Omega-3 supplementation for Reducing Externalizing Behaviour problems in Typically Developing children and adolescents: A meta-analysis

The University of Adelaide

This thesis is submitted in partial fulfilment of the Honours Degree of Bachelor of Psychological Science

School of Psychology

The University of Adelaide

October 2018

Word Count: 8935

Declaration

This thesis contains no material which has been accepted for the award of any other degree of diploma in any University, and, to the best of my knowledge, this thesis contains no material previously published except where due reference is made. I give permission for the digital version of this thesis to be made available on the web, via the University of Adelaide's digital thesis repository, the Library Search and through web search engines, unless permission has been granted by the School to restrict access for a period of time.

Kimberly Klassman

October 2018

Acknowledgements

I would like to thank my supervisor's Dr Jacqueline Gould and Dr Rachel Roberts for your support and guidance throughout the year – I have learnt a lot and are very grateful for your assistance with everything.

I would also like to thank Maureen Bell, the research librarian for meeting with me numerous times to assist with the development of my search terms – you are truly a very valuable asset to the university.

A big thank you to my dog Rico for your unknowing support and love provided to me throughout the year – without you, I really don't know how I would have gotten through this year.

Abstract

BACKGROUND: Externalizing behaviour problems represent a leading cause for referrals to childhood mental health services and have widespread impacts on individuals, families and society. Omega-3 deficiency has been implicated with externalizing behaviours. Whether increasing omega-3 intake may can alleviate deficiency and thus improve EB in children and adolescents warrants investigation. Omega-3 fatty acids have shown to improve behavioural outcomes in neurodevelopmental disorders however consensus on whether this extends to a typically developing population remains unknown. OBJECTIVE: The objective was to evaluate the efficacy of omega-3 supplementation for reducing externalizing behaviour problems in typically developing children and adolescents across parent, teacher and self-rated measures. DESIGN: Three electronic databases were searched. Randomized controlled trials comparing omega-3 to a placebo for behavioural problems were included in this review. Risk of bias in included trials was assessed, and the results compared in meta-analyses. RESULTS: 12 Randomised controlled trials involving 2461 participants were included in the review. Standardised mean differences and associated 95% confidence intervals, p values, and heterogeneity statistics were calculated. Risk of bias analysis was conducted to determine the quality of the randomised controlled trials. No differences were observed in oppositional, anti-social and aggressive behaviours compared to control across parent, teacher and self-rated measures. No differences were also found for hyperactivity. The quality of trials varied. CONCLUSION: The evidence does not conclusively support or refute that omega-3 supplementation reduces externalizing behaviour problems in typically developing children and adolescents.

TABLE OF CONTENTS

Tables

Figures

1. Introduction

The importance of nutrition and healthy outcomes is well established across the literature (Gow & Hibbeln, 2014; Oddy et.al, 2009; Prado & Dewey, 2014). Nutrition is especially important during developmental periods with inadequate nutrition being implicated with poor developmental outcomes, and in particular behaviour problems… For example, Malnutrition at age three has been associated with later externalizing behaviours (Liu et al., 2004). Omega-3 has been implicated to improve cognitive and behavioural outcomes in children with neurodevelopmental disorders, however whether this extends to a typically developing population remains unknown. For this reason, the following paper will be interested in determining the effectiveness of omega-3 supplementation for reducing externalizing behaviour problems in typically developing children and adolescents.

1.1 Externalizing behaviour problems

Behaviour problems in children are often a normal part of development; all children can be defiant and refuse parental directions however some children may experience abnormal patterns of challenging behaviours that may be considered outside the norm for their age and level of development (Matthys & Lochman, 2017). Behavioural problems in children are most typically categorized into internalizing and externalizing problems. Internalizing behaviour problems are difficulties that primarily affect the internal psychological environment and may include being withdrawn, anxious, and depressed. In contrast, externalizing behaviour's (EB) can be defined as a grouping of behaviour problems that are outwardly expressed and reflect an individual acting negatively on the external environment (Eisenburg et al., 2001). EB's include oppositional, anti-social and aggressive behaviour's (Matthys & Lochman, 2017) and are behavioural domains associated with oppositional defiant disorder and conduct disorder; disruptive behaviour disorders recognized in the Diagnostic statistical manual of psychiatric disorders (DSM-V; American Psychiatric Association, 2013). Hyperactivity is also often referred to as an externalizing behaviour due to the co-occurrence and overlapping of symptoms (Matthys & Lochman, 2017), however the DSM-V classifies hyperactivity as a behavioural domain associated with attention deficit disorder; a neurodevelopmental disorder characterized by different developmental trajectories. For this reason, this analysis will consider oppositional, anti-social and aggressive behaviour's as externalizing behaviours and thus the primary outcomes. Due to the co-occurrence of hyperactivity with EB, hyperactivity will be included as a secondary outcome.

1.1.1 Oppositional behaviours

In 2014, 5.1% of Australian children and adolescents had oppositional problem

behaviours which is equivalent to an estimated 204,000 children and adolescents across Australia (Lawrence et al., 2015). Oppositional behaviours are noncompliance based behaviours when a child is disobedient and resists authority figures. These may include arguing with authority figures, refusing to comply with requests or rules and blaming others for misbehavior (American Psychiatric Association, 2013). Children with a persisting pattern of angry, irritable, argumentative, and vindictive behavior may be diagnosed with oppositional defiant disorder and may act as a precursor for more severe behavioural disorders like conduct disorder (American Psychiatric Association, 2013; Matthys & Lochman, 2017).

1.1.2 Anti-social behaviours (conduct problems)

Anti-social behaviours (also referred to as conduct problems) can be considered as a more severe form of oppositional behaviour and are those which violate basic norms, rights and rules. For example, being defiant, destructive, threatening, lying, cheating, stealing and frequent school truancy would be considered antisocial behaviours (Liu, 2004; Matthys & Lochman, 2017). Children who exhibit repetitive and persistent manifestations of these types of severe behaviour problems may be diagnosed with conduct and adult anti-social personality disorder (Matthys & Lochman, 2017) In 2014, Conduct disorder effected an estimated 2.1% of children and adolescents, equating to 83,600 prevalence in the last 12 months (Lawrence et al., 2015).

1.1.3 Aggressive behaviours

Aggressive behaviours are those that harm or threaten to harm others, including children, adults, and animals and are physical or verbal in nature **(**American Psychiatric Association, 2013). Verbal aggression includes threats or provocation of another individual

with physical aggression involving using physical force to harm another individual, for example hitting (Matthys & Lochman, 2017). Aggression is often largely associated with anti-social behaviour's and thus characteristic of CD, however if aggressive behaviours persist and deviate beyond normality, an individual may be diagnosed with "intermittent explosive disorder," which is a form of clinical aggression in the DSM-V(American Psychiatric Association, 2013) Studies have shown that childhood aggression may act as a strong predictor of future adult crime and violence (Liu, 2004)

1.1.4 Hyperactivity

Hyperactive behaviour is typically characterized by constant activity, being easily distracted, impulsiveness, fidgeting, constant moving or wandering (American Psychiatric Association, 2013). Whilst hyperactivity is a core characteristic of ADHD, there is a high rate of co-occurrence of hyperactivity alongside EB problems

1.2 Risk factors

The development of behaviour problems is complex and likely involves the interplay of social, psychological and biological factors (Baker, Raine, Liu & Jacobson, 2008). Biological factors may include poor environmental conditions and exposure to neurotoxins during pregnancy and development (malnutrition, smoking, drugs) and a genetic predisposition to externalizing behavior (Baker, Raine, Liu & Jacobson, 2008). Psychosocial risk factors may include but are not limited to high psychosocial stress in early life, poor maternal attachment, ineffective parenting, poverty or individuals living in rural or lower socioeconomic areas (Liu, 2004; Thijssen, 2016). Gende may also act as moderating factor with males more likely to be exhibit externalizing behaviours problems compared to girls (Baker, Raine, Liu & Jacobson, 2008; Liu, 2004). Liu (2004) describes a model to explain the

relationship between psychosocial and biological factors and describes the complex relationship and interaction between them (see figure 1). In the model, psychosocial risk factors can give rise to biological risk factors and vice versa. For example, ineffective parenting may act as a psychosocial risk factor which can give rise to biological risk factors (eg malnutrition) which in turn can lead to behaviour problems with gender acting as a moderator.

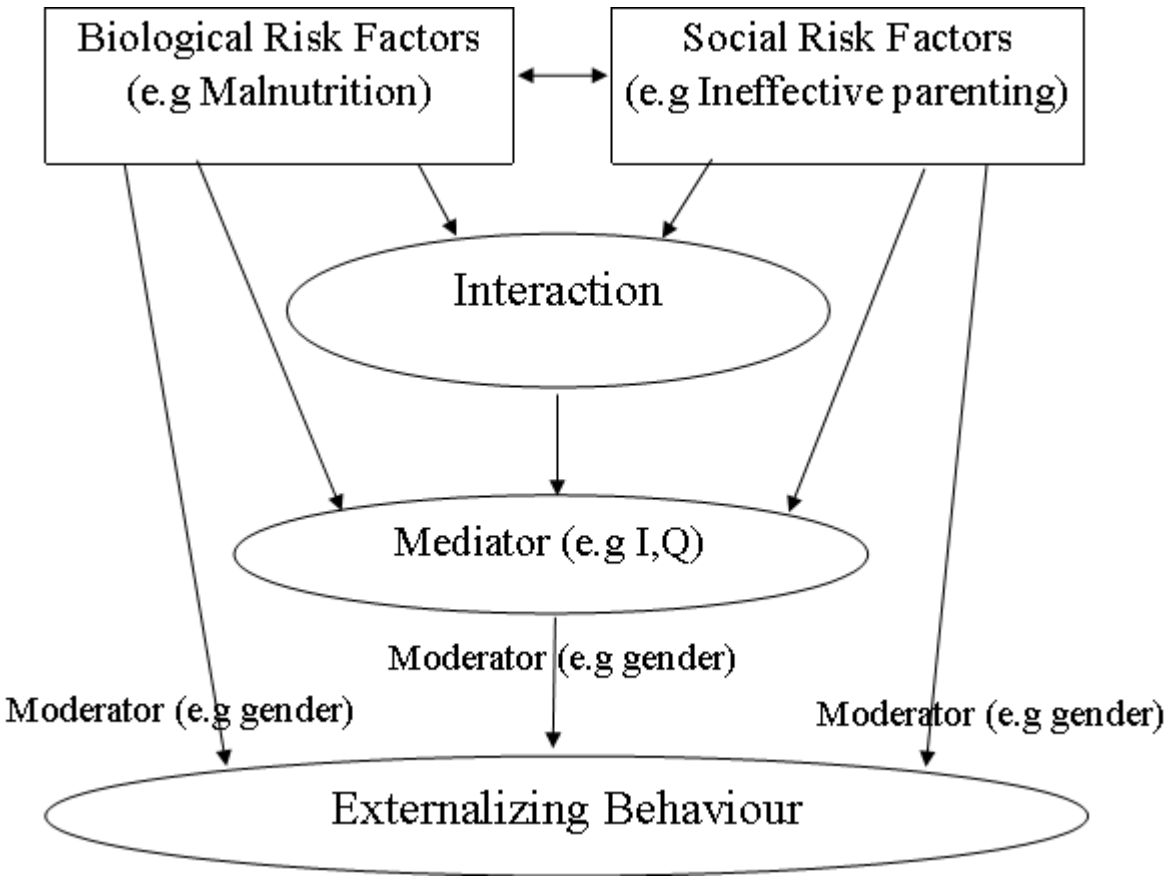

Figure 1. Development of externalizing behaviours; adapted from Liu (2004)

