; a twenty-two carbon atom chain with six double bonds, the final double bond being

three positions from the methyl group. The other two predominant n-3 fatty acids are alpha-linolenic acid (ALA, 18:3n-3) and eicosapentanoic acid (EPA, 20:5n-3). Arachidonic acid (AA, 20:4n-6) is the most abundant n-6 fatty acid in brain and retina. Linoleic acid (LA, 18:2n-6) is the eighteen-carbon n-6 FA precursor to AA.

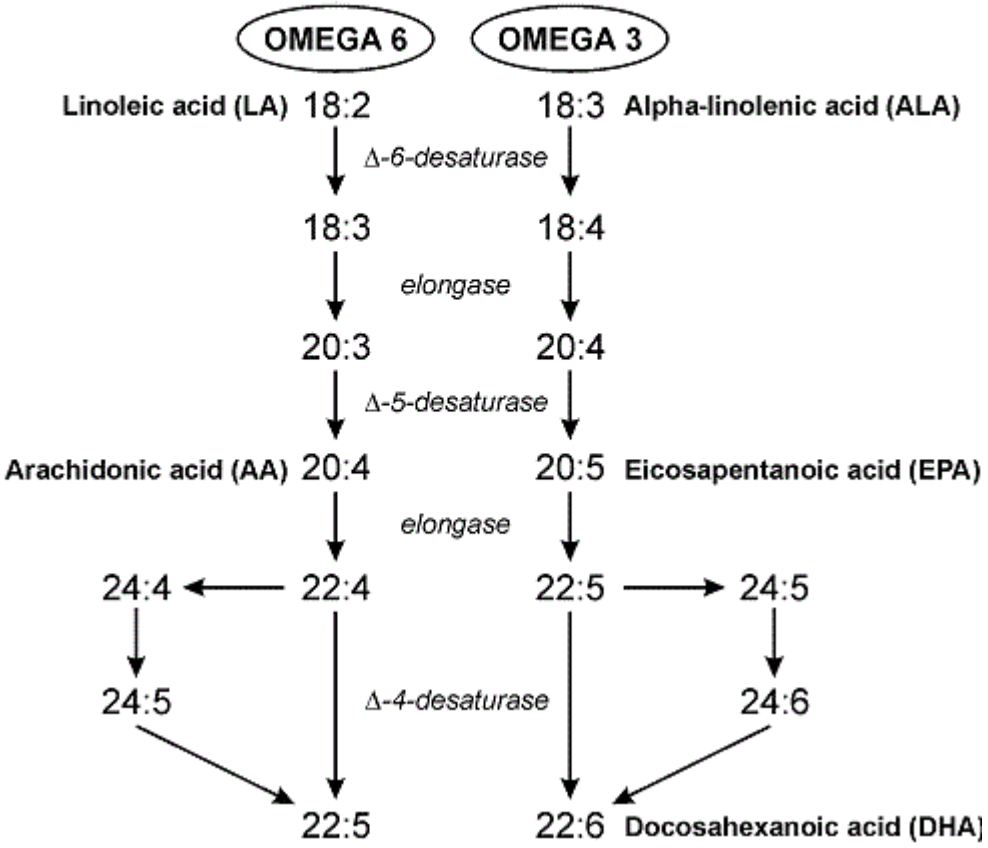
Vertebrate animals cannot add double bonds or desaturate fatty acids before the ninth carbon atom from the methyl end of the carbon chain (Jumpsen & Clandinin 2005). The n-3 and n-6 fatty acids contain double bonds that begin at the third and sixth carbon atoms respectively. The n-3 and n-6 fatty acids are considered essential fatty acids as they cannot be synthesized by the body and must be obtained from the diet.

1.3.2 Biosynthesis

Longer-chain PUFA are biosynthesised in the endoplasmic reticulum from shorter-chain precursors with the aid of position-specific desaturases (Sprecher 2000) (Figure 1.1). The first six steps of biosynthesis occur in the endoplasmic reticulum. ∆6-desaturase converts LA and ALA to *y*-linolenic (18:3n-6) and stearidonic acid (18:4n-3) respectively. Two carbon atoms are subsequently added to elongate 18:3 and 18:4 to 20:3 and 20:4 respectively. ∆5-desaturase converts the elongated n-6 fatty acid to AA and the n-3 fatty acid to EPA. Earlier research suggested biosynthesis was unidirectional and occurred exclusively in the endoplasmic reticulum (Brenna 2002). 22:5 and DHA were thought to be produced with the aid of ∆4-desaturase. Sprecher and other researchers suggest

Δ4-desaturase does not exist in mammalian tissues (Sprecher 2000). The current view holds that EPA is elongated twice, converted to 24:6 with the aid of Δ6-desaturase, then subsequently transported to peroxisomes and retroconverted by β-oxidation to DHA (Sprecher 2000).

Figure 1.1 PUFA Biosynthesis Pathways



Adapted from Haag, M. (2003). Essential Fatty Acids and the Brain. The Canadian Journal of Psychiatry 48: 195-203.

Synthesis of longer chain PUFA is mediated by intake of shorter chain precursors and the ratio of dietary n-6 to n-3 intake (Schuchardt et al. 2010; Simopoulos 2000). As LA intake increases, blood plasma levels of AA increase (Simopoulos 2000; Williams & Burdge 2006). To increase blood plasma DHA levels is more complex (Simopoulos 2000; Williams & Burdge 2006). LA and ALA compete for the delta-6 desaturation enzyme necessary for conversion of shorter chain PUFA to longer chain PUFA. Affinity of delta-6 desaturase is greater for ALA than for LA, however, higher LA intake leads to greater AA synthesis at the expense of longer chain n-3 production (Burdge & Calder 2005; Williams & Burdge 2006).

Studies demonstrate EPA concentrations increase in plasma and cell lipids relative to ALA intake (1-3 grams/day) in a dose-dependent manner (Barcelo-Coblijn & Murphy 2009; Burdge & Calder 2005). DHA intake does not show this relation to ALA intake (Barcelo-Coblijn & Murphy 2009; Burdge & Calder 2005). To increase endogeneous levels of DHA, smaller amounts of n-6 must be consumed in addition to greater intakes of DHA.

The manner in which brain and retina acquire necessary levels of PUFA has remained a subject of controversy until recently (Barcelo-Coblijn & Murphy 2009; Crawford et al. 1994; Innis et al. 1999). PUFA synthesis is thought to occur largely in liver after which longer-chain fatty acids are exported and incorporated into other cells (Innis et al. 1999). The brain is a unique organ. Earlier research suggested the brain is autonomous in producing most of its required lipids in

astrocytes (Barcelo-Coblijn & Murphy 2009; Innis 2000). More recent research demonstrates that DHA in free form is rapidly absorbed through the blood brain barrier and incorporated into brain phospholipids (Barcelo-Coblijn & Murphy 2009).

1.3.3 Conversion

Though humans are incapable of interconverting n-3 and n-6 FA due to the absence of the converting enzyme n-3 desaturase, humans are capable of synthesizing longer chain n-3 and n-6 from respective precursors (Brenna 2002; Simopoulos 2000). However, the conversion process is not efficient. The first step of the conversion process is rate-limiting (Brookes et al. 2006). Conversion of ALA to EPA has been found to be only two to ten percent and conversion of ALA to DHA only one to five percent (Barcelo-Coblijn & Murphy 2009; Brenna 2002; Brookes et al. 2006; Burdge & Calder 2005; Candela et al. 2011; Luchtman & Song 2013). A significant percentage of ALA is β-oxidized for use as energy by tissues in the body or recycled as a carbon source to produce other FA, amino acids, and sterols (Barcelo-Coblijn & Murphy 2009; Brenna 2002). Desaturase enzymes are regulated by a negative feedback loop, suggesting that composition of dietary FA intake is important (Brookes et al. 2006). If the dietary intake ratio of n-6 to n-3 FA is unbalanced, excess of one type of FA could suppress conversion of the other (Brookes et al. 2006).

The conversion process is regulated by various factors including genetic factors, stress hormone levels, and dietary intake of SFA and vitamin and mineral cofactors (Schuchardt et al. 2010). Minerals such as magnesium and zinc are crucial desaturase enzyme cofactors. Lack of these micronutrients leads to inhibition of enzyme activity (Schuchardt et al. 2010).

Recently, genetic association studies have found gene variants significantly influence PUFA composition of human tissues (Brookes et al. 2006; Glaser et al. 2011; Lattka et al. 2013; Standl et al. 2012; Steer et al. 2013). Single nucleotide polymorphisms (SNPs) within fatty acid desaturase enzymes 1 and 2 (FADS 1 and 2) determine the efficiency of the conversion process of n-3 and n-6 FA (Lattka et al. 2013; Standl et al. 2012). FADS genes 1 and 2 encode delta-5 and 6 desaturase enzymes respectively (Lattka et al. 2013; Steer et al. 2013).

FADS genotypes modulate DHA levels in maternal red blood cells and may influence the supply of DHA to the foetus during pregnancy (Lattka et al. 2013). The FADS gene cluster may be associated with formation of prefrontal white matter which is important for the development of higher-order cognitive functions (Peters et al. 2014). Proper synthesis of n-3 FA is required for healthy white matter formation. Deficiencies in white matter development are implicated in the pathophysiology of psychiatric disorders (Peters et al. 2014). Recent studies suggest SNPs modulate the effect of dietary FA intake on complex phenotypes such as cognitive and behavioural outcomes (Brookes et al. 2006; Lattka et al.

2013). The above research strongly indicates that FA status is influenced by both diet and endogenous metabolism (Glaser et al. 2011; Lattka et al. 2013). SNPs of low FADS activity could be aetiological factors in the n-3 deficiency observed in children with AD/HD (Brookes et al. 2006).

1.3.4 Mechanisms of Action

The specific mechanisms of action of DHA on symptoms of AD/HD are unknown (Ramakrishnan et al. 2009; Yehuda et al. 1999). However, theories have been proposed for the mechanisms of action of n-3 FA on cognition and behaviour (Kaplan et al. 2007; Yehuda et al. 1999). LCPUFA are incorporated into cell membranes, particularly the lipid bilayer of the plasma membrane (Candela et al. 2011). Long chain n-3 are structural and functional components of cell membranes which in turn mediate CNS processes such as attention, alertness, and motor coordination (Gustafsson et al. 2010; Schuchardt et al. 2010).

DHA is necessary for optimal visual and cognitive development (Carlson et al. 1996; Clandinin et al. 2005; Drover et al. 2009; Richardson 2008; Uauy et al. 2001). During the last trimester of gestation, there is rapid accumulation of DHA in the body (Clandinin et al. 1980) and DHA continues to accumulate in large amounts in the grey matter of the brain until five years of age (Levitt 2003). Research has shown high levels of DHA in phospholipids of the nervous system, reproductive organs, and retina (Carlson et al. 1996; Innis et al. 1999, 2004; Jumpsen & Clandinin 1995). The brain and retina continue to develop throughout

early childhood (Levitt 2003). Therefore, adequate intakes of DHA are required during early development for optimal visual and cognitive function.

Increases in DHA in neuronal cell membranes have been shown to improve neuronal maturation, membrane fluidity, and metabolic function (Clandinin et al 1994; Gustafsson et al. 2010; Luchtman & Song 2013; Riediger et al. 2009; Yehuda et al. 1999). Healthy neuronal development is important for efficient neurotransmission (Clandinin et al 1994; Riediger et al. 2009; Yehuda et al. 1999). DHA is concentrated at nerve cell synapses and is necessary for efficient neural cell signaling (Riediger et al. 2009; Yehuda et al. 1999). The importance of DHA in neuronal health and function is suggested by rigid maintenance of high amounts of DHA in membrane composition and low levels of other PUFA including EPA, ALA, and LA (Luchtman & Song 2013).

Adequate amounts of DHA are necessary in the phospholipid fraction of cell membranes for efficient activity of key enzymes necessary for signal transduction (Kaplan et al. 2007; Luchtman & Song 2013; Raz & Gabis 2009; Riediger et al. 2009; Yehuda et al. 1999). Co-enzymes regulate important cellular functions. The position, integration, and functioning of co-enzymes are affected by membrane fluidity and thus FA composition (Luchtman & Song 2013). For example, Folate (vitamin B9) and Pyridoxine (vitamin B6) are converted into co-enzymes necessary for synthesis of Dopamine from L-Tyrosine (Rolfes et al. 2006). Deficiencies in DHA may alter the structure and function of membranes,

leading to inadequate synthesis and/or binding of co-enzymes necessary for the production of dopamine (Milte et al. 2009; Riediger et al. 2009; Yehuda et al. 1999).

Research over the past decade has demonstrated that quantitative changes in endogenous n-3 FA often co-occur with quantitative changes in monoamine concentrations in brain (Chalon et al. 2001; Zimmer et al. 1999). Dopamine is the neurotransmitter essential for working memory and regulation of attention, motivation, and emotion (Chalon et al. 2001; Levitt 2003). Neurons responsible for the production of dopamine are composed of up to 80% DHA (Sadiq 2007 as cited in Rucklidge et al. 2009). Deficiencies in DHA may impede production of dopamine, leading to alterations in cognitive and behavioural functioning (Milte et al. 2009; Yehuda et al. 1999).

Ratio of n-6 to n-3 FA is important to cellular functioning and long-term health (Glaser et al 2011). The optimal ratio for healthy cellular functioning is 3 to 1 omega 6 to omega 3 fatty acids (Candela et al. 2011; Kirby et al. 2010; Simoupolos 2000). In North America, the ratio has increased to between 10 and 30:1 n-6 to n-3 FA (Simoupolos 2000).