1.3 Impact of Externalizing Behaviour problems

EB problems represent a leading cause for referral to child mental health services and have widespread impacts on individuals, families and society (Ogundele, 2018). These types

of behaviours generally arise in childhood and have the potential to develop into more severe behavioural disorders and substantially impact on a child's development, educational and psychosocial outcomes (Liu, 2004). A study by Kim-cohen and colleagues showed that conduct disorder in young children aged five predicted significant behavioural and educational difficulties five years later (Kim-Cohen et al., 2009). Childhood EB problems are viewed as a public health problem as they are a major risk factor for later juvenile and adult delinquency, and violence (Farrington & Loeber, 2000; Sourander et al., 2006). EB have been associated with a range of adverse outcomes including poorer educational outcomes, and more suspensions and expulsions compared to peers without behaviour problems (Lawrence et al, 2015; Liu 2004). Individuals with externalizing behaviour problems during childhood also show an increased risk of substance abuse and other mental disorders (Drabick, Gadow, & Sprafkin, 2006; Hopfer et al., 2013).

1.3.1 Impact on family/carers

Parents and caregivers of children and adolescents with EB problems often experience significant burden and distress associated with the caretaking of the child. These may include but are not limited to financial burden, family conflict, effect on family social life, interruption at work, mental health and physical problems (Meltzer, Ford, Goodman, & Vostanis, 2011; Simpson, Cohen, Bloom, & Blumberg, 2009). Children with EB problems require more supervision and attention than those without and therefore many parents avoid taking these children to public places, relatives and friends due to embarrassment and shame resulting from their child's behaviours. This may result in feelings of isolation and resentment towards the child which inevitably can exacerbate EB problems (Meltzer, Ford, Goodman, & Vostanis, 2011).

1.3.2 Impact on society

EB problems are a major social and financial burden on society. In 2010, conduct disorder was among the fifteen leading causes of the global disease burden among children aged 5-19 years. together with ADHD, conduct disorder contributed for 0.8% of the total global disability and accounted a total 6.24 million Years lost to Disability(DALY) to the total global burden of disease (Erskine, 2014). Furthermore, a study by Scott and colleagues (2001) investigated the financial costs of children with behaviour problems for public services use where they followed ten year-old children up to the ages of twenty eight. The costs for individuals with conduct disorder were ten times higher than children with no problems and three and a half times higher than children with milder behavioural problems. The fact that externalizing behavior problems can have significant negative long-term consequences, highlights the need for preventative programs as well as evidence-based intervention options for children and adolescents.

1.4 Current Interventions for behaviour problems

1.4.1 Psychotherapeutic interventions

Psychotherapeutic interventions are currently one of the most common treatment options as they have a strong evidence base for reducing EB problems(Sampaio, 2016). A meta-analysis found a large effect for psychotherapy for reducing behaviour problems and were associated with improvements in parent-child relationship, improved classroom behaviour, reduced frequency or aggression outbursts and a reduction in overall behaviour outcome scores (Espstein et al., 2015). A later review on parenting interventions for behaviour problems showed a similar finding (Tully & Hunt, 2016). Despite psychological interventions being an effective treatment option for behaviour problems, there are often barriers to accessing therapy, especially for those from regional

areas and lower socioeconomic backgrounds, both where behaviour problems are more prevalent (Reiss, 2015). For example, individuals from regional or lower socioeconomic areas are less likely and to access therapy and psychological interventions due to financial barriers, distance and accessibility of services (Lawrence et al, 2015)

1.4.2 Pharmacological Interventions

Pharmaceutical agents are also considered to be an intervention option for externalizing behaviours. Epstein and colleagues (2015) reviewed the effect for pharmaceutical interventions for EB problems and produced mixed findings. They reviewed trials of antipsychotics, ADHD medications, and anti-epileptic medication and were not able to find strong effects for treating EB problems in children or adolescents without a neurodevelopmental disorder. Of the trials that did show an effect, generalizability of results is limited as many participants also had a co-morbid diagnosis of ADHD and therefore it is difficult to determine the true effect for EB problems for those without a diagnosis of ADHD (Espstein et al., 2015). An additional review found similar findings (Pringsheim, Hirsch, Gardner, & Gorman, 2015). Furthermore, pharmacological interventions may also be associated with adverse side-effects including weight gain (Pringsheim, Lam, Ching, & Patten, 2011), involuntary muscle contractions, abnormal electrocardiography readings and paranoid tendencies (Espstein et al., 2015; Pringsheim, Hirsch, Gardner, & Gorman, 2015), highlighting the need for interventions with a lower side effect profile (Ipser & Stein, 2007)

1.4.3 Dietary supplements

Another form of intervention are dietary supplements (DS), which are a type of complementary and alternative medicine. DS are often used in adjunction to and/or in replacement of traditional intervention options. Many individuals consume DS due to the

importance of good nutrition and associations with healthy outcomes (Gow & Hibbeln, 2014; Oddy et.al, 2009). There has been an increasing use of DS such as fish oil, for mental health problems and behavioral management (Sinha and Efron 2005; Barnes et al. 2008). According to the Australian Bureau of Statistics, fish oil is the one of the most-commonly taken over the counter DS in Australia in children aged between 2-18 (Australian Bureau of Statistics, 2014). DS are considered more accessible than psychotherapy and individuals may perceive them to be safer than pharmacological treatments (Dodge, 2016). However, DS should be used with caution as there is insufficient evidence to be considered an evidence-based intervention for EB problems (Catala-Lopez et al., 2017). This highlights the need for further investigation of the effect of dietary supplementation as an intervention for behaviour problems in children and adolescents.

1.5 Importance of nutrition

To determine the effect of dietary supplements as an intervention, it is important to address the relationship between nutrition status and behavioural outcomes. The significance of adequate nutrition in early life is well established (Gow & Hibbeln, 2014; Oddy et.al, 2009), with recommended daily intake guidelines existing for a nutritious and balanced diet (Australian Dietary Guidelines, 2013). However, contemporary western diets often contain large amounts of grains and processed foods which are overloaded with refined sugars, sodium and saturated fats and do not contain adequate quantities of important nutrients, and in particular omega-3 fatty acids (Prado & Dewey, 2014). This means that many individuals are not eating their recommended daily intake and are at risk of being malnourished (Gow & Hibbeln, 2014). For example, many Australian children and adolescents are reported to consume very low quantities of omega-3 fatty acids and thus may not be achieving their recommended intake (see Meyer, 2016). This may have adverse effects

due to the association between inadequate nutrition status during developmental periods and adverse developmental outcomes (Oddy et.al, 2009). For example, poor nutrition during pregnancy may lead to antisocial behaviours in later life (Neugebauer, Hoek, & Susser, 1999), Furthermore, research has shown that malnutrition in early life is associated with later problematic behaviours (see Liu, Raine, Venables & Mednick, 2004).Given the potential link between inadequate nutrition during development and later behaviour problems, raises the enquiry as to whether increasing nutrition intake, and of particular interest omega-3 can help to improve EB problems in children.

1.6 Omega fatty acids

Omega fatty acids are essential dietary fats necessary for typical development and functioning of the brain and immune system. They cannot be made by the body and must be consumed through diet or supplementation (Gow & Hibbeln, 2014). There are various types of essential fatty acids and they are classified by the position of their double carbons bonds which determines whether the molecule is an omega-3 fatty acid or omega-6 fatty acid. For example, the first double bond of omega-3 fatty acids are located at the third carbon atom and include docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and α-linolenic acid (ALA). Linoleic acid (LA) and arachidonic acid (AA) are omega-6 fatty acids and are located at the sixth carbon atom (See figure 2;Schuchardt, Huss, Stauss-Grabo & Hahn, 2010). Primary dietary sources of DHA and EPA include cold water fatty fish, milk and eggs fortified with DHA, and fish oil or algal supplements (Simopoulos, 2016

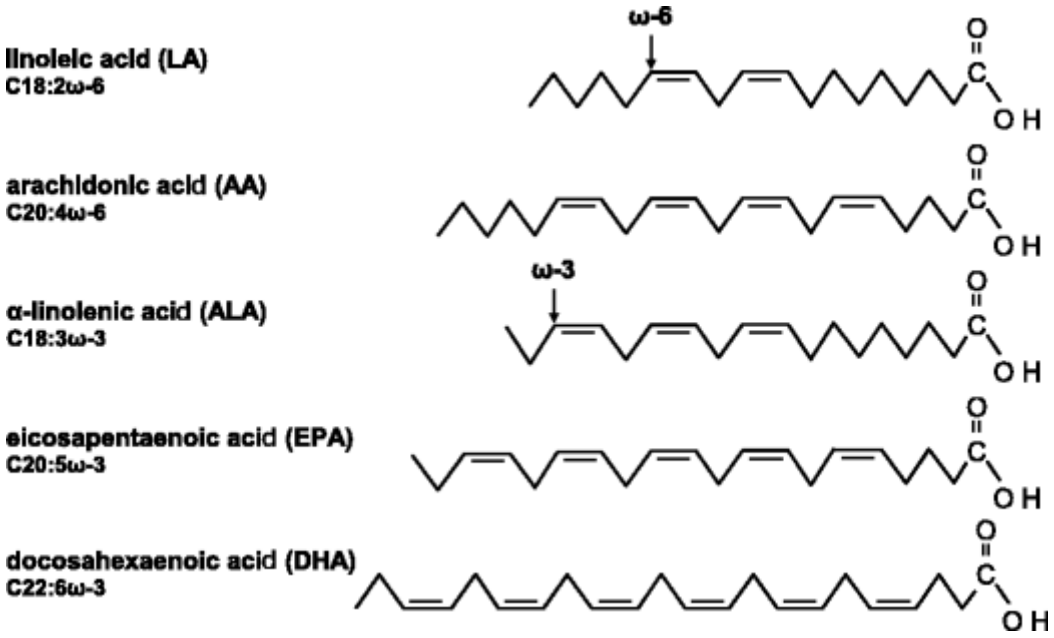

Figure 2: Structure of Omega-3 and Omega-6 fatty acids. Taken from (Schuchardt, Huss, Stauss-Grabo & Hahn, 2010, p. 2).

Omgea-3's are important for a range of metabolic functions. It is estimated that about sixty percent of the dry weight of an adult brain is comprised of lipids, making up eighty percent of nerve cells (Benton & Europe, 2008; Gow & Hibbeln, 2014). Omega-3's are major structural components in cell membranes in the brain and body. They act by helping to preserve and support the membrane by maintaining its fluidity and the activity of proteins contained within these membranes (enzymes, receptors, transporters, voltage-gated ion channels, etc; Choy & Raine 2018). They are also associated with many important neurological functions including neurotransmission, stimulating neurite outgrowth, and enhancing synaptic functioning and dendritic branching, gene expression, and myelination (Gow & Hibbeln, 2014; Hibbeln, Ferguson, & Blasbalg, 2006).

1.7 Omega-3 and brain development

The mechanisms of action by which inadequate omega-3 intake during development may be implicated with EB problems is important to determine the potential efficacy of omega-3 supplementation. Omega-3's play important roles during developmental periods; Infancy, childhood and adolescence are critical periods of development where the brain undergoes substantial structural and functional changes. Omega-3 is said to contribute to the establishment of connections between the frontal lobe regions in the brain that are responsible for important functions including attention, executive function and decision making (McNamara, Vannest & Valentine, 2015). Research has shown that structural and functional deficits in the prefrontal cortex are associated with externalizing behavior (Yang & Raine, 2009). Thus, inadequate intake of omega-3 during development may lead to reduced connectivity between these regions and increased risk of EB (Hibbeln et al., 2006). Humans studies on infants have reported that infants born preterm have reduced levels of DHA which has been associated with significant reductions in volume of various regions of the brain and reduced connectivity between them, which may be mitigated by postnatal high-dose DHA supplementation. Moreover, increases in DHA during development has been associated with active periods of a range of neurobiological functions including synaptogenesis, grey matter expansion and neurogenesis where new brain cells are formed (see Gow & Hibbeln, 2014; Hibbeln, Nieminen, Blasbalg, Riggs & Lands 2006). Omgea-3 deficiency may impair many these functions leading to disruption of neuronal pathways that regulate behaviour which may result in a residual predisposition toward aggressive and atypical behaviours (Hibbeln et al., 2006; McNamara, Vannest & Valentine, 2015). For example, animal studies have shown that reduced levels of omega-3's during early development leads to dysregulated neurotransmitter function which may be associated with later behaviour problems (McNamara, Vannest & Valentine, 2015). However, whether this effect extends to humans remains unclear. Nonetheless, omega-3 supplementation is suggested to reduce externalizing behaviours

through improved regulation of neurotransmitters and hormones, specifically serotonin and dopamine in the frontal cortex (Gow & Hibbeln, 2014). The potential role for omega-3 intervention during childhood and adolescence could be deemed plausible; research suggests that although by six years of age the brain is about 95% of its final size, expansion of the grey matter, especially in frontal regions, continues to increase throughout puberty and adolescence (Benton, 2008). Since omega-3 plays important roles in grey matter expansion and the development of the frontal cortex, omega-3 intervention during childhood and adolescence may be effective. Given the association between inadequate omega-3 during development and EB problems, whether an intervention of omega-3 during development periods can correct deficits and thus risk for behaviour problems warrants further investigation.

1.8 Omega-3 and neurodevelopmental disorders

Numerous studies have demonstrated a connection between omega-3 and various neurodevelopmental disorders due to the association between lower levels of omega-3 and individuals with psychiatric symptoms compared to individuals without (Hibben, Hawkey & Nigg, 2014). Whether these irregularities are due to low dietary intake of omega-3 or individuals experience abnormality in omega-3 metabolism is difficult to determine. For this reason and the growing awareness of the role of nutrition in neural development, research has been focused on determining whether omega-3 supplementation can rectify developmental deficits and thus related outcomes in these populations. Various randomized controlled trials have investigated this effect in individuals with ADHD (Gustafsson et al., 2010; Hirayama, Hamazaki & Terasawa, 2004; Johnson, Ostlund, Fransson, Kadesjo & Gillberg, 2009; Raz, Carasso & Yehuda, 2009; Voigt et al., 2001) Gustafsson and colleagues showed improvement in behaviour problems in children with ADHD after 15-week EPA treatment. Johnson et al.,

also showed positive findings for reducing behaviour problems (2009). However, Voigt and colleagues (2001) did not reproduce such an effect. A Cochrane review also reported mixed findings (Gillies, Sinn, Lad, Leach, & Ross, 2012). This effect has been investigated in individual's with autism spectrum disorders (ASD) with findings also showing mixed results (see Cheng et al., 2017; Hovarth, Łukasik, & Szajewska, 2017).

1.9 Omega-3 and typically developing populations

Despite research into the effectiveness of omega-3 supplementation for reducing behavioural outcomes in individuals with neurodevelopmental disorders, little is known about whether this effect extends to a typically developing population (TDP). Of the research that has been conducted, results have been mixed. For example, Richardson and colleagues conducted a well-known randomized controlled trial and found that n-3 supplementation reduced parent-rated externalizing behavior problems significantly compared to placebo (Richardson, Burton, Sewell, Spreckelsen & Montgomery, 2012) however this effect was not found for teacher reported data. Montgomery and colleagues attempted to replicate this effect, however no significant results were found (Montgomery, Spreckelsen, Burton, Burton, & Richardson, 2018). Whilst some reviews have focused on EB problems in a TDP (Choy & Raine 2018; Kuratk, Barrett, Nelson & Salem, 2013) there is yet to be a review that collates these studies into a meta-analysis to determine whether there is an effect. Gajos & Beaver, (2016) conducted a meta-analysis on omega-3 supplementation for aggression, however they included both TDP and individuals diagnosed with ADHD and other psychiatric disorders, as well as both children, adolescents and adults. This makes it difficult to determine the true effect of omega-3 in children and adolescents of a TDP. They also looked at the construct of aggression as a whole, combining EB domains (aggression, oppositional and antisocial/conduct behaviour) rather than looking at individual constructs.

Whilst these constructs are similar and belong to a similar domain of behavioural problems, they are also distinct constructs and do not always present synonymously. For example, a child may display oppositional behaviours and not show any forms of aggression or anti-social behaviour (Matthys & Lochman, 2017). It is therefore difficult to determine whether these behaviour domains respond differentially to supplementation. Furthermore, they combined teacher, parent rated and self-rated outcomes. Due to the potential effects that the rater may have on the outcome, it is important to examine EB behaviour's separately due to differences in perspectives of ratings. For example, a parent may have a stronger insight into their child's behavioural problems and may be more sensitive to small changes compared to a teacher's ratings (Kirby, Woodward, & Jackson, 2010).

1.10 Importance of this review

Given that EB problems represent a leading cause for referrals to childhood mental health services and have widespread impacts on individuals, families and society. In addition to the possible relationship between omega-3 deficiency during developmental periods and EB, highlights the need to determine whether omega-3 supplementation can alleviate deficiency and thus improve EB in children and adolescents. Because omega-3 supplementation is a highly accessible intervention, an analysis of randomized controlled trials is needed to establish the evidence base of omega-3 supplementation. Whilst there have been numerous studies reviewing the efficacy of omega-3 supplementation for reducing behaviour problems in children and adolescents with neurodevelopmental disorders, there is limited evidence on whether this effect extends to children and adolescents from a typically developing population. Increasingly, RCTs are being conducted to examine whether there is a benefit in a typically developing population however these have shown mixed results. In addition, there is yet to be a review within this area that collates these studies into a meta-

analysis to determine whether there is an effect on EB problems. Meta-analyses are considered the highest level of evidence and are important to determine the evidence base of an intervention (Haidich, 2010). Therefore, this analysis sought to expand on previous research (Gajos & Beaver, 2016) and recognize the need for a comprehensive systematic review of RCTs for which results are quantified in a meta-analysis is conducted according to the Cochrane handbook (Higgins & Green, 2011). This review is needed as it will examine randomized controlled trials to determine the evidence base of omega-3 supplementation for reducing externalizing behavioural problems (oppositional, conduct and aggressive behaviours) across various respondents (parent, teacher and self-rated) in TD children and adolescents, which to the best of our knowledge has not been done.

1.11 Aims

The current review will use meta-analytic techniques to determine the effectiveness of omega-3 supplementation for reducing externalizing behaviours in typically developing children and adolescents. It was broadly hypothesized that there would be significant differences in externalizing behaviour scores across respondents (parent, teacher and self-rated) and externalizing behavioural domains (oppositional, anti-social and aggressive behaviour) in children and adolescents receiving omega-3 supplementation compared to placebo.

The following are the review aims:

1. Determine whether there is a significant difference in primary outcomes (oppositional, anti-social and aggressive behaviour) across parent, teacher and self-rated in groups receiving omega-3 supplementation compared to placebo

2. Determine whether there is a significant difference in secondary outcomes (Hyperactivity) in groups receiving omega-3 supplementation compared to placebo
3. Examine risk of bias of included trials and the degree of study heterogeneity.

2. Method

2.1 Search strategy

Eligible studies that examined omega -3 supplementation for behaviour problems were sourced using a systematic search strategy from the databases in July 2018 shown in table 1. Search was undertaken with no date restrictions. A manual search of the reference lists of eligible studies and reviews was also conducted to determine additional studies that may not have been captured on the literature search.

Table 1:

Databases searched

Database	**Date searched**
EMBASE (Ovid)	23/7/2018
PubMed	23/7/2018
PsycINFO	23/7/2018
Cochrane	24/07/2018
Eu clinical trials register	24/07/2018
Clinical trials.gov	24/07/2018
Australian New Zealand Clinical Trial Registry	24/07/2018
Australian Clinical Trials	24/07/2018

Search terms included a combination of keywords for the search strategy such as “omega-3” “Behaviour” and “Randomized controlled trials” figure 3 shows example of PubMed search

strategy (see appendix 1 for full list of search terms for each database). A research librarian assisted with the development and reviewing of the search strategy to ensure all relevant articles were found. The reference lists of reviews and eligible articles identified by the search were also screened. The search was last conducted in July 2018, email alert was set up on the search engines to send any new relevant articles on a weekly basis until September 2018.