Production of eicosanoids and docosanoids is mediated by the endogenous ratio of n-6 to n-3 FA (Luchtman & Song 2013; Simoupolos 2000). Eicosanoids are key regulators of inflammation and are important in sleep and neurotransmitter

release (Luchtman & Song 2013). Increased endogenous levels of EPA leads to increased production of anti-inflammatory eicosanoids, in contrast to AA which is the precursor to predominantly inflammatory eicosanoids. Docosanoids are synthesized from DHA and are neuroprotective and anti-inflammatory. Production is influenced by dietary intake of both n-6 and n-3 fatty acids (Luchtman & Song 2013).

Reductions in inflammatory response may be important in ameliorating cognitive function (Milte et al. 2009, 2011; Riediger et al. 2009; Simopoulos 2000) and physical symptoms of EFA deficiency such as skin problems which are more prevalent in children with AD/HD compared to controls (Kaplan et al. 1989; Riediger et al. 2009; Stevens et al. 2003).

1.3.5 Dietary Sources

The n-3 and n-6 FA are naturally present in a variety of foods. ALA is the most common n-3 fatty acid in the diet (Kris-Etherton et al. 2000). Soybean, canola, and flaxseed oil are good sources of ALA (Kris-Etherton et al. 2000; Broadhurst et al. 2002). Rich plant sources of ALA include green leafy vegetables, flaxseed, walnuts, hempseed, and pumpkin seed (Broadhurst et al. 2002; Kris-Etherton et al. 2000).

EPA and DHA are synthesized by marine algae and are found in cold water fish and other marine sources. Mackerel, herring, and salmon are the three richest

marine sources of DHA (Kris-Etherton et al. 2000). Mackerel contains between 2 and 5 percent n-3 FA by weight, herring contains 1 to 3 percent n-3 FA by weight, and salmon contains 1.5 percent n-3 FA by weight. For comparison, shrimp contains approximately 0.5 percent n-3 FA by weight and cod contains 0.2 percent n-3 FA by weight.

The n-6 fatty acids LA and AA are abundant in Western diets (Broadhurst et al. 2002). LA is found in plant oils such as sunflower, safflower, and corn oils, nuts, seeds, animal fat, and whole grain bread. AA is present in meat, poultry, and egg yolks. The n-6 and n-3 fatty acids are also found in human breast milk (Clandinin et al. 1982).

1.3.6 Dietary Intake

Physiological cell function is optimal at a 3:1 ratio n-6 to n-3 (Candela et al. 2011; Kirby et al. 2010; Schuchardt et al. 2010; Simoupolos 2000). In North America, this ratio has increased to between 10 and 30:1 n-6 to n-3 FA, leading to increased inflammatory response as well as cognitive and behavioural deficits (Schuchardt et al. 2010; Simopoulos 2000).

To increase dietary intake of n-3 FA, food manufacturers enrich products with n-3 FA and market the health benefits of this important class of FA (Riediger et al. 2009; Simopoulos 2000). Some of the most common n-3 FA enriched products are infant formula, eggs, and bread (Riediger et al. 2009; Simopoulos 2000).

1.3.7 Recommended Dietary Intake

In Canada, nutrient dietary reference intakes are calculated for healthy individuals and medical conditions such as cardiac disease (Health Canada 2010; Rolfes et al. 2006). Values are based on experimentally derived international levels or mean nutrient intakes of groups of individuals (Simopoulos 2000). Two sets of values are calculated: Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) (Health Canada 2010; Rolfes et al. 2006). RDA values are average daily amounts of nutrients required for the healthy functioning of most individuals. If insufficient evidence exists to calculate an RDA, an AI is set.

RDA values for PUFA have not been calculated for children, however, AI values for the two main PUFA in the diet have been set (Health Canada 2010; Rolfes et al. 2006). It is recommended that children between the ages of four and eight years consume 10 grams of LA and 0.9 grams of ALA daily for healthy development (Health Canada 2010). Current research suggests children are meeting these recommendations (Health Canada 2010; Lien, 2005; Meyer et al. 2003).

There is a paucity of research examining AA and DHA intake in children (Lien 2005; Meyer et al. 2003). A few studies have been conducted with typical children. Lien (2005) conducted a study with 78 children between the ages of four and seven years. Mean dietary intakes of AA and DHA were 57 mg and 37 mg respectively. Meyer and colleagues (2003) reported mean dietary intakes of 47

mg and 22 mg of AA and DHA respectively in 799 Australian children between the ages of four and seven years. A study examining DHA intake in children three to five years of age in the Vancouver Canada Coastal Health Authority Region where seafood is more readily available found mean dietary intakes of AA and DHA to be higher at 226 mg and 96 mg/day respectively (Innis et al. 2004).

Research examining PUFA intake in children with AD/HD is limited. Studies have found similar PUFA intakes in children with AD/HD compared to typical children (Burgess et al. 2000; Richardson 2006; Stevens et al. 2003).

1.3.8 Assessment of Dietary Intake

Recently, researchers and clinicians have put a growing emphasis on dietary intake in paediatric populations. Links between lifestyle choices in childhood and conditions experienced in adulthood such as obesity, cardiovascular disease, and osteoporosis have received increased attention in recent years (Magarey et al. 2011; Rockett & Colditz 1997; Wilson & Lewis 2004). The important relationship between early nutritional practices and later health outcomes underlines the need for accurate dietary assessment in nutrition research to inform effective interventions (Fregapane & Asensio-Garcia 2000; Jardack 2006; Magarey et al. 2011; Ngo et al. 2009).

There are four main assessments employed in nutrition research to measure dietary intake in children: the diet history, 24 hour recall, food frequency

questionnaire (FFQ), and diet record (Magarey et al. 2011). This section will describe each of the methods and provide a rationale for the assessment method employed in the current study.

In 1938, Burke and Stuart developed the diet history method for use in longitudinal studies on child health and development (Block 1982; Rockett & Colditz 1997). A six to twelve-month history of typical dietary intake including portion sizes is obtained during an interview between the participant and investigator. Because of the significant time required to obtain diet histories, other less time consuming assessments are often employed.

The 24 hour recall is a self-administered assessment of the participant's dietary intake over the previous 24 hours. The assessment can be administered quickly reducing respondent burden present with other methods. Research has found that children's diets vary considerably from day to day, therefore, a single 24 hour recall does not accurately reflect usual dietary intake (Block 1982; Wilson & Lewis 2004). Seven-day recalls may be administered to obtain data more representative of usual intake. However, research suggests that recall beyond a period of several days may not be accurate due to memory lapses and lack of motivation (Block 1982; Cade et al. 2002). Use of multiple 24-hour recalls randomised throughout the study are more representative of typical dietary intake (Cade et al. 2002; Holmes & Nelson 2009).

Many epidemiological and clinical studies employ the FFQ to assess dietary intake (Jardack 2006). The FFQ is a relatively quick, self-administered assessment used to obtain information about typical dietary intake over the previous six to twelve months. Food items are grouped into categories. Respondents select how often a particular food category or item is eaten, ranging from never to multiple times a day. Early FFQs were purely qualitative, limiting analysis of nutrient and energy intake (Fregapane & Asensio-Garcia 2000). Currently, FFQs are semi-quantitative, asking respondents to either select portion sizes from a list for food items eaten or calculate individual portion sizes.

FFQs have been found to underestimate actual nutrient intake due to the imprecision associated with recalling precise food intake over many months (Bergman et al. 1990; Holmes & Nelson 2009; Magarey et al. 2011; Taylor & Goulding 1998). FFQs are not recommended for use in studies where actual nutrient and energy calculations are important. Research suggests that specific information about items consumed is necessary for accurate analyses of nutrient and energy intake (Briefel et al. 1992; Emmett 2009). Brand names, methods of preparation, and portion sizes allow for calculations of actual dietary intake.

Diet records are considered by many to be the ‘gold standard’ against which other dietary assessments are compared (Okuda et al. 2009; Michels et al. 2004; Wilson & Lewis 2004). Food records are often employed in paediatric research to assess energy and nutrient intakes (Holmes & Nelson 2009; Wilson & Lewis 2004).

Widdowson and McCance developed the weighed food record in the early 1930s (Rockett & Colditz 1997). Precise information about food items consumed, including descriptions, brand names, methods of preparation, and portion sizes are recorded. The number of days that meals are recorded varies depending on the study. Food records have shown good agreement with actual mean intake values and individual values over the first few days of the record (Block 1982; Togo et al. 2003). Because of the significant burden placed on respondents and investigators while recording and analysing diet record assessments, these types of assessments are not recommended for large studies (Emmett 2009; Holmes & Nelson 2009; Neuhouser et al. 2009).

1.4 DHA & AD/HD

An increasing body of research suggests that perinatal deficiencies in DHA may constitute a risk factor for later psychopathology (Lattka et al. 2013; McNamara & Carlson 2006). Early animal research found a causal relationship between essential fatty acid deficiency and neurological alterations in brain (Caldwell & Churchill 1966; Galli et al. 1971). These findings suggest that decreased levels of LCPUFA in brain affect cognitive functioning.

Omega 3 and omega 6 fatty acids are vital for brain, constituting 30 to 35 percent of total brain fatty acids (Youdim, Martin, & Joseph 2000, as reviewed in Luchtman & Song 2013). Studies confirm that EFA deficiency, namely DHA deficiency, leads to functional and structural deficits (Burgess et al. 2000; Carlson & Neuringer 1999; Jumpsen & Clandinin 1995; McNamara & Carlson 2006). Impairments in

retina and brain are observed, specifically in the frontal cortex and dopaminergic system.

Research with animals implicates a deficiency of DHA in cognitive and behavioural deficits (Burgess et al. 2000; Carlson & Neuringer 1999; Carrie et al. 2000; Drover et al. 2009; McNamara & Carlson 2006). Research conducted with n-3 FA deficient monkeys pre-and postnatally found lower n-3 FA levels in brain and retina and abnormal retinal function (Burgess et al. 2000; Carlson & Neuringer 1999). Low n-3 levels also increased look duration times in a visual attention test as compared to healthy controls. In infants, longer look duration correlates positively with poorer performance on later cognitive tests (Carlson & Neuringer 1999). Look duration may be a measure of information processing speed. Carlson and Neuringer (1999) found that EFA deficiency affects the temperament, motivation, and sensation of monkeys, which in turn affects cognition.

Studies with mice have also provided evidence for the importance of DHA (Carrie et al. 2000). Phospholipids in mice frontal cortex are highly enriched in EFA. Chronic ALA deficiency leads to significant reductions in n-3 FA in frontal regions, as well as in the striatum. Supplementation of ALA increases n-3 FA to control levels in striatum but not in prefrontal cortex, suggesting that chronic n-3 deficiency may lead to long-term impairments in prefrontal cortex functioning.

Research has also noted learning impairments, reduced exploratory activity, and increased anxiety in n-3 PUFA deficient mice (Carrie et al. 2000). EFA supplementation reverses the above deficits. Another study found that mice reared on omega-3 FA deficient diets for two generations behaved differently and had different side effects to behaviour-affecting medicines than controls (Hamazaki et al. 1999). The above studies with mice suggest that n-3 deficiency leads to alterations in behaviours.

A relationship between levels of brain PUFA and functioning of the dopaminergic system has been shown to exist (Takeuchi et al. 2002; Zimmer et al. 1999). ALA-deficient rats have decreased dopamine levels and Dopamine 2 (D2) receptor binding in the prefrontal cortex. This has been shown to cause visual and behavioural problems, as measured by abnormal responses on a variety of learning tests.

Inadequate storage of dopamine has been implicated in the aetiology of these problems. During learning tasks, the dopamine system is stimulated. Inadequate levels of this neurotransmitter may lead to cognitive impairments, as the brain is unable to sustain a satisfactory level of dopamine release.

Clinical trials over the past two decades with pre-term and term infants have shown supplementation of infant formulas with DHA leads to improvements in visual acuity (Birch et al. 2000, 2007; Carlson et al. 1999; Drover et al. 2009; Lien

2005), speed of information processing (Werkman et al. 1996), psychomotor and cognitive development (Birch et al. 2000; Clandinin et al. 2005; Drover et al. 2009), and enhanced growth (Clandinin et al. 2005). Gale and colleagues (2009) found that mothers who consumed oily fish in early pregnancy had a decreased risk of their children displaying hyperactivity at age nine.

Studies have found that, compared to controls, many children with AD/HD have low plasma phospholipid levels of DHA and increased n-6 to n-3 ratios (Antalis et al. 2006; Colter et al. 2008; Germano et al. 2007; Gow et al. 2009; Kaplan et al. 1989, 2007; Milte et al. 2011; Richardson 2006). In 1995, Stevens and colleagues observed significantly lower levels of AA, EPA, and DHA in plasma and blood cell lipids and increased n-6 to n-3 ratios in fifty-three boys with AD/HD, compared to controls. Lower n-3 status was associated with higher scores on the Conner's Parent Rating Scales.

The following year, the above researchers observed higher incidences of conduct problems, hyperactivity/impulsivity, temper tantrums, and sleep deficits in boys with AD/HD, compared to controls. The children with AD/HD urinated more often, had greater thirst, and exhibited more dry skin than their typical peers. EFA dietary intake did not differ between the two groups.

In 2000, Burgess and colleagues found that children who presented with more severe symptoms of AD/HD had lower total n-3 levels, including DHA than

children with milder symptoms. These results confirm those observed by Mitchell and colleagues (1987). Mitchell and colleagues (1987) found AA and DHA levels were lower in the plasma of forty-eight hyperactive children, compared to controls. The children with hyperactivity had significantly more language, reading, and learning difficulties and displayed more visual and auditory deficits.

Kaplan and colleagues found that halitosis, rhinitis, headaches, and night awakenings were reported significantly more often in children with AD/HD than in children without the disorder (Kaplan et al. 1989, 2007). Sleep latency was also found to be affected in children with AD/HD.

Recently, Milte and colleagues conducted a trial with 75 children diagnosed with AD/HD between the ages of seven and twelve years (2011). Levels of n-3 were inversely correlated with anxiety and shyness scores. Increased DHA predicted better reading outcomes. Reading improved by one to three quarters of a standard deviation for each percent increase in DHA.

Gow and colleagues (2009) found lower levels of DHA were associated with poor responses to facial expressions of fear in adolescents with AD/HD. Low n-3 concentrations have been linked to higher scores of cognitive impulsivity, motor control, and neuroticism. These findings are significant as the above listed symptoms are strongly associated with conduct problems, including violent behaviour in children with AD/HD.

A study conducted in Taiwan found lower RBC AA and DHA and total n-3 FA in 58 children diagnosed with AD/HD compared to 52 control children (Chen et al. 2004). Higher n-6:n-3 FA ratios were observed in the experimental group compared to controls. The children were between the ages of four and twelve years.

The above studies suggest that compared to typically functioning children, many children with AD/HD have lower concentrations of DHA in plasma phospholipids and elevated n-6:n-3 ratios and these may contribute to symptoms of AD/HD.

The importance of DHA for healthy cognitive and behavioural functioning led to supplementation studies of DHA with children with symptoms of AD/HD. One of the first studies was a double-blind randomized controlled trial (RCT) conducted by Voigt and colleagues in 2001. Sixty-three children with AD/HD between the ages of six and twelve years were randomly assigned to receive either an algae source of DHA or placebo for four months. The experimental group received 345 mg of DHA daily. Tests of inattention and impulsivity and parent behavioural rating scales were the outcome measures. Though plasma levels of DHA increased significantly in the experimental group compared to controls, no effects of treatment were observed.

These results are similar to a double-blind RCT conducted by Hamazaki and colleagues (1999). Forty children with AD/HD between the ages of six and twelve

years were randomly assigned to receive either PUFA or placebo for two months. The PUFA supplement consisted of 14 mg of DHA and 100 mg of EPA from fish oil and fermented soybean oil. No treatment effects were found in the experimental group compared to controls.

These two trials may not have observed treatment effects for several reasons. Hamazaki's trial had a duration of two months. Research suggests a minimum of three months to observe effects of DHA supplementation on behaviour (Richardson 2006). Voigt's trial was four months in length, however, more recent research suggests the doses of DHA administered in both Voigt's and Hamazaki's studies may not have been high enough to observe treatment effects in AD/HD populations (Richardson 2006; Sinn & Bryan 2007; Johnson et al. 2009).

A number of studies offer support for the role of DHA in ameliorating symptoms of AD/HD in children (Germano et al. 2007; Johnson et al. 2009; Richardson 2006; Sinn and Bryan 2007). A twelve-week RCT was conducted in the UK with 41 children between the ages of eight and twelve years with dyslexia and symptoms of AD/HD (Richardson & Puri 2002). Half the children received an EFA complex containing 186 mg EPA, 480 mg DHA, 96 mg GLA, 42 mg of AA, and 60 IU vitamin E per day and half the children received an olive oil placebo. Significant improvements were observed in the treatment group on CPRS scales of Cognitive Problems, Anxiety/Shyness, Inattentiveness, Hyperactivity/Impulsiveness, and the Conner’s AD/HD Index.

Three years later, the same research group conducted a twelve-week RCT with a one-way crossover design (Richardson & Montgomery 2005). All but four of the 117 children presented with symptoms of AD/HD in the clinical range. Symptoms of AD/HD were assessed by teachers only. Significant improvements were found in the treatment group in 4 out of 6 CTRS subscales, including Inattentiveness and Hyperactivity/Impulsiveness, 7 out of 7 global scales, and in objective assessments of reading and spelling. One hundred children completed the three-month crossover phase of the study. All children received the EFA complex. Similar outcomes to the treatment group were observed in the placebo group following supplementation with EFA.

An RCT was conducted with 50 children between the ages of six and thirteen years diagnosed with AD/HD and all presenting with EFA deficiency symptoms (Stevens et al. 2003). Children were randomized to receive either an EFA complex composed of 480 mg DHA, 80 mg EPA, 96 mg GLA, 40 mg AA, and 56 IU Vitamin E or placebo for four months. Significant treatment effects were observed on parent ratings of aggressive behaviour and teacher-rated attention difficulties. Significant increases in plasma phospholipid DHA and EPA were found in the EFA group compared to placebo. Increases in phospholipid DHA and EPA were significantly negatively correlated with parent and teacher ratings of behaviour.

Sinn and colleagues (2007) conducted a fifteen-week RCT with 167 children, ages seven to twelve years. The study was of similar design to Richardson and colleagues. To help ensure all children displayed symptoms of AD/HD, inclusion criteria included scoring a minimum of two standard deviations above the population mean on the Conner's Abbreviated AD/HD Index. Children were randomized into three groups: EFA, EFA + Multivitamin/Mineral, and Placebo (palm oil). No significant differences in treatment effects were observed in the EFA+Multivitamin/Mineral group compared to the EFA only group. When these two groups were combined, significant treatments effects were observed compared to placebo. Scores on CPRS scales of Cognitive Problems, Inattention, Restlessness/Impulsiveness Oppositional Behaviour, and the AD/HD Index improved in the EFA groups versus placebo.

Sinn and Bryan conducted a fifteen-week second phase to their study including 109 children from their first sample (2007). The trial was single-blind in that only the researchers were aware that all children were placed in the EFA+Multivitamin/Mineral group. The former placebo group showed improvements on the same scales as the EFA groups had in phase one. Both groups continued to show improvement on CPRS scales.

Johnson and colleagues (2009) conducted a three-month RCT with 75 children and adolescents with AD/HD between the ages of eight and eighteen years. The experimental group consumed six capsules a day of Equazen™ eye q for a total

of 558 mg EPA,174 mg DHA, 60 mg GLA, and 10.8 mg vitamin E daily. The placebo group consumed olive oil capsules. A significant increase in n-3 FA and a significant decrease in the n-6:n-3 ratio were observed. Changes in FA status were associated with treatment response. Significant treatment effects were observed on the Clinical Global Impression Scale (CGI) which assesses functional impairment and symptom severity. A subgroup with reading and writing difficulties were among the strongest responders. The subgroup comprised of 26 percent of the treatment group displayed a 25 percent reduction in AD/HD symptoms and a drop of CGI scores to near normal range. The supplementation phase was followed by a three-month open-label phase of n-3 FA supplement for all children. Similar results were observed in the second phase with 47 percent of children experiencing significant reductions in CGI scores and symptoms of AD/HD.

Germano and colleagues (2007) conducted an open-label trial with 31 children with AD/HD between the ages of three and a half and sixteen years. Children took 2.5 grams of EPA/DHA/kg of body weight daily (52% EPA, 28% DHA) for eight weeks. Inattention and Hyperactivity scores on the CPRS improved significantly.

Sorgi and colleagues (2007) conducted an eight-week open-label study with nine children with AD/HD between the ages of eight and sixteen years. Children received 10.8 grams of EPA and 5.4 grams of DHA daily. Significant improvements were found on the Inattention, Hyperactivity, and Oppositional

Defiant behaviour scales. Clinical improvement correlated with decreases in the AA:EPA ratio.

The current two studies were conducted to address a number of questions still present in the EFA supplementation literature involving children with AD/HD. Researchers have identified a number of issues to consider for future n-3 supplementation research with children with AD/HD (Raz & Gabis 2009; Richardson 2006; Schuchardt et al. 2010). Firstly, studies vary in the primary diagnosis of children. Additional studies are needed examining children with formal diagnoses of AD/HD. Following best practice guidelines, administration of behavioural assessments in more than one setting is needed to confirm results (Raz & Gabis 2009). Optimum compositions and dosages of LCPUFA supplements and length of treatment must be identified. Richardson (2006) suggests more studies examining biological markers of n-3 fatty acid status are required to answer important questions about mechanisms of action of n-3 FA and identify which children would benefit most from nutritherapy with n-3 FA.

The current research sought to identify subgroups of children with AD/HD with impaired FA metabolisms and examine the effects of n-3 FA supplementation on both physical and behavioural symptoms of AD/HD. The next two chapters discuss the present research examining n-3 FA status and the benefits of therapeutic doses of n-3 fatty acids on symptoms of AD/HD in children diagnosed with the disorder.

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Chapter II The Relationship Between Dietary Intake, Plasma Phospholipid Levels Of DHA, And Physical Symptoms Of EFA Deficiency In Children Diagnosed With AD/HD

2.1 Introduction

AD/HD is the most prevalent neurodevelopmental disorder in paediatric populations (AAP 2011; Barkley 2006; Milte et al. 2009; Rader et al. 2009). Children with AD/HD present with chronic neuropsychological and cognitive deficits that severely impact social and academic functioning (AAP 2011; Barkley 2006, 2013; DuPaul et al. 2011; Hale et al. 2011; Milte et al. 2009; Rader et al. 2009). The disorder is associated with considerable psychological and financial burden (AAP 2011; Barkley 2006, 2013; Milte et al. 2009; Rader et al. 2009). Up to 80 percent of children with AD/HD struggle academically and in their personal lives (DuPaul et al. 2002; Jitendra et al. 2008). Billions of dollars are incurred annually by school systems to manage symptoms of AD/HD and health care costs are more than double in this population (Barkley 2006, 2013; Mash & Barkley 2006; Schab et al. 2004). Because of the significant impact of AD/HD, examining aetiological factors to devise more effective treatments is necessary.

Recent research suggests deficiencies in DHA may be involved in the aetiology of AD/HD (Antalis et al. 2006; Burgess et al. 2000; Gow et al. 2009; Kirby et al. 2010; Konikowska et al. 2012; Richardson 2006, 2008; Sinn & Bryan 2007). DHA is necessary for optimal visual and cognitive development (Drover et al. 2009; Carlson

et al. 1996, 2013; Clandinin et al. 2005; Latka et al. 2013; Milte et al. 2009; Uauy et al. 2001). Omega 3 and omega 6 fatty acids are vital for brain development; they constitute 30 to 35 percent of total brain fatty acids (Luchtman & Song 2013). The importance of DHA in neuronal health and function is suggested by rigid maintenance of high amounts of DHA and AA in membrane composition and low levels of other PUFA including EPA, ALA, and LA (Luchtman & Song 2013).

Dopaminergic projections in frontal cortex develop and mature predominantly between midgestation and birth (McNamara & Carlson 2006) and are composed of up to 80% DHA (Sadiq 2007 as cited in Rucklidge et al. 2009). During the last trimester of gestation, there is rapid accumulation of DHA in the body (Clandinin et al. 1980) and DHA continues to accumulate in large amounts in the grey matter of the brain until five years of age (Levitt 2003).

Research has shown high levels of DHA in phospholipids of the nervous system, reproductive organs, and retina (Carlson et al. 1996, 2013; Clandinin et al. 1980). The brain and retina continue to develop throughout early childhood (Levitt 2003). Therefore, adequate intakes of DHA are required during early development for optimal visual and cognitive function.

An increasing body of research suggests that perinatal deficiencies in DHA may constitute a risk factor for later psychopathology (Lattka et al. 2013; McNamara & Carlson 2006). Deficiencies in DHA may contribute to physical and behavioural

impairments observed in children with AD/HD (Gow et al. 2009; Kirby et al. 2010; McNamara & Carlson 2006; Mitchell et al. 1987; Richardson 2008; Stevens et al. 2003). Research has found subsets of children with the disorder have lower plasma and red blood cell levels of DHA, increased n-6 to n-3 fatty acid ratios, and exhibit symptoms of EFA deficiency (Carlson & Neuringer 1999; Colter et al. 2008; Germano et al. 2007; Milte et al. 2011; Richardson & Puri 2002; Stevens et al. 2003). Kaplan and colleagues found that headaches, night awakenings, halitosis, and rhinitis were reported significantly more often in children with AD/HD compared to typically functioning children (Conte et al. 1987; Kaplan et al. 1987_b, 1989, 2007). Sleep latency was also shown to be affected in children with AD/HD (Kaplan et al. 1987_a).

Mitchell and colleagues reported children with hyperactivity had lower levels of AA and DHA in serum phospholipids than non-hyperactive children (Mitchell et al. 1987). Children with hyperactivity had significantly more language, reading, and learning difficulties and displayed more visual and auditory deficits compared to controls.

In 1995, Stevens and colleagues reported that compared to controls, children with AD/HD had significantly lower levels of AA and DHA in their plasma and blood cell lipids and increased n-6 to n-3 fatty acid ratios (Stevens et al. 1995, 2003). Children with AD/HD urinated more often, had greater thirst, and exhibited more dry skin than children without AD/HD.