AND ⇨

OR ⇩

Omega -3	Behaviour problems	Randomised controlled trial	Child
Fish oil*	Behaviour*	Randomized controlled trial	Child*
Omega-3	Behavior*		Adoles*
Cod liver oil	Oppositional behav*	Randomised controlled trial	Teen*
Marine oil*			Paediatric
Algal oil*	Externalizing behav*	Rct*	Pediatric
Algae oil*		Placebo*	
Long chain polyunsaturated	Conduct problem*	Clinical trial*	
Pufa	Disruptive behav*	Randomise*	
Eicosapentaenoic acid*	Defiant behav*	Randomized*	
Epa	Anti-social behav*	Double blind*	
Docosahexaenoic acid*	Aggressive behav*	Supplement*	
Docosahexenoic acid*	Aggression		
Docosahexanoate*			
Docosahexaenoate*[tiab]			
Dha			
Fatty acid*			
N-3			

Figure 3: PubMed Search terms

2.2 Eligibility Criteria for Studies

Studies were included in the current meta-analysis if they met the following criteria: Were (A) randomised controlled trials comparing the efficacy of omega-3 supplementation compared to a placebo for externalizing behaviours problems which could include any form of omega-3 supplementation (DHA, EPA and ALA) with or without additional vitamins. Participants were included if they were (B) typically developing school aged children and adolescents aged between 4-18. Studies included used (C) psychometrically validated measures of externalizing behaviour problems and had to (D) provide quantitative end point data (i.e. group means and *SD*'s, *SE'*s etc) and be (E) published in English. Studies were excluded if they: Were (A) not a randomized controlled trial (i.e observations studies, case studies), (B) Participants had a diagnosis or suspected diagnosis (including high scores on a diagnostic measure) of ADHD, Autism, intellectual disability or a severe psychiatric disorder (psychosis, schizophrenia or bipolar), had been taking omega-3 supplements or medication that can influence behaviour, prior to the trial.

2.3 Screening of Studies

Screening of studies was conducted using Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia) a program designed to screen searches in accordance with the Preferred Reporting Items for Systematic Reviews and Meta- analysis (PRISMA) guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009).

2.4 Data extraction

In alignment with the PRISMA guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009), data was systematically collected using a data extraction form that had been adapted from the Cochrane Collaboration pregnancy group (see appendix 2). Extracted data included study characteristics (country, setting, measures used), risk of bias analysis, participant

demographics (e.g. mean age, gender and socioeconomic status) and statistical data (e.g. means and SDs for measures of behaviour problems). Intention to treat data was used where available, a method recommended for dealing with missing data in RCTs (see Gupta, 2011). End point data was also used as recommended by the Cochrane handbook (Higgins & Green, 2011). As a meta-analysis is based on assumptions of normality, continuous data was checked for skewness prior to being included in the meta-analysis (Higgins & Green, 2011) which was conducted by reading the results of the trials.

2.5 Risk of bias analysis

Risk of bias analysis was undertaken using the method recommended by the Cochrane collaboration for assessing the validity of randomized controlled trials (Higgins & Green, 2011). Trials were assessed on the following criteria (A) the adequacy of sequence generation (selection bias); (B) allocation concealment(selection bias); (C) the blinding of participants and personnel(performance bias); (D) the blinding of outcome assessors(detection bias); E) incomplete outcome data(attrition bias); and (F) selective outcome reporting(reporting bias) and other potential sources of bias. Trials was assessed according to 'low risk of bias', 'high risk of bias' or 'unclear risk of bias' as per the Cochrane handbook (Higgins & Green, 2011).

2.6 Data Preparation

Prior to data analysis, recalculation was required for two studies. Hamazaki reported two subscales of aggression (verbal and physical). These scores were averaged to provide an overall aggression score (see Cochrane handbook on this method; Higgins & Green, 2011). Dean (2010) reported standard error and mean which was converted to SD

using Review Manager calculator (Version 5.2.3, ©2014 Cochrane Collaboration, Copenhagen).

2.7 Statistical Analysis and Interpretation

The standardized mean difference (SMD) was used as the summary statistic for the meta-analysis which is calculated by pooling the standardized mean behavioural reduction of each study outcome using Review Manager 5.3 (Version 5.3, ©2014 Cochrane Collaboration, Copenhagen). SMD was used over weighted mean differences as it allows the assessment of the same outcome from different psychometric scales (Higgins & Green, 2011). In the SMD approach, the standard deviations are used to standardize the mean differences to a single scale, as well as the computation of study weights (Higgins & Green, 2011). A random effects model was be used which is consistent with other meta-analysis on omega-3 trials (see Gillies et al, 2012). Unlike the fixed effects model, the random effects model assumes that the true effect is related but not identical across studies because of sampling and methodological differences (Borenstein, Hedges, Higgins, & Rothstein, 2009; Higgins, Thompson, Deeks, and Altman, 2003). The random effects model is based on the inverse-variance approach, where adjustments to the study weights are made according to the extent of the variation (or heterogeneity; Higgins & Green, 2011). Heterogeneity is the variability in the intervention effects and manifests when observed intervention effects are more different from each other than what would be expected due to random error. Heterogeneity statistics, Cochran's Q and I^2 index, will be calculated for analysis's that include two or more RCT's. Calculation of the I^2 index is based on the following formula provided by Higgins, Thompson, Deeks, and Altman (2003):

$$I^2 = \left(\frac{Q - df}{Q}\right) \times 100\%$$

Q is Cochran's (chi-square) heterogeneity statistic and *df* is the degrees of freedom. This describes the variability in effect estimates due to heterogeneity. I^2 quantifies inconsistency across studies and is expressed as a percentage. Interpretation of heterogeneity is described in table 2 below.

Table 2: Heterogeneity interpretation

I^2 Percentage	**Interpretation of heterogeneity**
0% to 40%:	Low heterogeneity
30% to 60%:*	Moderate heterogeneity
50% to 90%:*	Substantial heterogeneity*;
75% to 100%:*	Considerable heterogeneity*.

Note: *The importance of the observed value of I^2 depends on (a) the magnitude and direction of effects and (B) strength of evidence for heterogeneity (e.g. P value from the chi-squared test, or a confidence interval for I^2) (Higgins & Green, 2011).

In order to determine the precision of each summary statistic, 95% confidence intervals (CIs) was calculated for both individual SMD and the combined SMD of trials. CIs that do not include the value of zero are said to be statistically significant (Thompson, 2007). Rosenthal, (1979) and Orwin (1983) suggest assessing the potential for publication bias influencing the results of a meta-analysis by calculating the 'fail-safe N'. However, the Cochrane Collaboration advises against the use of 'fail-safe N' for reviews following the Cochrane handbook and therefore this statistic will not be used in this analysis (Higgins & Green, 2011). Post outcome standard deviations and means from scores from standardized measures will be used to determine the SMD. If a RCT provided multiple behavioural subdomain for one behavioural outcome (e.g. multiple subscale scores for a single outcome) a mean effect was calculated for that study. Differences across groups was considered significant if (a) the standardized mean difference represents a small 0.3 to large effect 0.8 (Cohen 1988), consistent with other meta-analysis on omega-3 trials (see Gillies et al, 2012),

and (b) the associated 95% CI does not span zero. Heterogeneity and risk of bias will also be considered when interpreting the results obtained from this meta-analysis

Results

3.1 Study selection

11 Randomized controlled trials were included in this review. The initial database search produced 358 articles and after removal of duplicates, this number was reduced to 311. Title and abstracts were screened for relevance using inclusion and exclusion criteria which resulted in 31 studies. The full-text of these studies were screened against eligibility criteria and excluded with reasons provided (see appendix 3). Thirteen of these studies included participants from clinical populations, eight of these studies were not RCT's, three of these studies did not include behavioural outcomes and two were literature reviews. Furthermore, four studies did not contain outcome data and were not included; Two of which were pilot studies or protocol's (Damsguard, 2016; Sinn, 2011), one study was an ongoing study which was confirmed by contacting the author (Fung, 2018) and one of these studies did not contain post outcome data (Parletta, 2014) and therefore the main author was contacted to see if data was available. However, data was not able to be retrieved as teacher's who completed baseline questionnaires left the school and therefore for this reason was this trial was excluded from the review. This resulted in 11 eligible trials to be included in this review (refer to Figure … for a summary of the search process). During data extraction, two of these studies were identified to not be able to include in the meta-analysis and therefore were instead included in the qualitative review (Itomura, 2005; Raine, 2015). This resulted in nine studies being included in the meta-analysis (see figure 4 below for the search process.