Recently, Milte and colleagues conducted a trial with 75 children diagnosed with AD/HD between the ages of seven and twelve years (2011). Levels of n-3 were inversely correlated with anxiety and shyness scores. Increased DHA predicted better reading outcomes. Reading improved by one to three quarters of a standard deviation for each percent increase in DHA.

A number of studies suggest that a subset of children with AD/HD have lower DHA status, increased n-6:n-3 ratios, and exhibit more physical and behavioural impairments compared to control children (Colter et al. 2008; Germano et al. 2007; Sinn 2008; Sinn & Bryan 2007). The current study examined whether children with AD/HD exhibit physical symptoms of EFA deficiency and whether severity of symptoms correlates with dietary intake of DHA and/or plasma phospholipid levels of the fatty acid.

2.2 Study Design:

The purpose of the present study was to examine the relationship between dietary intake, plasma phospholipid levels of DHA, and physical symptoms of EFA deficiency in children diagnosed with AD/HD.

It was hypothesized that children with AD/HD would present with lower DHA status than typically functioning children and would exhibit symptoms of EFA deficiency. Blood and buccal samples were obtained and compared to four-day diet record and Medical Symptom Questionnaire results.

The current study was approved by the Health Research Ethics Board of the Department of Medicine at the University of Alberta. Parent(s)' written consent and each child's written assent were obtained prior to enrolment in the study (Appendix A).

2.3 Methods:

2.3.1 Recruitment of Participants:

One hundred and three boys and girls between the ages of five and twelve years diagnosed with AD/HD were randomly recruited as volunteers from Edmonton and other areas of Alberta. Recruitment occurred through a number of different sources including articles in main newspapers, radio and television newscasts, circulating pamphlets describing the study to all Edmonton Public Elementary schools, and through a local mental health services organization that sent information regarding the study to parents of children who met inclusion criteria.

2.3.2 Inclusion Criteria:

Children diagnosed by a qualified healthcare professional (Pediatrician, Psychiatrist or Psychologist) as having any of the sub-types of AD/HD, according to DSM-IV criteria were included (American Psychiatric Association 2000). AD/HD had to be the primary diagnosis but participants could present with co-morbid conditions. Comorbidities included Oppositional Defiant Disorder (ODD), Conduct Disorder (CD), anxiety disorders, mood disorders, and learning disorders. Children diagnosed with Fetal Alcohol Syndrome or Autism were excluded from the study.

Only children of average cognitive functioning according to parent report or scoring a full scale IQ of 70 or greater on a standardised test conducted by a healthcare professional within the past year were included in the study. Participants could be taking medication such as methylphenidate (Ritalin®) to treat AD/HD and/or other prescription medication. Specific medications each child was taking, including dose and length of treatment were recorded. Study participants could not be consuming any special diets or supplements that contained DHA.

2.3.3 Diet Records:

Following screening interviews conducted by telephone, families who met inclusion criteria were mailed diet record forms. Four-day diet records were completed to quantify the amount of DHA-containing foods such as meat, fish, eggs, nuts, and seeds children consumed (Appendix D). Parents were instructed to complete the form over four consecutive days, one of which was required to be a weekend day. Records were reviewed with the researcher during a meeting held at a mental health services organization in Edmonton, Canada. Diet records were analysed by a dietetic intern using Food Processor software (Esha Research, Salem Oregon).

2.3.4 Fatty Acid Analysis:

A blood sample was obtained from each child by a finger prick by a registered nurse. Blood samples were collected in microtainer tubes containing heparin and put on ice in a biosafety container for transport to the lab for analysis. Plasma was separated by centrifugation and frozen until analysis. Plasma lipids were extracted using the

modified Folch procedure (Folch et al. 1957) and the phospholipid fraction was separated by thin layer chromatography (Clandinin et al. 1997). Fatty acids of the phospholipid fraction were quantified by gas-liquid chromatography. Proportion of FA in plasma phospholipids is considered a highly reliable biomarker of n-3 status of body organs such as the heart and brain (Clandinin et al. 1999; Kuratko & Salem 2009 as cited in Bradbury 2011).

2.3.5 Height, Weight, and Buccal Swab:

The registered nurse measured each child's height and weight. Buccal swab samples were collected from each child using individually packaged sterile cotton swabs. Alcohol was added to the tubes to preserve samples during transport to the lab for analysis.

Total lipids were extracted from buccal cells according to the Folch method (Folch et al. 1957). Fatty acid methyl esters were prepared from the lipid fraction and were separated and analysed by gas-liquid chromatography. Fatty acid analysis of buccal cells has been shown to reflect dietary intake and blood and plasma levels of EFA (Birch et al. 2000).

2.3.6 Medical Symptom Questionnaire:

During the meeting with the researcher, parents completed a Medical Symptom Questionnaire about their child (Appendix F). The questionnaire consisted of the twelve most common physical symptoms of EFA deficiency observed in children with

AD/HD (Conte et al. 1987; Kaplan et al. 1987; Mitchell et al. 1987; Stevens et al. 1995). Symptoms included halitosis, rhinitis, headaches, sleep deficits (Conte et al. 1987; Kaplan et al. 1987, 1989), frequent urination, greater thirst, skin problems such as dry skin and eczema (Stevens et al. 1995, 2003), and visual and auditory deficits (Mitchell et al. 1987). A four-point Likert scale was used to determine severity of each symptom. A rating of 0 indicated absence of a symptom and a rating of 3 indicated presence of a symptom most of the time. The total possible score on the scale was 36. The Medical Symptom Questionnaire was created by the first author and is not a standardized assessment.

2.3.7 Statistical Analyses

Data were analysed using SPSS 16.0 (SPSS Inc., Chicago, 2007). Variables showed a normal distribution, as determined by the Shapiro-Wilk test. Pearson product correlations and t-tests were calculated.

2.4 Results:

Sample Demographics:

One hundred and three children participated in the study; ninety boys and thirteen girls. Seventy percent of subjects were between the ages of five and eight years and thirty percent were nine to twelve years of age. Seventy-eight percent of the sample were taking prescription medication to treat AD/HD (Table 2.1).

Table 2.1 Gender and Medication Status

Gender	Sample (% of total)	Taking Meds for AD/HD (% of gender)	5-8 year-olds (% of age group)	9-12 year olds (% of age group)
Male	90 (87.4%)	70 (78%)	64 (89%)	26 (84%)
Female	13 (12.6%)	10 (77%)	8 (11%)	5 (16%)
Total	103	80 (78%)	72 (70%)	31 (30%)

Dietary Intakes of DHA:

Participants' mean dietary intake of DHA over the four-day period was calculated. Values ranged from 1.0-424 mg. The mean dietary intake of DHA across all participants was 42 mg over the four-day diet record period (Table 2.2).

Fatty Acid Profiles:

In the current sample, mean plasma phospholipid levels of DHA and AA were 10.2 µg/ml (SEM 0.96) and 33.4 µg/ml (SEM 2.15) respectively (Table 2.2).

Table 2.2 Mean DHA Intake and Fatty Acid Profiles

	DHA Intake mg	Plasma DHA ug/ml (SEM)	Plasma AA ug/ml (SEM)
Mean n=79	42	10.2 (0.96)	33.4 (2.15)

Mean plasma AA and DHA levels were compared to values obtained in a clinical trial with typically functioning children (Lien 2005). The control children were four to eight years of age and were living in the same region as study participants. Mean AA and DHA levels in Lien's study were 61.7 µg/ml (SEM 2.64) and 16.6 µg/ml (SEM 0.82) respectively. Mean levels of DHA and AA in the current sample were compared to values obtained in Lien's study using the T-test. Mean DHA and AA levels in children with AD/HD were found to be significantly lower than levels found in typically functioning children (P<0.0001), Cohen's d=.83 and 1.58 respectively (Table 2.3) (Cohen 1988).

Table 2.3 AA and DHA Status in AD/HD and Control Children

Fatty Acid Content in Plasma Phospholipid (ug/mL)	**Control Sample n=26**		**ADHD Sample n=79**		**P value T-Test**
	Mean	**SEM**	**Mean**	**SEM**	
AA	61.7	2.64	33.4	2.15	P<0.0001
DHA	16.6	0.82	10.2	0.96	P<0.0001

Recently, genetic association studies have found single nucleotide polymorphisms (SNPs) within fatty acid desaturase enzymes 1 and 2 (FADS 1 and 2). FADS 1 and 2 determine the efficiency of the conversion process of n-3 and n-6 FA (Lattka et al. 2013; Standl et al. 2012). FADS genes 1 and 2 encode delta-5 and 6 desaturase enzymes respectively (Lattka et al. 2013; Steer et al. 2013). Research suggests that minor allele carriers of FADS genes 1 and 2 may exhibit reduced desaturase enzyme

activity, leading to lower conversion rates of longer-chain PUFA (Lattka et al. 2013; Miklavcic et al. submitted for publication; Standl et al. 2012).

Genotyping of the current sample was conducted as a post-hoc analysis by a collaborator (Miklavcic et al. submitted for publication). Post-hoc analyses of the current data using a t-test found that children who presented with two major alleles in FADS 1 and/or 2 had significantly lower levels of PUFA precursors in plasma phospholipids compared to children who presented with at least one minor allele. Children with two dominant alleles had lower levels of LA in plasma phospholipid in FADS 1 ($p<0.05$) and FADS 2 ($p<0.10$) compared to children who presented with at least one minor allele (Table 2.4). Major allele carriers of FADS 2 had lower levels of ALA in plasma phospholipid ($p<0.10$) compared to children who presented with at least one minor allele (Table 2.4). Across FADS 1 and 2, the mean level of LA was significantly lower ($p<0.05$) in major allele carriers compared to children with at least one minor allele (Table 2.5). All effect sizes were medium. Because minor alleles are associated with decreased desaturase enzyme activity, increased levels of ALA and LA in plasma phospholipids suggest lower conversion rates of precursors to longer chain PUFA.

Table 2.4 Mean Plasma Fatty Acid Values in FADS 1 and 2

PLASMA FATTY ACID	FADS1				FADS2			
	Major Allele Carrier [N=38]		Minor Allele Carrier [N=45]		Major Allele Carrier [N=52]		Minor Allele Carrier [N=31]	
	Mean (µg/ml)	SEM	Mean (µg/ml)	SEM	Mean (µg/ml)	SEM	Mean (µg/ml)	SEM
LA	71.62[a]	5.53	93.70[b]	8.51	76.67[c]	6.84	95.20[d]	8.39
ALA	1.66	.30	2.45	.65	1.60[e]	.30	2.90[f]	.87
AA	32.04	3.06	34.34	3.02	32.89	2.93	33.95	3.03
EPA	1.48	.28	1.76	.45	1.80	.37	1.35	.40
DHA	8.45	1.30	11.08	1.32	10.18	1.38	9.37	.98

Major allele carriers had significantly lower levels of LA in plasma phospholipid in FADS 1 [(a,b), p =0.04] and FADS 2 [(c,d), p=0.095} compared to carriers of at least one minor allele. Major allele carriers of FADS 2 had lower levels of ALA in plasma phospholipid [(e,f), p=0.098] compared to carriers of at least one minor allele, suggesting higher conversion rates of precursors to longer chain PUFA among major allele carriers. LA=Linoleic acid, ALA=Alpha-Linolenic acid, AA= Arachidonic acid, EPA= Eicosapentanoic acid , DHA= Docosahexaenoic acid.

Table 2.5 Mean Plasma Fatty Acid Values Across FADS 1 and 2

PLASMA FATTY ACID	FADS1 and FADS2			
	Major Allele Carrier [N=36]		One or More Minor Alleles Carrier [N=47]	
	Mean	SEM	Mean	SEM
LA	70.76[a]	5.81	93.42[b]	8.14
ALA	1.70	.31	2.39	.63
AA	32.44	3.21	33.93	2.91
EPA	1.50	.29	1.73	.43
DHA	8.62	1.37	10.84	1.27

Across FADS 1 and 2, mean level of LA was significantly lower [(a,b), p =0.036] in major allele carriers compared to carriers of at least one minor allele. Because major alleles are associated with increased desaturase enzyme activity, decreased levels of LA in plasma phospholipids suggest higher conversion rates of precursors to longer chain PUFA. LA=Linoleic acid, ALA=Alpha-Linolenic acid, AA= Arachidonic acid, EPA= Eicosapentanoic acid , DHA= Docosahexaenoic acid.

Buccal Swab Samples:

Mean AA and DHA levels in buccal cell phospholipid were 1.4 µg/ml and 0.23 µg/ml respectively. No significant correlations were found between AA and DHA levels in buccal swab and blood samples (data not shown).

Medical Symptom Questionnaire:

All 103 children with AD/HD in the study experienced symptoms commonly associated with essential fatty acid deficiency such as thirst, frequent urination, and skin conditions. The mean score on the questionnaire was 12/36 or 33% meaning that numerous symptoms were present to a mild or moderate degree or at least 4 symptoms were present to a severe degree.

Initial analyses found no significant correlations between MSQ scores and FA profiles. However, when all 12 items of the Medical Symptom Questionnaire were compared individually to plasma DHA levels, two of the twelve symptoms showed significant negative correlations with plasma DHA levels (Table 2.6). Children with higher plasma DHA levels scored significantly lower on the thirst ($p = .029$) and frequent urination ($p = .033$) scales. Although skin conditions showed a negative correlation with DHA levels, this did not reach significance ($p = .153$).

Table 2.6 MSQ[1] Scales and Plasma DHA Levels

Medical Symptom Questionnaire Scale N=79	Correlation with DHA in Plasma Phospholipid
Thirst	r = -.214* p=.029
Urination	r = -.207* p=.033
Skin Problems	r = -.117 p=.153

Participants with higher plasma DHA levels scored significantly lower on the thirst (p= .029) and frequent urination (p=.033) scales. Skin conditions showed a negative correlation with DHA levels (p=.153). [1]Medical Symptom Questionnaire *Significant at p<0.05, T-test, one-tailed

2.5 Discussion:

The current study suggests deficiencies in DHA may be involved in the aetiology of AD/HD. Dietary intake in the present study is consistent with research conducted with typically functioning children (Lien 2005; Lien et al. 2009; Meyer et al. 2003). The mean dietary intake of DHA in the present study was 42 mg/day. This is comparable to the mean dietary intake of 37 mg/day of DHA observed in Lien's study. Lien examined dietary intakes of DHA in 78 children from Alberta, Canada between the ages of four and seven years, employing a three-day diet record (Lien 2005). Average DHA intakes ranged from 0-350mg/day, which is similar to the 1.0-424

mg/day consumed by children with AD/HD in the present study.

Similarly, in a study by Meyer and colleagues conducted with 799 Australian children between the ages of four and seven years using a 24-hour recall, mean dietary intake of DHA was 47mg/day (Meyer et al. 2003). A study in the United States conducted with 31 children between the ages of four and twelve years reported mean DHA intakes of 58 mg/day for 4 to 6 year olds and 53 mg/day for 7 to 12 year olds (Hawthorne et al. 2009).

Though typically functioning children consumed similar dietary intakes of DHA to the children with AD/HD in our study (Lien 2005; Lien et al. 2009; Meyer et al. 2003), the latter participants have mean plasma phospholipid levels of DHA about half the values observed in Lien's study (Lien 2005; Lien et al. 2009). These results are consistent with recent research comparing EFA status in adolescents diagnosed with AD/HD to a sample of age-matched controls (Colter et al. 2008). Both groups of adolescents consumed similar amounts of DHA, however, the group with AD/HD had significantly lower levels of DHA in red blood cells compared to controls.

Medical symptom questionnaire results obtained in the present study are consistent with previous research suggesting children with AD/HD exhibit symptoms of EFA deficiency (Kaplan et al. $1987_{a,}$ 1987_{b}, 1989, 2007; Mitchell et al. 1987; Stevens et al. 1995, 2003). Both Mitchell's and Stevens' research with children with AD/HD reported increases in the same EFA deficiency symptoms as the current study: Thirst,

urination, and skin problems were more prevalent in children with symptoms of AD/HD compared to controls. The current study suggests severity of EFA deficiency symptoms is inversely correlated with plasma DHA status. This is significant as it may be possible to examine severity of EFA deficiency symptoms as a non-invasive predictor of DHA deficiency.

The current study comparing DHA status in children with AD/HD to typically functioning children offers support for theories linking impairments in EFA metabolism to symptoms of AD/HD (Kaplan et al. 2004, 2007; Lattka et al. 2013; Yehuda et al. 1999). Research suggests minor alleles in FADS 1 and 2 are associated with lower desaturase enzyme activity and therefore decreased AA and DHA levels in plasma phospholipids (Lattka et al. 2013; Miklavcic et al. submitted for publication). Children who presented with at least one minor allele of FADS gene 1 or 2 exhibited higher levels of shorter chain PUFA in plasma, suggesting lower conversion rates of precursors to AA and DHA. SNPs associated with lower desaturase enzyme activity within FADS 1 and 2 could be aetiological factors in the n-3 deficiency observed in children with AD/HD (Brookes et al. 2006).

Research indicates that FA status is influenced by both diet and endogenous metabolism (Glaser et al. 2011; Lattka et al. 2013; Miklavcic et al. submitted for publication). Research findings suggest that genotypes possessing minor alleles in FADS 1 and/or 2 coupled with significantly low PUFA intakes are potential risk factors for neurodevelopmental disorders, such as AD/HD (Lattka et al. 2013).

The present study offers several strengths compared to similar studies. All children in the study were diagnosed with AD/HD by a health care professional. This is in contrast to other studies that have included children with symptoms of AD/HD but who were not diagnosed with the disorder (Kaplan et al. 1989; Mitchell et al. 1987; Stevens et al. 1995, 2003). Over three quarters of the present sample (78%) were taking medication to treat symptoms of AD/HD, suggesting a severely affected AD/HD population. Children from both urban and rural areas of Alberta participated in the study, allowing for a more representative sample. Multiple outcome variables were examined in the current study. This allowed for analysis of correlations between dietary intake of DHA and biological markers of n-3 fatty acid status. These analyses are required to inform research examining mechanisms of action of n-3 FA and to identify which children would benefit most from nutritherapy with n-3 fatty acids.

There are limitations associated with the present study. This was a correlational study, therefore, no cause and effect relationships can be inferred. However, recent randomized controlled trials (RCTs) supplementing children with AD/HD with n-3 fatty acids suggest a causal relationship between plasma phospholipid levels of DHA and symptoms of AD/HD (Richardson 2006; Sinn and Bryan 2007). The Medical Symptom Questionnaire examined the twelve most common EFA deficiency symptoms identified in the literature, however, it was not a standardized assessment. The current study was a human trial examining a challenging population. Though recruitment occurred randomly through a number of different sources, the one and a half years required to obtain the sample was a considerable length of time. A greater

number of males participated in our study (87% of the sample) than females. This is consistent with current research that suggests a prevalence ratio of 3:1 males to females in AD/HD populations (APA 2000; Barkley 2006). Increasing the number of female participants in future studies will increase the generalizability of results to this gender.

Omega 3 fatty acids are heavily involved in brain and nervous system processes. Deficiencies or imbalances in n-3 FA may lead to impaired neurological functioning which presents in children as developmental and behavioural disorders. It is an important question whether deficiencies in omega-3 fatty acid metabolism are involved in the aetiology and/or progression of AD/HD and other psychiatric disorders, as low n-3 FA status is associated with them. Future studies examining impairments in metabolic processes and subsequent supplementation of therapeutic doses of n-3 fatty acids are needed to help ensure healthy cognitive function in children with AD/HD.

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Chapter III Supplementation With Omega 3 Fatty Acids Ameliorates Symptoms Of Inattention In Children Diagnosed With AD/HD

3.1 Introduction

Attention Deficit/Hyperactivity Disorder is a severe and chronic neurodevelopmental disorder requiring a multi-modal treatment approach (AAP 2001, 2011; Barkley 2006, 2013; CME 2006; Konikowska et al. 2012; Rader et al. 2009). Psychostimulants are the first line treatment indicated for AD/HD (AAP 2011; Biederman et al. 2004; Davis & Williams 2011; DuPaul et al. 2013; Rader et al. 2009). However, medications do not address aetiological factors of the disorder and up to 40 percent of children do not respond favourably to pharmacological treatment (AAP 2001, 2011; Berne 2002; Davis & Williams 2011).

Research suggests many families are not comfortable with pharmacotherapy as a treatment option for their children with AD/HD (Vitiello & Sherrill 2007). Side-effects of stimulant use can be difficult to manage and safety concerns associated with chronic use of medication by children can be disconcerning (Davis & Williams 2011; Vitiello & Sherrill 2007). Alternative or adjunct treatments to medication are needed to address symptoms of AD/HD in children (AAP 2001, 2011; Hechtmann et al. 2004).

Deficiencies in DHA may be involved in the aetiology of AD/HD (Antalis et al. 2006; Burgess et al. 2000; Richardson 2006, 2008; Sinn & Bryan 2007). An increasing body of research suggests that deficiencies in DHA in childhood may constitute a risk

factor for later psychopathology (Konikowska et al. 2012; McNamara & Carlson 2006).

The importance of DHA for healthy cognitive and behavioural functioning has led to supplementation studies of DHA with children with symptoms of AD/HD (Germano et al. 2007; Johnson et al. 2009; Richardson 2006; Sinn & Bryan 2007). Supplementation of therapeutic doses of n-3 FA ameliorates symptoms of AD/HD according to parent and teacher standardized assessments (Germano et al. 2007; Johnson et al. 2009; Richardson 2006; Sinn & Bryan 2007).

Richardson & Puri conducted an RCT with 41 children between the ages of eight and twelve years with dyslexia and symptoms of AD/HD (2002). Half the children received an EFA complex containing 186 mg EPA and 480 mg DHA and half the children received an olive oil placebo. Significant improvements were observed in the treatment group on CPRS scales of Cognitive Problems, Anxiety/Shyness, Inattentiveness, Hyperactivity/Impulsiveness, and the Conners AD/HD Index.

Three years later, the same research group conducted a twelve-week RCT with a one-way crossover design with 117 children with symptoms of AD/HD (Richardson & Montgomery 2005). Significant improvements were found in the treatment group in 4 out of 6 CTRS subscales, including Inattentiveness and Hyperactivity/Impulsiveness and in objective assessments of reading and spelling. One hundred children completed the three-month crossover phase of the study. All children received the

EFA complex. Similar outcomes to the treatment group were observed in the placebo group following supplementation with EFA.

In 2007, Sinn and colleagues conducted a fifteen-week RCT with 167 children, ages seven to twelve years. Children were randomized into three groups: EFA, EFA + Multivitamin/Mineral, and Placebo (palm oil). No significant differences in treatment effects were observed in the EFA+Multivitamin/Mineral group compared to the EFA only group. When these two groups were combined, significant treatments effects were observed compared to placebo. Scores on CPRS scales of Inattentiveness, Hyperactivity/Impulsiveness, Oppositional Behaviour, Cognitive Problems, and the AD/HD Index improved in the EFA groups versus placebo. Sinn & Bryan conducted a fifteen-week second phase to their research, which included 109 children from the first sample (2007). The trial was single-blind in that only the researchers were aware that all children were placed in the EFA+Multivitamin/Mineral group. The former placebo group showed improvements on the same scales as the EFA groups had in phase one. Both groups continued to show improvement on CPRS scales.

Germano and colleagues (2007) conducted an open-label trial with 31 children with AD/HD between the ages of three and a half and sixteen years. Children took 2.5 grams of EPA/DHA/kg of body weight (52% EPA, 28% DHA) for eight weeks. Inattention and Hyperactivity scores on the CPRS improved significantly.

Supplementation with n-3 FA increases DHA and EPA levels in plasma phospholipid. Increases in endogenous n-3 FA levels show significant negative correlations with parent and teacher ratings of behaviour (Johnson et al. 2009; Sorgi et al. 2007; Stevens et al. 2003). Stevens et al. (2003) conducted an RCT with 50 children between the ages of six and thirteen years diagnosed with AD/HD. All children presented with EFA deficiency symptoms. Children were randomized to receive either an EFA complex containing 40 mg AA, 80 mg EPA, and 480 mg DHA or placebo for four months. Significant treatment effects were observed on teacher-rated attention difficulties and parent ratings of aggressive behaviour. Significant increases in plasma phospholipid DHA and EPA were found in the EFA group compared to placebo. Increases in phospholipid DHA and EPA were significantly negatively correlated with teacher and parent ratings of behaviour.

In 2009, Johnson and colleagues conducted a three-month RCT with 75 children and adolescents with AD/HD between the ages of eight and eighteen years. The experimental group consumed six capsules a day of Equazen™ eye q for a total of 558 mg EPA and 174 mg DHA daily. The placebo group consumed olive oil capsules. A significant decrease in the n-6:n-3 ratio and a significant increase in n-3 FA were observed. Changes in FA status were associated with treatment response. Significant treatment effects were observed on the Clinical Global Impression Scale (CGI) which assesses symptom severity and functional impairment. Following the supplementation phase, a three-month open-label trial was conducted. All children received the n-3 FA supplement. Similar results were observed in the second phase

with 47 percent of children experiencing significant reductions in CGI scores and symptoms of AD/HD.

Sorgi and colleagues (2007) conducted an open-label study with nine children with AD/HD between the ages of eight and sixteen years. Children received 10.8 grams of EPA and 5.4 grams of DHA daily. Significant improvements were found on the Inattention, Hyperactivity, and Oppositional Defiant behaviour scales. Clinical improvement correlated with decreases in the AA:EPA ratio.

The above research suggests supplementation with therapeutic doses of DHA leads to significant improvements in behaviours associated with AD/HD. Increased dietary intake of DHA improves physiological levels of the fatty acid which is inversely correlated with behaviour difficulties.

Though the above research is promising, a number of questions still exist surrounding use of n-3 fatty acids as a treatment option for AD/HD (Clayton et al. 2007; Kirby et al. 2010; Richardson 2006; Rucklidge et al. 2009). It remains unclear what dosages are the most therapeutic, whether DHA or EPA or a combination leads to amelioration of symptoms, and whether small amounts of n-6 must be included with n-3 fatty acids to observe treatment effects. The present study was designed to examine the above questions surrounding the benefits of n-3 FA on symptoms of AD/HD in children. Although the doses of n-3 fatty acids supplemented in the current study were high relative to average intake, the dosages are safe daily intake values of n-3 fatty acids.

The present trial is the second phase of the research study discussed in the previous chapter, examining dietary intake of DHA and FA status in children diagnosed with AD/HD.