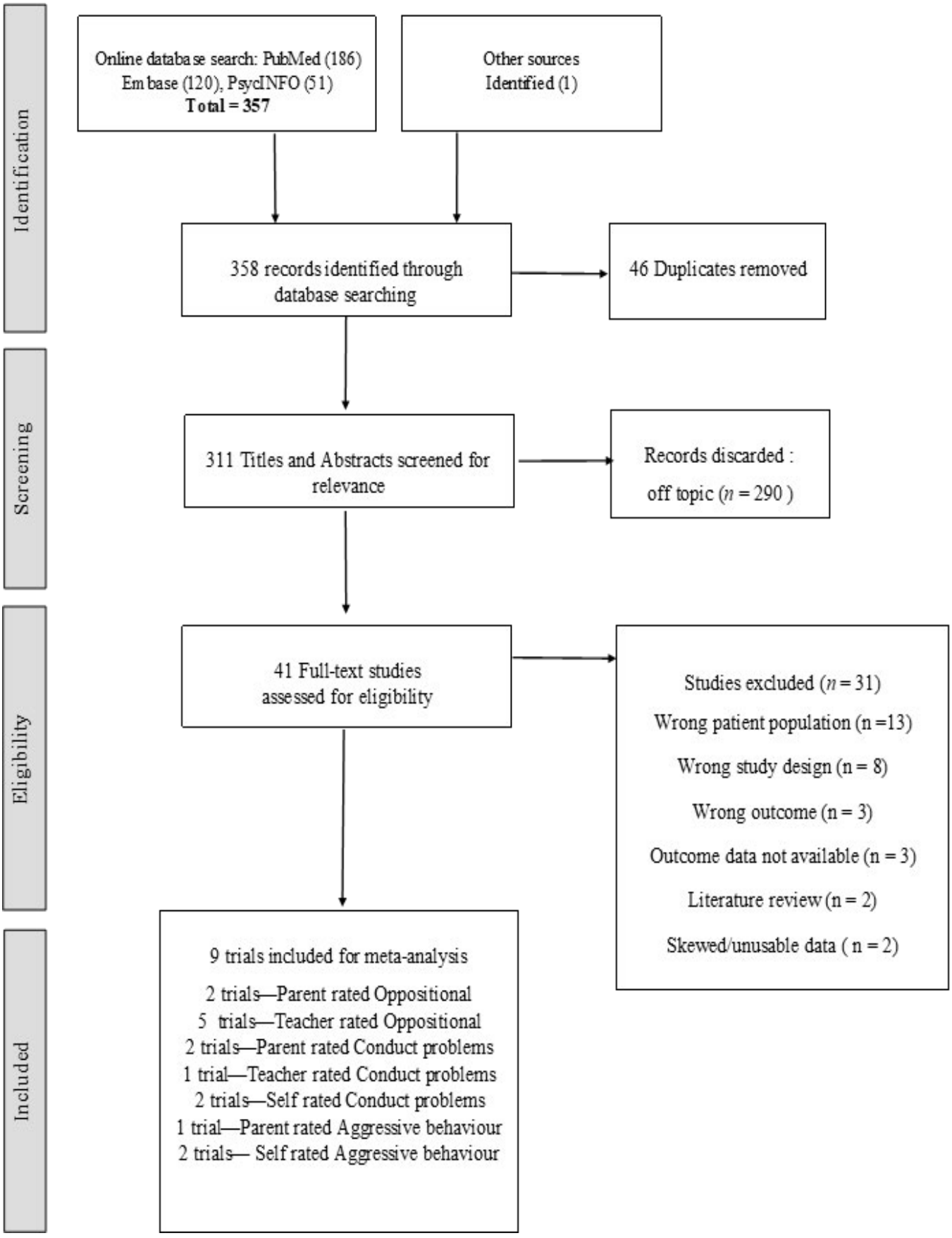

Figure 4: PRISMA flowchart of study selection process

3.2 Study characteristics

See table 3 for characteristics of trials included in this review. Majority of trials reported a double-blind randomization design, with the exception of one study which reported a single blind design where participants were aware of what intervention they are receiving (Raine, 2016). Most of trials were parallel design, with two as cross-over (Dean, 2010; Kirby, 2010) and one factorial design (Smuts, 2015). Most studies utilised intention to treat analysis(refs) and three used per protocol data (Itomura, 2005; Tamman, 2016; Smuts, 2015). Trials were published in peer-reviewed journals, with publication dates ranging from 2005 to 2018. Sample sizes of trials ranged from 21 to 450. All of the studies relied on psychological measures for the assessment of behavioural problems including the Child Behavioural Checklist (CBCL) (Raine, 2015; Raine, 2016) subscales of the Conners Parent rating scales (Montgomery, 2018; Richardson, 2012) and Conners Teacher Rating Scales (Montgomery, 2018; Richardson, 2005; Richardson, 2012, Tamman, 2016; Smuts, 2015) the Hostility-aggression questionnaire for children (HAQ-C) (Hamazaki, 2008; Itomura, 2005) and the Strengths and difficulties questionnaire (Dean, 2010; Kirby, 2010). Many of these measures show strong psychometric properties (Achenbach, 1978, Buss and Perry, 1992, Conners, Sitarenios, Parker & Epstein, 1998; Goodman, 2001). Majority of studies reported means and SD's allowing calculating the calculation of effect sizes. One trial reported standard error (Dean, 2010). Raine (2016) reported unadjusted mean and 95% confidence intervals which could not be used and therefore the lead author was emailed to determine if additional data was available to be used in the meta-analysis. One study (Itomura, 2005) reported data that was skewed and therefore was excluded from the meta-analysis. Most trials were conducted in high-income countries including the United Kingdom, USA, Australia and Japan, and three trials were conducted in lower income countries; Indonesia, South Africa and Mauritius.

Table 3: Study Characteristics

Study	Setting	Study design	N	Age	Duration	Outcome measurement	Outcome of interest	Type of supplement	Type of placebo
Kirby 2010	UK	Cross-over	450	8-10	4 months	Strengths and Difficulties Questionnaire self-report	Conduct problems	Fish oil capsule DHA – 200mg EPA – 28mg	Italian Olive Oil
Richardson 2012	UK	Parallel	362	7-9	4 months	Conners rating scale – Teacher report	Oppositional and hyperactive behaviour	Algal oil capsule DHA 600mg	Corn/soybean Oil
Hamazaki 2008	Indonesia	Parallel	233	9-12	3 months	Hostility-Aggression Questionnaire for Children	Physical aggression Verbal aggression	Fish oil Capsule DHA 650mg EPA 100mg	Soybean Oil
Itomura 2005	Japan	Parallel	166	9-12	3 months	Hostility-Aggression Questionnaire for Children	Physical aggression Verbal aggression	Fish oil fortified food DHA 514mg EPA 120mg	Soybean oil grapeseed oil fortified food
Montgomery 2018	UK	Parallel	376	7-9	4 months	Conners rating scale – parent and teacher report	Oppositional and hyperactive behaviour	Algal oil capsule DHA 600mg	Corn/soybean Oil
Raine 2015	Mauritius	Parallel	200	8-16	6 months	Child behaviour checklist - Parent and youth self-report	Rule breaking and aggression	Fish oil and multivitamin fruit juice DHA 300mg EPA 200mg ALA 400mg DPA 100mg Vitamin D Antioxidants	Fruit juice with vitamin D and antioxidants
Raine 2016	USA	Cross over	290	11-12	3 months	Child behaviour checklist - Parent and youth self-report	Rule breaking and aggression	Fruit juice with vitamin D and antioxidants DHA 300mg EPA 200mg ALA 400mg	Fruit juice with vitamin D and antioxidants

Dean 2010	AUS	Prospective	21	6-17	3 months	Strengths and Difficulties Questionnaire self-report	Conduct problems	Fish oil Capsule DHA 2000mg EPA 400mg	Olive oil 10mg fish oil
Tamman 2016	UK	Parallel	196	13-16	4 months	Conners rating scale – Teacher	Oppositional and hyperactive behaviour	Fish oil capsule DHA 116mg EPA 165mg	Sunflower oil, olive oil 10mg fish oil
Richardson 2005	UK	Parallel	117	5-12	3 months	Conners rating scale – Teacher	Oppositional and hyperactive behaviour	Fish oil 80% Primrose oil 20% Capsule	Olive oil
Smuts 2015	South Africa	Factorial	20	6-11	8.5 months	Conners rating scale – Teacher	Oppositional and hyperactive behaviour	DHA 175mg EPA 558mg ALA 60mg	Medium chain tag

3.3 Participants

The trials involved a total of 2461 participants. Participants age ranged from 4-17 years. Majority of the trials included participants from a typically developing school aged population. However, some studies did not include exclusion criteria and therefore it is difficult to determine whether these children would meet the criteria of a neurodevelopmental disorder such as ADHD or autism (Hamazaki, 2008; Itomura, 2005). Two studies included participants who were in the lower percentile in reading (Richardson, 2012; Montgomery, 2018), one study included participants with a developmental co-ordination disorder (Richardson, 2005) and one study included participants who had a diagnosis of a behaviour disorder such as CD or ODD (Dean, 2010).

3.4 Intervention

All the included trials used oral based intervention of omega-3 including functional foods, fortified juice or capsules. Majority of the studies used fish oil as the primary source of omega-3, with one trial sourcing from algal oil and primrose oil (Richardson, 2005). The types of omega-3 and quantities varied. For example, doses of omega-3 varied from 184mg/d to 2400mg/d with interventions including both EPA and DHA in majority of studies and two studies using DHA alone (Richardson, 2012; Montgomery, 2018). Three studies also included ALA (Raine, 2015; Raine, 2016; Richardson, 2005). Four of the included studies included additional vitamins as part of the intervention, these included Vitamin D (Raine 2015; 2016), a combination multivitamin (Tamman 2016), and vitamin E (Richardson 2005). All studies included a control group however varied with the type of placebo used. For example, placebo types included soybean oil used in four studies (Itomura, 2005; Hamazaki, 2008; Montgomery, 2018; Richardson, 2012) corn oil (Montgomery, 2018; Richardson, 2012), sunflour oil (Tamman, 2016) juice with multivitamin (Raine, 2015; Raine, 2016), Olive oil

(Dean, 2010; Richardson, 2005) and medium chain TAG (Smuts, 2016). The length of intervention varied from 3 months to 8.5 months. Whilst some studies reported follow up data, these were not included as part of the meta-analysis.

3.5 Risk of bias

Risk of bias was assessed using the Cochrane Collaboration's tool (Higgins 2011). A summary of the risk of bias associated with each trial is shown in figure 3. All trials reported adequate randomization methods. However, some trials lacked clarity on allocation concealment methods (Hamazaki 2010; Itomura, 2005; Raine, 2015 Smuts, 2015) or reported high risk methods including allocating participants to the next consecutive number which allows researchers to predict allocation (Dean 2010), increasing the risk of selection bias. Most of the trials adequately blinded participants and assessors to the treatment allocation and outcome assessment and therefore were rated as low risk. However, two studies were rated as high risk due to quality of blinding methods (Hamazaki, 2005; Itomura, 2005) and one study was rated high due to being a single blind open trial, where participants were aware what intervention they were receiving. One trial was rated as unclear as they did not address blinding methods (Smuts, 2015). Most trials used matching interventions and placebos and because parent and teacher rated measures were used, were rated as a low risk bias for blinding of outcome assessment; two studies were rated as high risk as they did not adequately match the intervention and placebo (Raine, 2016; Itomura, 2005) and two study did not adequately describe methods which was classified as unclear risk for blinding of outcome assessment (Hamazaki, 2005; Smuts, 2015). Attrition rates varied between studies, some studies reported attrition rates that would be considered high risk (>20%, Kirby, 2010; Richardson, 2012; Montgomery, 2018) as they utilized intention to treat analysis; studies were thus rated as low risk with the exception of one study which reported attrition rates of

85-90% which was rated as high risk (Richardson, 2005). Two remaining studies were rated as high risk of attrition bias; Smuts 2015 did not report baseline data and therefore it was difficult to determine attrition rates and another study reported high rates of attrition (Kirby, 2010). All trials reported outcomes that was described in the method or protocol, except for when there were issues with missing data. Other risk of bias was determined to be unclear for most trials except for one study which was substantially underpowered (Dean, 2010).