3.2. Study Design:

The purpose of the present study was to assess effects of supplementation with DHA on physical and behavioural symptoms of AD/HD in children diagnosed with the disorder. It was hypothesized that DHA supplementation would increase endogenous levels of the fatty acid and lead to improvements in EFA deficiency symptoms and behaviours associated with AD/HD. The current study was a randomized, double-blind, placebo-controlled 16-week supplementation study. Half the children received an n-3 supplement containing DHA and eicosapentanoic acid (EPA). The other half of the children received an olive oil supplement. Blood samples and diet records were obtained at baseline and 16 weeks. Standardized behavioural assessments and a questionnaire assessing physical symptoms of EFA deficiency were completed at baseline, 8 weeks, and 16 weeks.

The study was approved by the Health Research Ethics Board of the Department of Medicine at the University of Alberta. Parent(s)' written consent (Appendix B) and children's written assent (Appendix C) were obtained prior to enrollment in the trial. Children nine years and older were required to sign a Child Consent Form to participate in the study.

3.3 Methods:

3.3.1 Recruitment of Participants:

The present study is the second phase of the research trial discussed in Chapter Two. Of the 103 children who participated in the previous trial, 79 children had plasma levels of DHA below the mean level obtained during a study examining levels of DHA in typically functioning children (Lien 2005). Parents of the 79 children were contacted to participate in the supplementation study. All families had previously consented to being contacted for future research. Fifty-six families agreed to participate in the present study, however, due to time constraints and parents' concerns about their children's compliance rates throughout the study, 39 families attended the first interview. The recidivism rate during the study was 33 percent for a total of 26 families who completed the trial.

3.3.2 Inclusion Criteria:

Children diagnosed by a qualified healthcare professional (Pediatrician, Psychiatrist or Psychologist) as having any of the sub-types of AD/HD, according to DSM-IV criteria were included (American Psychiatric Association 2000). AD/HD had to be the primary diagnosis but participants could present with co-morbid conditions. Comorbidities included Oppositional Defiant Disorder (ODD), Conduct Disorder (CD), anxiety disorders, mood disorders, and learning disorders. Children diagnosed with Fetal Alcohol Syndrome or Autism were excluded from the study.

Only children of normal intelligence according to parent report or scoring a full scale IQ of 70 or greater on a standardised test conducted by a healthcare professional within the past year were included in the study. Participants could be taking medication such as methylphenidate (Ritalin) to treat AD/HD and/or other prescription medication. Specific medications each child was taking, dose, and length of treatment were recorded. Study participants could not be consuming any special diets or supplements that contained DHA.

3.3.3 Assessments:

All assessments except diet records and the Conners 3 Teacher Rating Scale were completed during meetings arranged at a mental health services organisation in Edmonton. The same parent and teacher of each child completed the assessments at all time points.

3.3.4 Fatty Acid Analysis, Height, and Weight:

A registered nurse measured each child's height and weight at baseline and 16 weeks. The nurse collected a blood sample at baseline and 16 weeks from each child by a finger prick. Blood samples were collected in microtainer tubes containing heparin and put on ice in a biosafety container for transport to the lab for analysis.

Plasma was separated by centrifugation and frozen until analysis. Plasma lipids were extracted using the modified Folch procedure (Folch et al. 1957) and the phospholipid fraction was separated by thin layer chromatography (Clandinin et al 1997). Fatty

acids of the phospholipid fraction were quantified by gas-liquid chromatography. Quantity of n-3 FA in plasma phospholipids is considered a highly reliable biomarker of n-3 FA status of body organs such as the heart and brain (Salem et al. 2001).

3.3.5 Conners 3 Rating Scales:

Parents completed the short form of the Conners 3 Parent Rating Scale (Conners, 2008) at baseline, 8 weeks, and 16 weeks. Teachers completed the short form of the Conners 3 Teacher Rating Scale (Conners, 2008) at baseline and 8 weeks. Teacher assessments were unavailable after 8 weeks due to summer vacation. Contact information for the primary teacher of each child was obtained from parents. Rating forms and letters of instruction were sent to and returned by the teachers by mail.

The Conners 3 Rating Scales (CRS-3) measure socio-behavioural functioning in children three to seventeen years of age (Conners 2008). The CRS-3 consists of long and short parent and teacher forms and a youth self-report form. The short parent form consists of 43 items measuring seven factors including hyperactivity, inattention, social problems, and psychosomatic complaints. The teacher short form consists of 41 items measuring the same factors as the parent form, except psychosomatic concerns. The CRS-3 is one of the most widely used standardized assessments with children with AD/HD (Smith & Handler 2007).

The Conners 3 was employed in the current study for a number of reasons. The CRS-3 is a standardized assessment with large normative data on both genders

encompassing the age range of the study's sample (Conners 2008). The CRS-3 measures socio-behavioural functioning in children with AD/HD and has been found to have strong reliability and validity (Conners 2008; Smith & Handler 2007). The current study was designed to assess improvements in social and behavioural symptoms of AD/HD in children following supplementation with DHA. The Conners 3 is the most widely used behavioural assessment with children with AD/HD and has been found to have strong test-retest reliability (Conners 2008; Smith & Handler 2007).

Consistent with best practice guidelines, the Conners 3 measures functioning in multiple settings across multiple informants. The short parent and teacher forms are self-administered and take five to ten minutes to complete, minimizing respondent burden. The CRS-3 was chosen because the scales reliably and easily assess improvements in symptoms of AD/HD found to respond to treatment with DHA.

3.3.6 Medical Symptom Questionnaire:

Parents completed a Medical Symptom Questionnaire at baseline and 16 weeks regarding their child (Appendix G). The questionnaire consisted of the twelve most common physical symptoms of EFA deficiency observed in children with AD/HD. Symptoms included halitosis, rhinitis, headaches, sleep deficits, frequent urination, greater thirst, skin problems, and visual and auditory deficits. A four-point Likert scale was used. A rating of 0 indicated absence of a symptom and a rating of 3 indicated presence of a symptom most of the time. The total possible score on the scale was

36. The medical symptom questionnaire was created by the first author and is not a standardized assessment.

3.3.7 Diet Records:

Families completed four-day diet records at baseline and 16 weeks to assess intakes of DHA, EPA, and AA (Appendix E). Diet records were mailed to parents and reviewed during meetings arranged at a mental health services organization in Edmonton.

3.3.8 Supplementation:

The current study is the first supplementation trial to account for body weight in determining doses of omega-3 fatty acids. Children's current weights were obtained from parents. Two supplementation groups were determined based on the weights of the children. It was hypothesized that heavier and hence often older children would require higher doses of n-3 fatty acids to observe similar effects of the lighter often younger children. Children 40 to 79 pounds were coded as the Lower Weight (LW) group. Children 80 to 140 pounds were coded as the Upper Weight (UW) group. An assistant who had no contact with the families prepared the supplements. Employing a computer-generated random numbers program, the assistant randomly placed children from each weight group into either the experimental or control group.

Children in the experimental group received Omega 3 Think® (Genuine Health, Toronto, Canada). Omega 3 Think® is an n-3 fatty acid supplement containing 250

mg of DHA and 100 mg of EPA per enteric-coated capsule. Children in the lower weight experimental group (n=7) consumed 2 capsules daily for a total of 700 mg of n-3 fatty acids (500 mg of DHA and 200 mg of EPA). Children in the upper weight experimental group (n=6) consumed 3 capsules per day for a total of 1050 mg of n-3 fatty acids (750 mg of DHA and 300 mg of EPA). The other half of the children (n=13) received a placebo containing olive oil. Both the supplement and placebo capsules were counted and put into identical opaque Nalgene® pill bottles by the assistant and numerically coded. Only the assistant had access to the code throughout the study. All other staff and study participants were blinded to whether a child was a part of the experimental or placebo group.

Families received the first eight weeks supply of n-3 supplement or placebo for their child at baseline and the final eight weeks of supplement or placebo at the two-month mark meeting. Two one-month calendars were given to parents at baseline and eight weeks that corresponded to the first and last two months of the study respectively. The calendars included instructions on when to administer the supplement, as well as the daily and weekly dosages. The parents were asked not to exceed the recommended daily dose, to record any days their child consumed less than the required dose, and what was consumed on those days. If the child experienced any illness more severe than a cold over the two-month period, the parent was asked to record information about the illness on the days of the calendar that corresponded to the days the child was ill.

This study was not conducted on an intent-to-treat basis. At the completion of the study, parents were informed whether their child had received the Omega-3 Think® supplement or placebo. Families received a pamphlet describing Omega 3 Think® and other omega fatty acid supplements available from Genuine Health. Information regarding doses of n-3 fatty acids that may have beneficial effects on symptoms of AD/HD was discussed with parents.

3.3.9 Statistical Analyses

Data were analysed using SPSS 16.0 (SPSS Inc., Chicago, 2007). One-Way Analyses of Variance (ANOVA) were conducted. Data followed a normal distribution as determined by the Shapiro-Wilk test.

3.4 Results

Dietary Intake

Parents were instructed not to alter their child's typical diet for the duration of the study. The mean dietary intakes of DHA in the experimental group were 159 mg at baseline and 58 mg at the completion of the study (Table 3.1). DHA intakes in the placebo group showed a similar trend from 72 mg at baseline to 26 mg at the end of the trial. The mean dietary intake ratio of AA:DHA at baseline across all participants was 3:1 AA:DHA. Six families did not return the final Diet Record at the completion of the study; therefore, their dietary intake results could not be included in the analyses.

Table 3.1 Dietary Fatty Acid Intake

Experimental Group	Subject ID #	*B DHA Grams	**P DHA Grams	B EPA Grams	P EPA Grams	B AA Grams	P AA Grams
	2	0.01	0.06	0.02	0.01	0.31	0.4
	7	0.3	0.01	0.02	0.01	0.18	0.1
	9	0.05	0.05	0.03	0.03	0.39	0.37
	10	0.15	0.01	0.03	0.01	0.44	0.16
	15	0.03	0.01	0.01	0.01	0.3	0.1
	23	0.06	0.06	0.04	0.02	0.41	0.25
	26	0.02	0.03	0.01	0.03	0.15	0.23
	53	0.77	0.04	0.55	0.01	0.59	0.23
	67	0.13	0.07	0.01	0.01	0.22	0.36
	80	0.07	0.24	0.01	0.19	0.81	0.48
	Means	0.159	0.058	0.073	0.033	0.38	0.268
Placebo Group							
	22P	0.1	1.29	0.02	0.99	0.52	0.27
	24P	0.06	0.09	0.01	0.03	0.37	0.36
	33P	0.03	0.64	0.02	0.47	0.27	0.13
	58P	0.01	0.14	0.1	0.13	0.17	0.38
	70P	0.04	0.04	0.01	0.01	0.18	0.32
	78P	0.03	0.01	0.01	0.01	0.33	0.35
	87P	0.13	0.08	0.05	0.01	1.14	0.84
	101P	0.01	0.01	0.01	0.01	0.08	0.09
	104P	0.3	0.26	0.18	0.05	0.07	0.04
	105A	0.01	0.04	0.01	0.01	0.14	0.42
	Means	0.072	0.26	0.042	0.172	0.327	0.32

*B= Baseline **P=Post

Fatty Acid Profiles:

Levels of DHA increased from baseline levels in plasma phospholipids over 16 weeks in the supplement group but not in the placebo group (Table 3.2). Mean levels of DHA at baseline were 8 µg/ml and 11 µg/ml of plasma in the experimental and placebo group respectively. Following the 16-week supplementation period, plasma

DHA increased to 19 µg/ml in the supplementation group and remained constant in the placebo group at 11 µg/ml. Because of the small sample size, results were non-significant. Compliance rates for supplement and placebo intakes were 81% and 84% respectively.

Table 3.2 Fatty Acid Profiles in Plasma Phospholipid

Experimental Group	Subject ID#	*B DHA ug/ml	**P DHA ug/ml	B EPA ug/ml	P EPA ug/ml	B AA ug/ml	P AA ug/ml
	2	5.35	30.84	1.34	15.97	17.17	54.32
	9	9.73	24.94	4.26	7.24	82.1	123.37
	10	2.61	26.54	3.19	17.38	23.22	39.51
	26	8.19	38.55	10.77	3.09	10.4	53.07
	67	16.52	6.7	8.42	3.11	60.36	5.3
	74	3.83	2.38	6.83	1.78	43.46	7.53
	80	15.62	12.1	7.07	4.55	92.23	49.33
	84	4.78	12.86	3.95	27.53	54.45	81.41
Means		8.33	19.36	5.73	10.08	47.92	51.73
Placebo Group							
	24	13.09	10.77	***INC	INC	53.26	13.14
	33	16.55	15.67	23	2.18	22.18	89.86
	58	7.91	7.02	INC	INC	21.59	55.45
	96	8.64	2.72	2.76	0.65	37.93	13.7
	101	21.29	10.26	11.24	39.25	131.05	21.09
	104	4.89	17.08	3.93	6.83	26.82	35.5
	105	1.88	11.75	2.8	4.93	9.54	59.67
Means		10.61	10.75	8.75	10.77	43.20	41.20

*B= Baseline **P=Post ***INC=Incomplete Analysis

Conners 3 Parent and Teacher Rating Scales

Preliminary analyses found no effect of treatment on behavioural symptoms of AD/HD. However, when DHA status was included as an independent variable, there was significant effect of treatment on the Inattention scale as determined by one-way

ANOVA (*F (3, 11*) *=3.640, p=.048*) at a power of 0.91 (Figure 3.1). The magnitude of change in scores on the CPRS was calculated by subtracting final scores from baseline scores. Positive end values were obtained when final scores were lower than at baseline indicating an amelioration of symptoms. Negative end values were calculated when final scores were higher than at baseline indicating no treatment effects.

The black boxes in Figure 3.1 indicate the mean difference in Inattention scores in children in both groups whose levels of plasma phospholipid DHA increased throughout the study. Children in the experimental group whose plasma DHA increased, experienced a mean reduction of three points on the Conners 3 according to parent report (95% CI: 0.09-5.9).

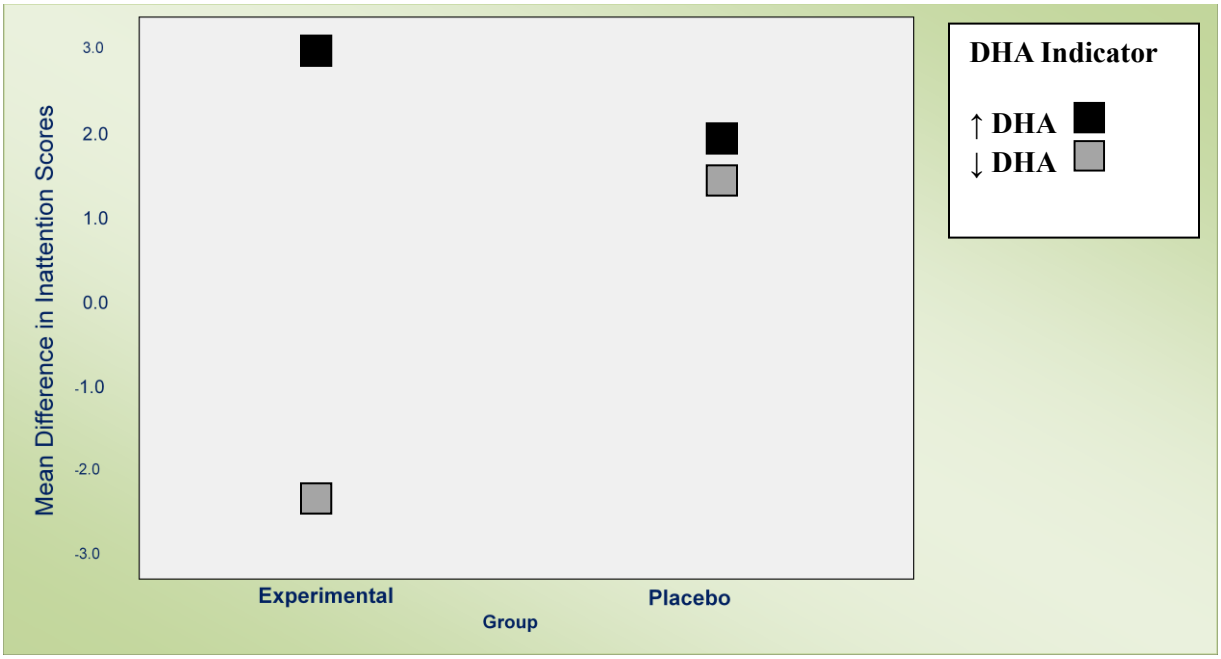
The grey boxes in Figure 3.1 illustrate the mean difference in Inattention scores in children in both groups whose levels of plasma phospholipid DHA decreased throughout the study. Children in the experimental group whose plasma DHA decreased during the study showed a negative mean difference of 2.5 points on the CPRS, suggesting no amelioration of symptoms (95% CI: -6.1-1.5).

Comparison of both subgroups of the experimental group showed a significant difference in scores. The mean difference in Inattention scores was statistically significantly higher in children in the Experimental group whose plasma DHA levels increased (*3.0 ± 2.35, p=.043*), compared to children in the same group whose

plasma DHA levels did not increase *(-2.33 ± 1.53*).

In contrast, there were no significant differences on the Conners 3 Parent Rating Scales in the placebo group. Children in the placebo group whose DHA increased showed a positive mean difference of 2 points on the Conners 3 according to parent report (95% CI: -1.3-4.5). Children in the placebo group whose DHA decreased showed a positive mean difference of 1.5 points on the Conners 3 according to parent report (95% CI: -23.4-27.4).

Figure 3.1 Mean Difference in Inattention Scores and DHA Status



Due to summer vacation, scores on the CTRS could not be obtained at the completion of the study. Examination of scores at baseline and eight weeks using paired-samples t-tests revealed a significant difference on the Inattention scale in the

experimental group (2 ± 3.0) compared to placebo (2.1 ± 1.4) when percent of difference between baseline and eight-week scores were analysed (t(11)=2.321, p=.04) *Cohen's d=1.4* (Cohen 1988*)*. A greater number of children in the experimental group experienced ameliorations in attention compared to the placebo group.

Medical Symptom Questionnaire

No significant improvements in symptoms of EFA deficiency were observed in the experimental group compared to the control group (Appendix I).

3.5 Discussion

The current study examined effects of supplementation with DHA on symptoms of AD/HD in children diagnosed with the disorder. Amounts of DHA in plasma phospholipids more than doubled from baseline values in the experimental group compared to controls following sixteen weeks of supplementation with DHA. These results are consistent with previous studies that found significant improvements in DHA status following supplementation with the fatty acid (Johnson et al. 2009; Sorgi et al. 2007; Stevens et al. 2003).

Compared to controls, significant improvement on the Inattention scale of the CPRS was observed in a subset of children in the experimental group following sixteen weeks of supplementation. Children whose plasma DHA increased in the experimental group experienced significant reductions in inattention according to

parent report. Previous research by Stevens and colleagues (2003) and Sorgi and colleagues (2007) found similar results. Degree of clinical improvement was related to level of increase in phospholipid DHA. Results from the current study suggest increases in plasma phospholipid levels of DHA may be associated with amelioration of symptoms of AD/HD in children diagnosed with the disorder.

Mean dietary intakes of DHA were found to be higher in this sample than in previous trials conducted with typically functioning children (Lien 2005; Lien & Clandinin 2009; Meyer et al. 2003). Lien examined dietary intakes of DHA in 78 children from Alberta, Canada between the ages of four and seven years, employing a three-day diet record (Lien 2005). Average DHA intakes ranged from 0-350mg/day. The mean dietary intake of DHA across all participants in Lien's study was 37 mg/day.

An Australian study examining dietary intakes of essential fatty acids was conducted with 799 children between the ages of four and seven years. A 24-hour recall was completed by parents. Mean dietary intake of DHA was 47mg/day (Meyer et al. 2003). This is in contrast to the mean dietary intake of 109 mg/day of DHA observed in the present study.

The current sample was composed of a subset of children from the researcher's previous study. Mean dietary intake of DHA in the previous study was 42 mg, which is consistent with similar trials. Since the previous trial, families may have incorporated more foods containing omega 3 fatty acids into their children's diet.

Two recent studies that observed treatment effects challenge conclusions drawn from reviewing other n-3 supplementation studies (Germano et al 2007; Richardson 2006; Sorgi et al. 2007). Both studies were two-month trials that supplemented with the highest doses of n-3 FA given to children with AD/HD to date. Germano and colleagues (2007) supplemented children with 5-11 grams of omega-3 per day and Sorgi and colleagues (2007) supplemented children with 16.2 grams of EPA/DHA concentrate per day. The Food and Drug Administration recommends an upper limit of three grams per day of dietary intake of n-3 FA (Brow & Panosh 2009).

Germano (2007) and Sorgi's studies (2007) suggest treatment effects of n-3 supplementation may be clinically evident within two months if dosages of n-3 are significantly high. Up to one gram per day of n-3 FA has been shown to be well tolerated in children (Raz & Gabis 2009). Current research suggests any combination of DHA and EPA may be equally beneficial at doses higher than 500 mg (Richardson 2006; Germano et al. 2007). Germano (2007) and Sorgi's studies (2007) support the present study suggesting that at doses higher than 700 mg of n-3, addition of n-6 to supplements is not required to observe treatment effects in children with AD/HD.

The current study is the first to observe treatment effects of FDA-approved doses of n-3 fatty acids without the inclusion of n-6 fatty acids in children with AD/HD. The current trial supplemented with two of the highest doses of DHA given to children with AD/HD to date. Results of the current study suggest that 700-1050 milligrams of n-3

fatty acids with a DHA:EPA ratio of 2.5:1 supplemented for 16 weeks leads to amelioration of symptoms of inattention in children diagnosed with AD/HD.

There are several strengths associated with the current study. All children were diagnosed with AD/HD by a healthcare professional. As current research suggests, additional trials are required examining the effects of omega-3 fatty acids on children diagnosed with AD/HD as compared to children with symptoms of the disorder but no formal diagnosis (Sinn et al. 2007). Consistent with best practice guidelines, the Conners 3, which is a standardized assessment, was administered to objectively measure behaviour change throughout the study. Future research employing cognitive assessments of attention and memory may yield positive treatment effects.

A few limitations are associated with the current study. Firstly, the sample size of the current study is small. Secondly, the Medical Symptom Questionnaire was a non-standardised assessment. Thirdly, Conners 3 Teacher Rating Scales scores were unavailable for the final 8 weeks of the study preventing the comparison of parent and teacher Conners Rating Scales scores at 16 weeks.

Most research examining treatments for AD/HD, such as pharmacology, is concerned with symptom reduction rather than cure. Research focusing on treatments with curative effects are needed. The current research is significant as it suggests deficiency in DHA may be an aetiological factor of AD/HD and supplementation with the essential fatty acid improves attention in children with the disorder. Future

research examining various metabolites of DHA may offer additional information on the roles of DHA in cognition and behaviour.

Attention is a main executive function needed to self-regulate responses for goal-directed behaviour. Up to 80 percent of children with AD/HD struggle academically due to poor executive functioning. Improvements in attention leads to stronger ability to plan, organise, and self-direct behaviour, thereby significantly increasing academic and social performance.

The significant academic, personal, and financial burden associated with AD/HD stresses the importance of early and effective management of symptoms of the disorder. Supplementation of therapeutic doses of DHA may improve attention, thus reducing the negative impact of AD/HD, and leading to better academic and social prognoses for children with the disorder.

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Chapter IV Significance of Research and Future Directions

4.1 Study One

The purpose of the present study was to examine the relationship between dietary intake, plasma phospholipid levels of DHA, and physical symptoms of EFA deficiency in children diagnosed with AD/HD. Levels of DHA and AA in plasma phospholipid were examined in 103 children between the ages of five and twelve years diagnosed with AD/HD. Fatty acid profiles were compared to those of typically functioning children. Mean plasma phospholipid levels of DHA and AA were half the values described in a comparable group of control children.

All 103 children with AD/HD experienced physical symptoms commonly associated with essential fatty acid deficiency. Two of the twelve symptoms showed significant negative correlations with plasma DHA levels. Children with higher plasma DHA levels scored significantly lower on the thirst and frequent urination scales. Severity of EFA deficiency symptoms may be inversely correlated with plasma DHA status, suggesting a neuroprotective effect of DHA.

In the current study, children with AD/HD consumed similar dietary intakes of DHA to typically functioning children, however, children with AD/HD had mean plasma phospholipid levels of DHA about half the values observed in studies conducted with typically functioning children. The post hoc analyses conducted on the current data offer unique contributions to the literature on AD/HD. Post hoc analyses found

children with AD/HD who presented with genotypes of one or more minor alleles in FADS 1 or 2 exhibited increased levels of LA and ALA in plasma, suggesting decreased conversion rates of PUFA precursors to longer-chain PUFA. SNPs associated with lower desaturase enzyme activity within FADS 1 and 2 could be aetiological factors in the n-3 deficiency observed in children with AD/HD.

Because deficiency in n-3 FA constitutes a risk factor for later development of psychopathology, identifying the aetiological factors of n-3 deficiency is important. The current research is significant as it suggests the deficiency in n-3 FA observed in children with AD/HD is due to genetic impairments in metabolic processes rather than dietary intake. Therapeutic intakes of DHA may be necessary in this population to help ensure optimal neurocognitive development.

4.2 Study Two

This RCT examined effects of supplementation with DHA on symptoms of AD/HD in thirty-nine children diagnosed with the disorder. Participants were between the ages of five and thirteen years.

Levels of DHA increased from 8 µg/ml to 19 µg/ml in plasma phospholipids over 16 weeks in the supplement group. Plasma DHA remained constant in the placebo group at 11 µg/ml. Children in the experimental group whose plasma DHA increased, experienced a significant reduction in symptoms of Inattention on the Conners 3 according to parent report.

This research is the first to observe treatment effects of FDA-approved doses of n-3 fatty acids without the inclusion of n-6 fatty acids in children with AD/HD. This result is significant as it agrees with North American findings supporting the increase of n-3 FA intake in the diet, while at the same time reducing n-6 FA intake. Reducing the n-6:n-3 ratio closer to an optimal 1:1 ratio has been found in this and other research to improve physiological and cognitive functioning.

AD/HD is the most common neurodevelopmental disorder in paediatric populations with prevalence rates from two to twenty percent. Children with AD/HD present with chronic neuropsychological and cognitive deficits that severely impact social and academic functioning. The significant academic, personal, and financial burden associated with AD/HD stresses the importance of early and effective treatment. Most treatments are concerned with acute symptom reduction, rather than treating the underlying aetiology of the disorder. The current research is significant as it suggests therapeutic intakes of DHA may ameliorate attention by improving neuropsychological health. Attentional control is an important executive function, necessary to self-regulate behaviour towards future-directed goals. Stronger executive functioning leads to improved academic and social performance. As attention becomes more focused on future-oriented behaviours and less on the immediate environment, professional and social prognoses ameliorate across the lifespan. The current findings suggest that therapeutic intakes of DHA improve one of the core symptoms of AD/HD; reducing the severity and hence the burden of the disorder. Supplementation with therapeutic doses of DHA may be effective as an

alternative or adjunct treatment to medication for managing symptoms of AD/HD in children diagnosed with the disorder.