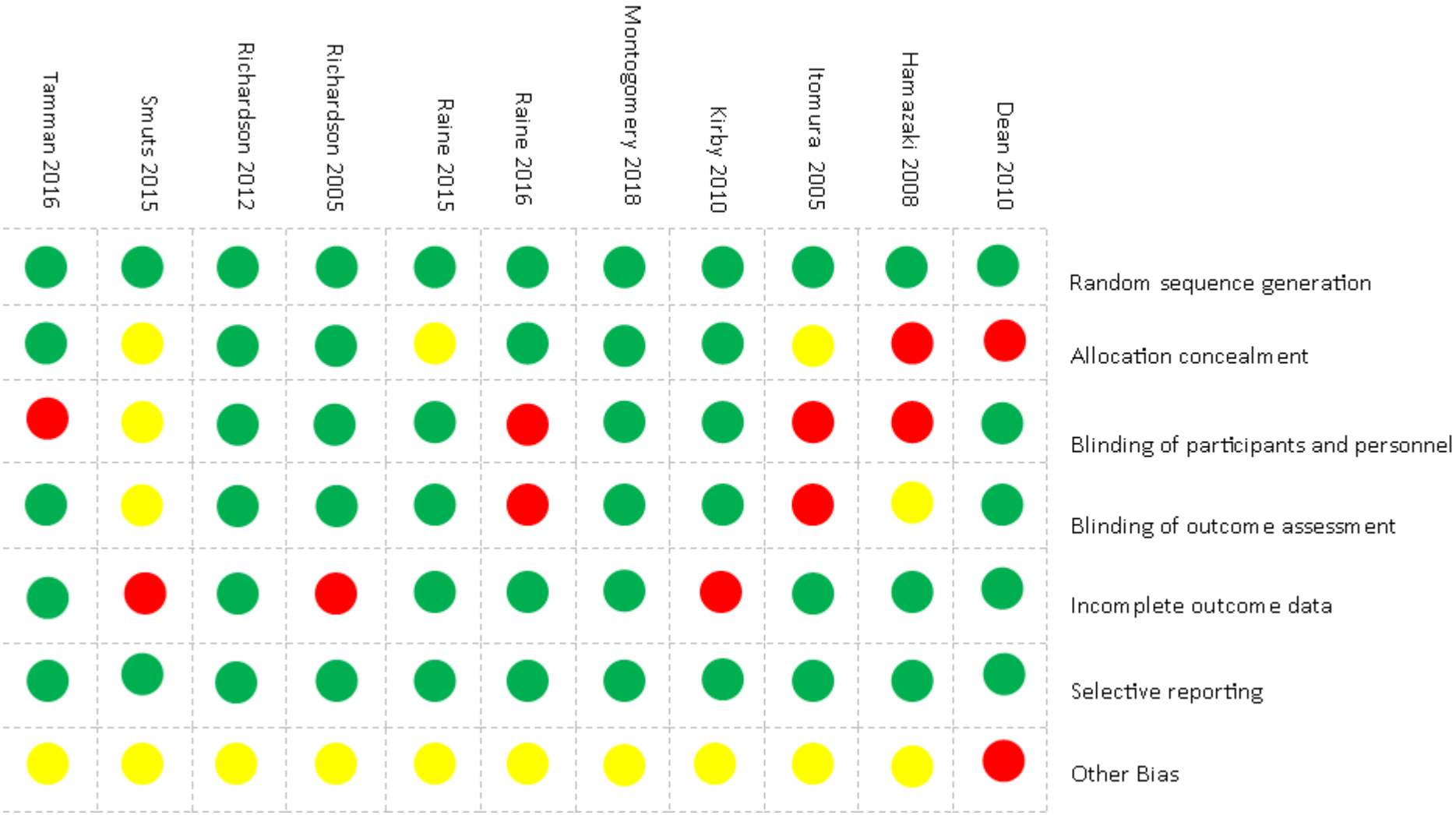

Figure 5: Risk of Bias results for included trials

3.6 Oppositional behaviours

Two trials reported parent rated oppositional behaviours using Conners Rating Scale (CRS) with no statistically significant differences found between experimental and control group (SMD -0.01; 95% CI -0.16, 0.13, n=738, p=0.88) heterogeneity was low (I^2 =0%). (figure 6). There were also no differences found for teacher rated oppositional behaviour in trials using the CRS, (SMD: -0.03; 95% CI -0.153, 0.09, n=1072, p= 0.58; Figure 7). Data was not available for self-rated oppositional behaviour.

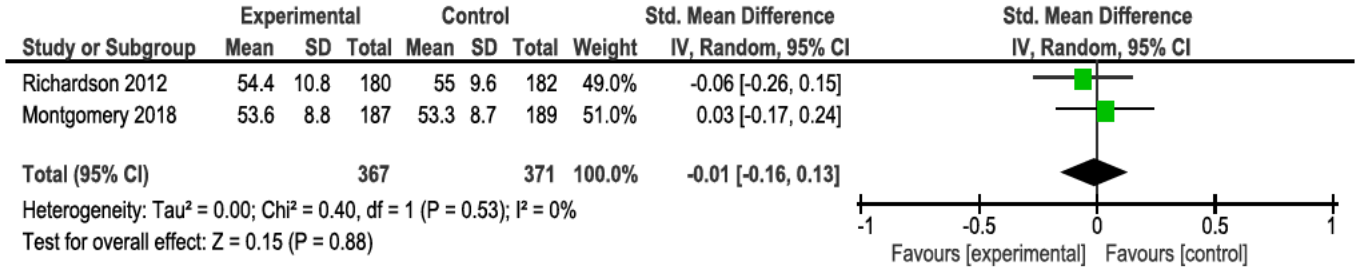

Figure 6: Parent rated Oppositional Behaviour

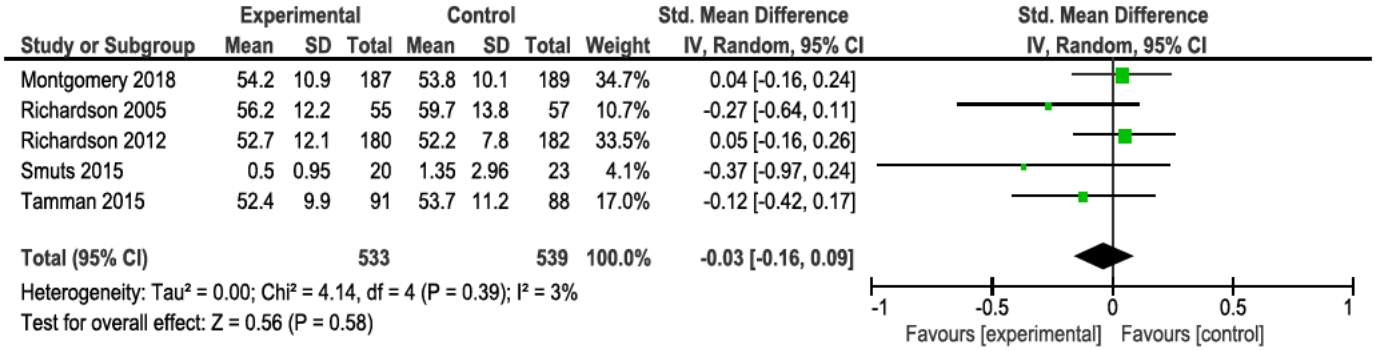

Figure 7: Teacher rated Oppositional Behaviour

3.7 Conduct problems/antisocial behaviour

Two trials reported parent rated, conduct problems using measures from the Child Behaviour Checklist (CBC) and the Strengths and Difficulties questionnaire (SDQ), finding no significant difference between the experimental and control group (SMD -0.02; CI -0.23, 0.19, n=350, p=0.83, Figure 8). Both these measures were also used for self-rated conduct problems where no difference was found between experimental group and control group

(SMD -0.13; CI -0.13, 0.40, *n*=221, *p*=0.33; Figure 9). One study reported teacher rated conduct problems measured with the SDQ showing no significant differences (SMD: 0.20; CI -0.03, 0.44, n = 287, *p*=0.09).

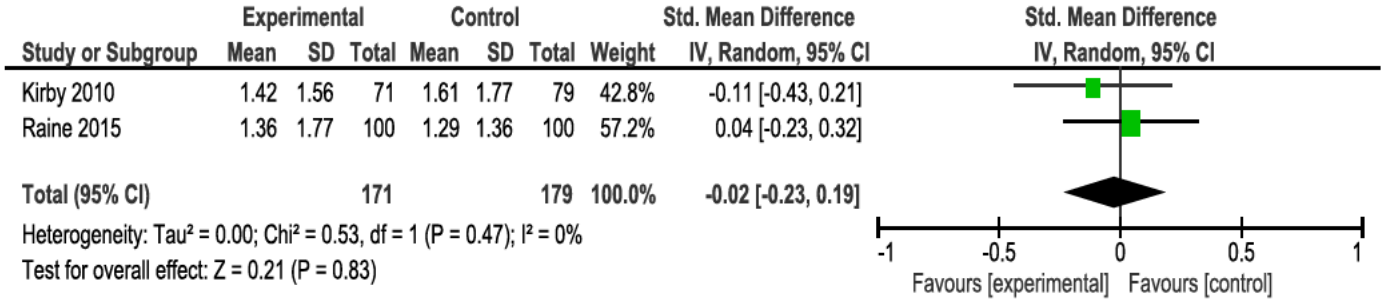

Figure 8: Parent rated Anti-social Behaviour

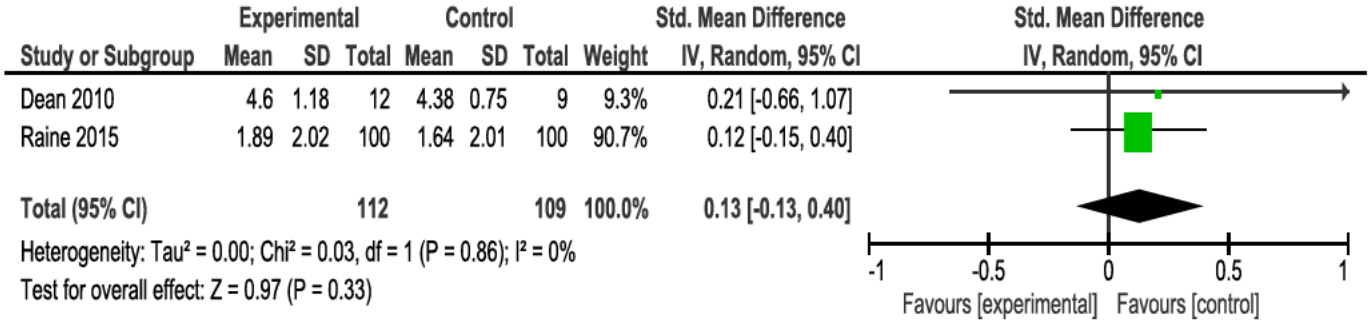

Figure 9: Self rated Anti-social Behaviour

3.8 Aggressive behaviour

Two trials reported parent ratings for aggressive behaviour using the Hostility-Aggression Questionnaire for Children (HAQ-C) however data was not able to be used for Raine (2016) as they did not report appropriate data and therefore only one trial was included in statistical analysis (Raine, 2015). There was no differences found (SMD: -0.11, CI -0.17,0.39, n=200, *p*=0.44; Figure 8). No data was available for teacher rated aggression scores. Two trials reported self-rated aggression with these results showing non-significant differences between the control and experimental condition (SMD 0.22; CI: - 0.18, 0.62, n=389, *p*=0.29; figure 9) using the CRS and HAQ-C. Heterogeneity between these two trials were also found to be high (I^2 =75%). Itomura, 2005 reported self-rated aggression scores and

was not able to produce an effect for omega-3 for reducing aggression compared to control group.

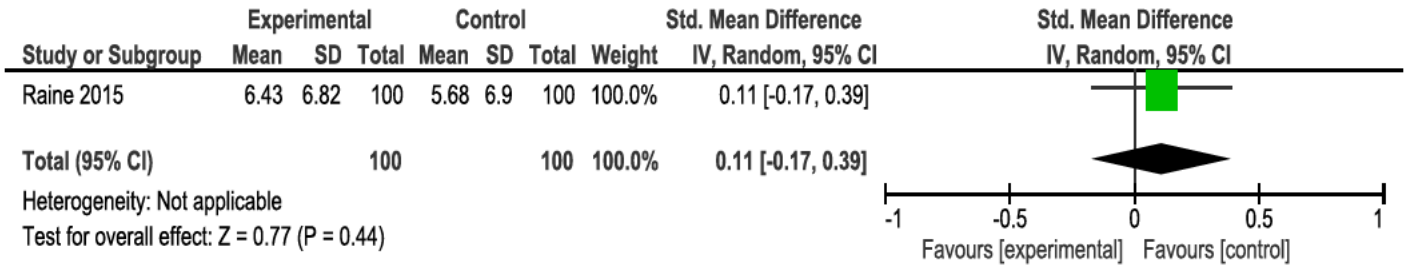

Figure 10: Parent rated Aggressive Behaviour.