4.3 Future Directions

Deficiencies in DHA are associated with development of later psychopathology. Single nucleotide polymorphisms (SNPs) within fatty acid desaturase enzymes 1 and 2 (FADS 1 and 2) may be aetiological factors in the n-3 deficiency observed in children with AD/HD. FADS genotypes modulate DHA levels in maternal red blood cells and may influence the supply of DHA to the foetus during pregnancy. Therefore, screening couples who plan to conceive and their offspring for SNPs may be an effective method for identifying children who are at increased risk of AD/HD. Supplementing at-risk couples and children with therapeutic doses of DHA may lessen the symptoms of, or even prevent the severe neurodevelopmental disorder AD/HD.

APPENDICES

A. STUDY ONE
PARENT CONSENT FORM

Part 1:

Title of Project: The effects of Arachidonic and Docosahexaenoic acid levels on children with Attention Deficit/Hyperactivity Disorder

Principal Investigator: Dr. Tom Clandinin, Professor of Nutrition,
Department of Medicine, The University of Alberta
Telephone: (780) 492-5188 E-Mail: tclandin@ualberta.ca

Part 2:

Do you understand that your child has been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet?	Yes	No
Do you understand the benefits and risks involved in allowing your child to take part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that you are free to refuse to participate or withdraw your child from this study at any time? You do not have to give a reason and it will not affect your child's care.	Yes	No
Has the issue of confidentiality been explained to you? Do you understand who will have access to your child's records?	Yes	No

This study was explained to me by: _____

I agree to allow my child to take part in this study.

_____		
Signature of Parent or Guardian	Date	Printed Name
_____	_____	_____
Signature of Research Participant	Date	Printed Name
_____	_____	_____
Signature of Witness	Date	Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to allow their child to participate.

_____	_____
Signature of Investigator or Designee	Date

May we keep your name and phone number on file and call you about other research studies? Yes No

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A COPY GIVEN TO THE RESEARCH SUBJECT

B. STUDY TWO PARENT CONSENT FORM

Part 1:

Title of Project: Assessment of supplementation with Arachidonic acid and Docosahexaenoic acid on plasma values and behaviour in children with Attention Deficit/Hyperactivity Disorder.

Investigators: Dr. Tom Clandinin, University Professor of Nutrition,
(780) 492-5188 tclandin@ualberta.ca

Ellen Ivity, Interdisciplinary Ph.D. Candidate
(780) 492-8463 eivity@ualberta.ca

Part 2:

Do you understand that your child has been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet?	Yes	No
Do you understand the benefits and risks involved in allowing your child to take part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that you are free to refuse to participate or withdraw your child from this study at any time? You do not have to give a reason and it will not affect your child's care.	Yes	No
Has the issue of confidentiality been explained to you? Do you understand who will have access to your child's records?	Yes	No

This study was explained to me by: _____

I agree to allow my child to take part in this study.

_____		
Signature of Parent or Guardian	Date	Printed Name
_____	_____	_____
Signature of Research Participant	Date	Printed Name
_____	_____	_____
Signature of Witness	Date	Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to allow their child to participate.

_____	_____
Signature of Investigator or Designee	Date

May we keep your name and phone number on file and call you about other research studies? Yes No

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A COPY GIVEN TO THE RESEARCH SUBJECT

C. STUDY TWO CHILD CONSENT FORM

Assessment of supplementation with Arachidonic acid and Docosahexaenoic acid on plasma values and behaviour in children with Attention Deficit/Hyperactivity Disorder.

Investigators: **Ellen Ivity, B.A. (Psychology)**

Interdisciplinary Ph.D. Candidate,

(780) 492-8463 eivity@ualberta.ca

Dr. Tom Clandinin, Ph.D.

University Professor of Nutrition

(780) 492-5188 tclandin@ualberta.ca

The University of Alberta is conducting a research study looking at omega-3 fatty acids and how they may help make the symptoms of Attention Deficit/Hyperactivity Disorder (AD/HD) better. Omega-3 fatty acids are fats that we need to eat to help us to focus and learn better. These fats are found in fish and seeds. Because you have AD/HD, you are being asked to participate in this study. About 60 other kids will also participate in this study.

What will you have to do?: If you and your parents agree to take part, we will ask you to come to the clinic 3 times over a period of four months.

During the first meeting, your parent or guardian will fill out two questionnaires and will be given a two-month supply of a natural supplement for you to take almost every day. A nurse will measure your height and weight. She will also take a blood sample from you by a fingerprick. You will be asked if you have any questions. Your teacher will also be mailed a questionnaire to fill out.

At the second meeting, which will occur two months after the first, your parent or guardian will return any supplement you did not take and will receive another two-month supply of supplement for you to take almost every day. You will be asked if you have any questions. You will need to take the supplement almost every day for four months.

At the last meeting, which will take place four months after the first, your parent or guardian will return any supplement you did not take and will fill out two questionnaires. Your teacher will be mailed one more questionnaire to fill out. A nurse will measure your height and weight. She will also take a blood sample from you by a fingerprick.

One group of children in this study will take a natural supplement with omega-3 fatty acids in it. Another group of children will take a natural supplement with no omega-3 fatty acids in it. Both supplements are healthy and safe. You can't decide which group you're in and no one will know which supplement you are taking. Your parent or guardian can find out which supplement you are taking in an emergency.

If any medicines you are taking change over the four months while you are in the study, please let Ellen Ivity know.

Will it help?: You may feel better while you are taking the supplement.

Will it hurt?: The supplements are natural and safe. They are made of healthy nutrients your body needs. The only part of the experiment that might hurt a little for a couple of minutes is the fingerprick. It may feel like a mosquito bite. You must tell your mom or dad or your guardian about anything you think is different while you are taking the supplement. If we find out anything new about the supplements we will tell you.

Can you quit?: You don't have to take part in the study and you can quit at any time. No one will be mad at you if you decide you don't want to be in this study or if you decide to stop part way through. You should tell your parent or guardian if you want to quit.

Who will know?: No one except your parents, your teacher, and your doctor will know you're taking part in the study unless you want to tell them. Your name and your questionnaires won't be seen by anyone except the investigators and the nurse during the study.

Your signature: We would like you to sign this form to show that you agree to take part. Your mom or dad or guardian will be asked to sign another form agreeing for you to take part in the study.

Do you have more questions? You can ask your mom or dad or guardian about anything you don't understand. You can also ask Ellen, if you have any questions.

I agree to take part in this study.

_____ _____

Signature of Research Participant Date

_____ _____

Signature of Witness Date

_____ _____

Signature of Investigator Date

D. Food Record Study One

Child's Name:

Parent or Guardian's Name:

Day: 1 2 3 4 **Day of Week and Date:**

Meal (ie, lunch, afternoon snack)	**Type of meat, fish, egg, nut, or seed** (ie, pork chop, chicken egg, cashew, flax seed)	**Brand name** (ie, Arby's, High Liner, Lucerne, Homemade)	**Description** (ie, fried, boiled, fresh, frozen)	**Amount and Size** (ie, deck of cards, large egg, 1 cup, 2 slices-10cm wide x 10 cm long x 2cm thick)	**Office use only**
NOTES					

E. Food Record Study Two

Child's Name:

Office Use Only
Participant's #:

Parent or Guardian's Name:

Day: 1 2 3 4

Day of Week and Date

Meal (ie, lunch, afternoon snack)	**Type of food or drink** (ie, pork chop, vegetable soup, cashew, yogurt, orange juice)	**Brand name** (ie, Arby's, High Liner, Lucerne, Homemade)	**Description** (ie, pan fried, boiled, fresh, frozen from concentrate)	**Amount and Size** (ie, deck of cards, large egg, 1 cup, 2 slices-10 cm wide x 10 cm long x 2cm thick)	**Office use only**

F. Medical History Questionnaire

Study One

Date:_____

Parent or Guardian's Name:

Child's Name:

Child's Gender: Child's Age:

Please answer the following items about your child, using the scale from 0 = Not at all to 3 = Very much.

0=Not at all 1=Just a little 2=Pretty much 3=Very much

1. Often thirsty	0	1	2	3	
2. Has to urinate frequently	0	1	2	3	
3. Any skin problems (please specify) (ie.,rashes, dry skin, eczema)		0	1	2	3
4. Dry hair or dandruff	0	1	2	3	
5. Brittle nails	0	1	2	3	
6. Frequent stomach aches	0	1	2	3	
7. Has had many earaches	0	1	2	3	
8. Often has bad breath		0	1	2	3
9. Often has a stuffy or runny nose		0	1	2	3
10. Sleep problems (please specify) (ie. difficulty falling or staying asleep)		0	1	2	3
11. Hearing difficulties (please specify)		0	1	2	3
12. Vision problems (please specify)		0	1	2	3

Parent or Guardian's signature:_____

G. Medical History Questionnaire

Study Two

Participant #

Date:

Parent or Guardian's Name:

Child's Name: Gender:

Age:

Please rate whether your child experiences any of the following items, using a scale from 0 to 3.

0=No 1=Sometimes 2=Often 3=Most of the time

1. Thirsty		0	1	2	3
2. Has to urinate		0	1	2	3
3. Any skin problems (please specify) (ie.,rashes, dry skin, eczema)		0	1	2	3
4. Dry hair or dandruff	0	1	2	3	
5. Brittle nails	0	1	2	3	
6. Stomachaches		0	1	2	3
7. Earaches	0	1	2	3	
8. Bad breath	0	1	2	3	
9. Stuffy or runny nose	0	1	2	3	
10. Sleep problems (please specify) (ie. difficulty falling or staying asleep)		0	1	2	3
11. Hearing difficulties (please specify)		0	1	2	3
12. Vision problems (please specify)		0	1	2	3

Parent or Guardian's signature:_____

H. Study One DHA Intake and Fatty Acid Profiles

Subject ID Number	DHA Intake mg	Plasma DHA ug/ml	Plasma AA ug/ml
1	1	10.7	78
2	123.1	5.2	28.2
3	95.4	8.6	38
5	5.6	3.1	26.2
7	48.7	8.7	21.5
9	3	5.9	34.1
10	5.7	0.8	3.8
11	15	13.7	55.5
15	26.6	16.6	90
16	423.9	6	34.9
19	1	3.7	33.2
21	17.3	7.1	23.2
22	2.1	6	22.3
23	36.2	1	6.4
24	33	2.2	3.8
25	133.8	3.4	14.5
26	36.6	4.4	21.8
29	4.7	21.7	25.9
30	33.5	16.8	48
32	66.5	17.3	36.5
33	38.9	11.7	44.9
34	16.8	10.6	34.1
36	1	9.2	14.2
37	1.8	6.6	23
38	35.9	17.8	24.9
41	32.4	11.9	33.3
42	10.1	4.3	2.4
43	222.7	4.1	12
44	9.7	6.6	29.2
45	41.7	9.9	23.6
46	6.4	7	1.5
48	13.5	3.4	3.7
49	25.7	9.8	34.6
51	1.3	6.6	48.4
52	20.5	16.8	58.6
53	19.2	13.7	34.8
54	9.3	12.7	37
55	36.8	13.4	34.1
56	10.5	10.4	37.7
57	152.5	5.1	45.5
58	18.3	5.3	45.5
59	1	6.5	50
60	9.4	3.1	22.1
61	11.6	5.2	35.5

62	33.1	0	42.5
63	14.2	5.2	2.8
64	11.1	2	25.7
65	1	4	38.5
66	54.8	9	60.1
67	5.9	12.8	32.8
68	124.7	7.6	35
69	48.8	15.9	43.2
70	98.8	3	14
71	14.7	2.6	11.5
72	22.8	3.7	22.7
73	2.7	5.4	19.3
74	19.9	3.5	16
75	3.6	2	18.4
76	1	5.2	28.9
78	275.5	19.2	49.2
80	1	11.4	38.4
81	5	10.4	33.1
82	11	10.7	24
83	7.3	21.2	28.5
84	1	10.1	35.9
85	65.6	10.5	42.9
86	1	16.1	35
87	4.3	15.9	67.1
89	17.3	7.3	23.5
90	29.8	9	28.3
91	61.6	10.9	34.8
92	39.1	15.5	43
93	43.9	15.6	27.4
94	11.3	5.8	21.2
96	109.9	34.6	43.8
97	122.3	48.6	86.3
98	1	3.3	40.9
99	67.4	45.2	101.3
101	9.2	24.7	44.7
Mean	**42**	**10.2**	**33.4**

I. Study Two Physical Symptoms of EFA Deficiency

Experimental Group	Subject ID #	B MSQ*	P MSQ**
	2	11	9
	9	4	8
	10	11	10
	15	4	5
	23	17	6
	26	10	11
	30	12	8
	53	8	11
	67	21	18
	74	21	11
	80	11	9
	84	5	7
Means		11.25	9.42
Placebo Group			
	22	14	12
	24	12	8
	33	13	11
	42	20	17
	54	13	10
	58	9	6
	70	14	6
	78	17	17
	87	8	10
	96	25	20
	101	13	13
	104	10	9
	105	14	13
Means		14	11.69

* Baseline Medical Symptom Questionnaire

** Post Medical Symptom Questionnaire