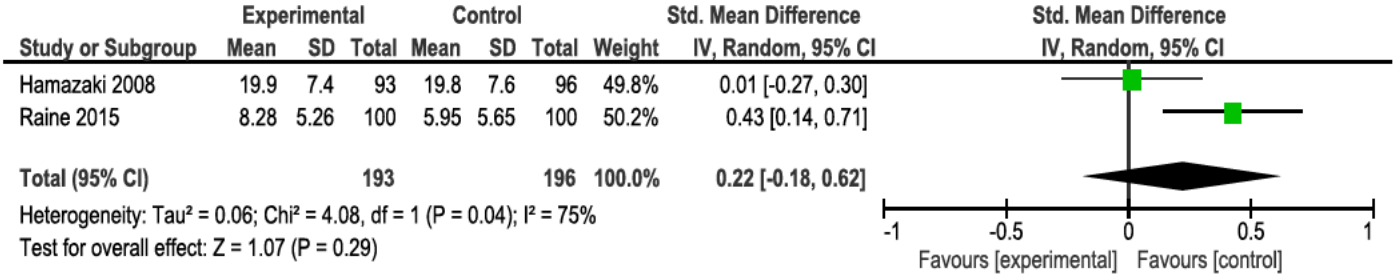

Figure 11: Self rated Aggressive Behaviour

3.9 Externalizing behaviours

Raine, 2016 reported data on self-rated and parent rated externalizing behaviour problems measured by the CBC which included both anti-social behaviour and aggression combined. Data was reported as unadjusted means and confidence interval and was not able to be included in a meta-analysis. At 3 months, the treatment group, scored lower according to parent and self-reported compared to the control group finding a small effect, however this was not found for the parent rated data. Omega-3 combined with cognitive behaviour therapy (CBT) also found reductions at 3 months for self-rated behaviour and at 12 months lower scores were shown for omega-3 group compared to CBT only (Raine, 2016)

3.10 Hyperactivity

Three trials reported parent rated hyperactivity indicating no significant differences between experimental and control group (SMD: 0.03; CI -0.12, 0.18, n = 888, p = 0.72;

figure 12) using the SDQ and CRS. Similar measures were used for teacher rated hyperactivity outcome data in five trials, no differences across groups (SMD:0.09, CI: -0.02, 0.20, N=1316, *p*=0.12; figure 13).

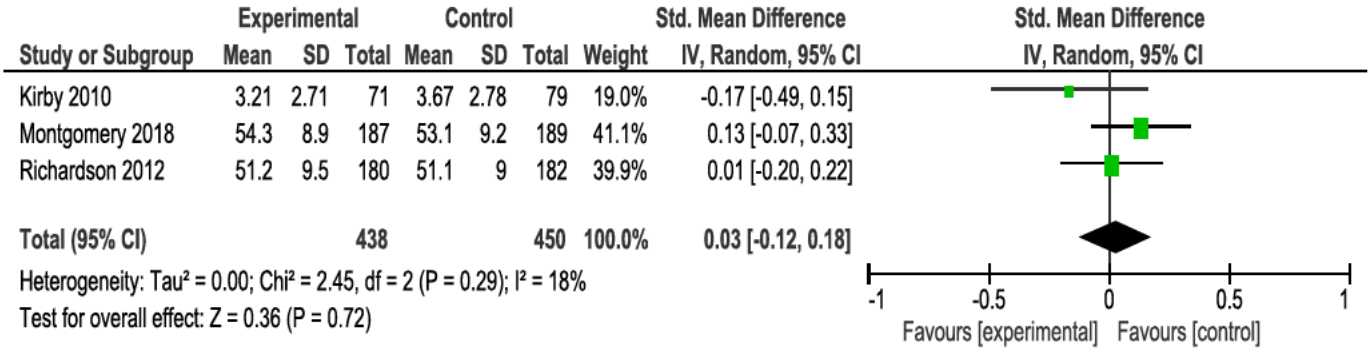

Figure 12: Parent rated hyperactivity

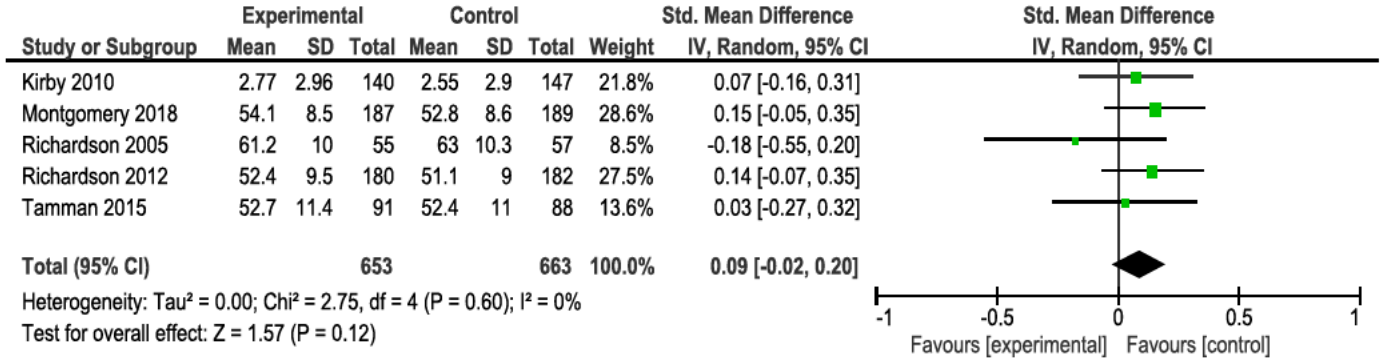

Figure 13: Teacher rated hyperactivity

Discussion

Various systematic reviews and meta-analyses have examined the efficacy of omega-3 consumption for improving symptoms in neurodevelopmental disorders, but no studies to date have explicitly examined this effect for reducing externalizing behaviour problems in a typically developing (TD) population using meta-analytic techniques. The purpose of the current meta-analysis was to examine RCTs to determine the effectiveness of omega-3 supplementation for reducing externalizing behaviour, and in particular individual externalizing behavioural domains in TD children and adolescents. Overall the results from this meta-analysis show that there is limited evidence that Omega-3 fatty acid supplementation provides a benefit for externalizing behavioural problems in children and adolescents across each respondent type (parent, teacher and self-rated) and behavioural domains (oppositional behaviour, conduct problems, aggression). Although some individual RCT's show positive findings for reducing externalizing behaviours in children and adolescents (Richardson, 2005; Richardson, 2012; Kirby, 2010) combined with other studies, this effect was not able to be found. There was also no indication of a beneficial effect of omega-3 on hyperactivity, a behavioural domain typically associated with ADHD. These results are consistent with the literature on omega-3 supplementation which have shown mixed results. For example, A meta-analysis by Bloch, (2011) found a small but significant reduction in ADHD symptoms with omega-3 supplementation. However, Gillies and colleagues (2012) found limited evidence for the effectiveness of omega-3 supplementation for behaviour problems in children and adolescents with ADHD. This is also been found for meta-analysis on omega-3 supplementation in pregnancy for cognitive outcomes (Gould, Smithers, & Makrides, 2013). Risk of bias analysis reported variation in the validity across trials, highlighting the differences across methodologies.

The findings reported in the meta-analysis could be the result of several factors. For example, many of the trials included had relatively small sample sizes and thus may not be adequately powered. Bloch and Qawasmi highlighted that in order to have sufficient power (β = 80%, 2-tailed $\alpha = 0.05$) to detect a significant difference (effect size of 0.31), RCTs of omega-3 interventions compared to placebo would require a sample size around 330 children (2011). Of the trials included in this review only three of the studies would be considered adequately powered according to this evaluation (Kirby, 2010; Montgomery, 2018 & Richardson, 2012). Bloch and Qawasmi suggest that the many of the sample sizes in RCTs to date are therefore underpowered and may account for some of the inconsistent findings in omega-3 research making them more susceptible to methodological flaws (2011). Another important factor to consider is that majority of trials utilised ITT analysis. Whilst this is typically viewed as an advantageous approach in RCTs, it may also underestimate the treatment effects (Hernan & Hernandez-Díaz, 2012).

Furthermore, inter-individual variability may play a role in determining the effectiveness of omega-3. Ghasemifard and colleagues highlight the importance of not using a fixed dose for individuals in omega-3 trials and instead adjusting the dosage to suit the individual. For example, the omega-3 dosage in many trials are not weight adjusted and therefore children and adolescents who weigh more may require higher dosages compared to those who weigh less for an effect to be determined (Ghasemifard, Turchini & Sinclair, 2014). A study on omega-3 and cardiovascular health highlights this inter-individual variability where 69 individuals ingested 1g per day of EPA and DHA for 3 months reporting a highly variable response to supplementation. It was shown that omega-3 blood levels were elevated 4-fold in some individuals while other participants showed a limited response (Von, 2010). Interestingly, a

study that administered weight-adjusted doses of DHA/EPA in a sample of children, found significant increases in Omega-3 blood levels as well as a significant improvement in motor skills (Beblo, Reinhardt, Demmelmair, Muntau, & Koletzko, 2007). Whether this effect was the result of weight adjusted doses warrants further investigation.

The optimum dosage of EPA and DHA for an effect to be determined is also unknown, especially within a typically developing population where smaller changes may be expected (Kuratko, Barrett, Nelson, & Salem, 2013). In the trials in this analysis, dosage ranged from 184mg per day to 2400mg, with EPA ranging from 28mg to 558mg p/day and DHA 116mg to 2000mg p/day across trials. Because the optimum dosage of omega-3 is unknown, the dosage in the active treatment in some trials may not be at an optimum level to have an effect on behaviour outcomes (Kirby, Woodward, & Jackson, 2010). This substantial variation between dosages could contribute for the variability in results in omega-3 supplementation trials and thus the lack of effect in this analysis (Frensham, Bryan & Parletta, 2012; Gajos & Beaver, 2016) Moreover, the variation in the length of supplementation may also play a role. Trials ranged from 3months to 8.5 months in this analysis. Early research suggests that it takes approximately 3 months for omega-3 levels to see effects from supplementation due to the slow turnover in the neuronal membrane (Kirby, Woodward & Jackson, 2010). However, this was based on rat studies which therefore is difficult to determine the time it takes for humans to build up omega-3 levels for an effect to be seen remains unknown. Furthermore, it is suggested that the turnover of omega-3's in the brain in children and adolescents is slower after 2 years of age and therefore longer periods of supplementation may be required to alter the omega-3 content of the CNS and thus see related effects (Ryan & Nelson, 2008). Future research is needed to determine the optimal dose and length of omega-3 supplementation needed to benefit a various psychological

outcomes across different age groups. There is also large variation in the makeup for the supplements with some trials containing purely DHA (Montgomery, 2018; Richardson, 2012) with remaining trials including DHA and EPA and some with ALA combined (Raine, 2015, Raine, 2016, Richardson, 2005) which could contribute to inconsistent results due to the differential effects of EPA and DHA (Dyall, 2015). Whilst a previous meta-analysis on aggression was able to find consistent results even when different types of omega-3 fatty acids were administered/assessed (Gajos & Beaver, 2016), Bloch and Qawasmi (2011) found that higher doses of EPA compared to ALA and DHA were significantly but modestly correlated with omega-3 efficacy in the treatment of ADHD. It is unclear why EPA improved ADHD symptoms while supplementation with DHA did not to the same degree. DHA is considered the most important omega-3 in the brain, and as a result has been the most studied, however emerging research is starting to determine the importance of EPA. Research from adult studies suggests EPA may be more effective in reducing a various symptom's in neurodevelopmental and psychiatric disorders compared to DHA (Gesch, Hammond, Hampson, Eves & Crowder, 2002). In this analysis, all trials that included EPA also included DHA as an intervention and therefore comparative analysis could not be done. Future research could be focused on comparing the efficacy of EPA to DHA in typically developing populations to determine whether it can improve behavioural problems. It is also important to note that many of the placebos used in the trials in this review used supplements which are made of other types of oil including olive, soybean, cornflour and sunflour oil which may be considered bioactive (…) For example, cornflour and sunflour oil may provide additional LA, soybean may provide ALA, or monounsaturated fatty acids and polyphenols from olive oil. (Dyall, 2011).

Despite substantial methodological inconsistencies, the effect observed in omega-3 trials in neurodevelopmental and psychiatric disorders trials, may not extend to a typically developing population. Much of the support for the efficacy of omega-3 supplementation relies on the notion that individuals with neurodevelopmental and psychiatric disorders have significantly lower omega-3 levels than those from a typically developing population. Whilst it is not known whether irregularities are due to low dietary intake of omega-3's or if these populations experience abnormality in PUFA metabolism, it highlights the fact that individuals in typically developing populations may not be deficit in omega-3 and therefore may account for the lack of effect from supplementation.

4.1 Clinical implications

Whilst there was no effect found for omega-3 supplementation for reducing externalizing behaviour problems in this analysis, it does highlight and draw attention to the lack of consistency across omega-3 RCT's research which may assist in formulating more consistent methodologies to determine whether there is an effect. Dyall, argues that until there is universal clarity in the reporting of techniques and consistency's in methodology in omega-3 research, results may continue to be mixed (2011). Furthermore, the lack of evidence for omega-3 found in this meta-analysis also adds to findings which have queried whether there is any support for the use of omega-3 supplementation. For example, controversial findings from a Cochrane review challenged a common acceptance that increased consumption of omega-3 can protect against heart disease, stroke or death, despite previous research finding a support (Von Schacky & Harris, 2007). The review found no support for the current recommendations for the use of omega-3 supplements in individuals with a history of coronary heart disease. An older review

also found a similar result (Hooper et.al, 2006). Non-significant findings are of equivalent importance than those that show an effect. Based on the current review, there is no evidence that omega-3 supplementation will be of benefit for reducing externalizing behaviours in typically developing children and adolescents.

4.2 Limitations

Whilst many of the methodological limitations in trials included in this review have been discussed, it is important to also address the limitations in the design of this review. Firstly, a protocol was not registered for this analysis, whilst the Cochrane handbook recommends registering a protocol prior to conducting a review, this was not done and thus may increase the risk of reporting bias. Secondly, the electronic search was limited to published studies only which may increase publication bias. Clinical trials.gov and related websites were searched to address this limitation, as they include clinical trials registrations and ongoing trials. Examination of reference lists of the included studies and reviews were also conducted. Furthermore, this analysis, did not include fail-safe N statistics which is typically used to determine publication bias in a meta-analysis. The Cochrane handbook advises against the use of Fail-safe N statistics and as this analysis followed the requirements of a Cochrane review, these statistics were thus not included (Handbook). Moreover, the criteria of participants in this review excluded individuals with a neurodevelopmental or severe psychiatric disorder, which was identified by either a diagnosis or elevated levels on a subjective measure. Due to the overlapping of various symptoms and co-morbidity of externalizing behaviour disorders with ADHD, it is difficult to determine whether individuals with EB also would meet the criteria of a diagnosis of ADHD. This may limit the generalisability of results to a typically developing

population. This study also did not include analysis of subgroups, whilst planned analysis of differential effects of age, comparative analysis of supplementation type (i.e DHA vs EPA etc) and length of intervention was intended to be completed, due to the low number of trials in EB domains, this was not possible. Lastly, follow up-data was also not analysed, this could be considered a limitation in omega-3 research due to the optimum length of intervention effect being unknown.

4.3 Strengths

The strengths in the design of this review include the separate analysis of parent, teacher and self-report data, this can been seen as a strength due to the subjective nature of outcome measures and the potential variability in responses across respondents. For example, parent report may been seen as superior to teacher ratings as they may be more sensitive to small changes (Kirby, Woodward, Jackson, Wang, & Crawford, 2010). The analysis of RCT's which included a placebo group can be considered a strength as this is the highest level of evidence to determine the evidence base of an intervention (see Centre for Evidence-Based Medicine, 2009) Furthermore, the adherence to the Cochrane handbook and PRISMA guidelines and the use of risk of bias analysis can also be considered a strength. For example, a previous meta-analysis on aggression (including externalizing behaviours combined) did not rate the quality of the studies included and therefore the interpretation of results is restricted (Gajos & Beaver, 2016).

Conclusion

This review does not support nor refute the evidence for omega-3 supplementation for reducing behaviour outcomes in typically developing children and

adolescents. Nonetheless, this review highlighted the methodological inconsistencies across trials in omega-3 research. More research is needed to determine the optimum quantity and length of dosage for an effect in a typically developing population. Further evidence from sufficiently powered trials with consistent methodologies is important to establish whether there is an evidence-based support for omega-3 supplementation for externalizing behaviour's in typically developing children and adolescents.

References

References marked with an asterick indicate studies included in the meta-analysis

Abdelhamid, A. S., Brown, T. J., Brainard, J. S., Biswas, P., Thorpe, G. C., Moore, H. J., & Song, F. (2018). Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Database of Systematic Reviews*, (7). doi: 10.1002/14651858.CD003177.pub3.

Achenbach, T. M., & Edelbrock, C. S. (1978). The classification of child psychopathology: a review and analysis of empirical efforts. *Psychological bulletin*, *85*(6), 1275. http://dx.doi.org/10.1037/0033-2909.85.6.1275

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (DSM-5®)*. American Psychiatric Publications.

Australian Bureau of Statistics. (2014). Australian Health Survey: Nutrition First Results-

Andrade, L., Caraveo-Anduaga, J. J., Berglund, P., Bijl, R., Kessler, R. C., Demler, O.,& Wittchen, H. U. (2013). Cross-national comparisons of the prevalence's and correlates of mental disorders.

Baker, L. A., Raine, A., Liu, J., & Jacobson, K. C. (2008). Differential genetic and environmental influences on reactive and proactive aggression in children. *Journal of Abnormal Child Psychology*, *36*(8), 1265-1278. Doi: 10.1007/s10802-008-9249-1

Beblo, S., Reinhardt, H., Demmelmair, H., Muntau, A. C., & Koletzko, B. (2007). Effect of fish oil supplementation on fatty acid status, coordination, and fine motor skills in children

with phenylketonuria. *The Journal of Paediatrics*, *150*(5), 479-484. doi: 10.1016/j.jpeds.2006.12.011

Benton, D. (2008). The influence of children's diet on their cognition and behaviour. *European Journal of Nutrition*, *47*(3), 25-37. doi: 10.1007/s00394-008-3003-x

Bloch, H. M., & Qawasmi, A. (2011). Omega-3 fatty acid supplementation for the treatment of children with ADHD symptomatology: systematic review and meta-analysis. *Journal of the American Academy of Child and Adolescent Psychiatry*, 50, 991-1000. doi: 10.1016/j.jaac.2011.06.008

Borenstein, M., Hedges, L. V., Higgins, J. P. T., & Rothstein, H. R. (2009). *Introduction to meta-analysis*: John Wiley & Sons, Ltd.

Catala-Lopez, F., Hutton, B., Nunez-Beltran, A., Page, M. J., Ridao, M., Saint-Gerons, D. M., & Moher, D. (2017). The pharmacological and non-pharmacological treatment of attention deficit hyperactivity disorder in children and adolescents: a systematic review with network meta-analyses of randomised trials. *Public Library of Science*, *12*(7), e0180355. doi.org/10.1371/journal.pone.0180355

Cochrane Collaboration. (2014). Review Manager (RevMan)[Computer Program] Version 5.2. 3. Copenhagen: The Nordic Cochrane Centre; 2012. *HEALTH PSYCHOLOGY REVIEW*, *17*.

Conners, C. K., Sitarenios, G., Parker, J. D., & Epstein, J. N. (1998). The revised Conners' Parent Rating Scale (CPRS-R): factor structure, reliability, and criterion validity. *Journal of Abnormal Child Psychology*, *26*(4), 257-268.

Conners, C. K., Sitarenios, G., Parker, J. D., & Epstein, J. N. (1998). Revision and restandardization of the Conners Teacher Rating Scale (CTRS-R): factor structure, reliability, and criterion validity. *Journal of Abnormal Child Psychology*, *26*(4), 279-291.

Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia. Available at: www.covidence.org

Dean, A., Adam, K., Bor, W., & Bellgrove, M. (2010). A randomized controlled, trial of fish oil in the treatment of overt aggression in children and adolescents with disruptive behaviour disorders-clinical and cognitive outcomes. In International Journal of Neuropsychopharmacology (Vol. 13, No. Supp. 1, pp. 166-166). Cambridge University Press.*

Dodge, T. (2016). Consumers' perceptions of the dietary supplement health and education act: implications and recommendations. *Drug testing and analysis*, *8*(3-4), 407-409. doi: 10.1002/dta.1857.

Dyall, S. C. (2011). Methodological issues and inconsistencies in the field of omega-3 fatty acids research. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 85*(5), 281-285. https://doi.org/10.1016/j.plefa.2011.04.009

Eisenberg, N., Cumberland, A., Spinrad, T. L., Fabes, R. A., Shepard, S. A., Reiser, M., & Guthrie, I. K. (2001). The relations of regulation and emotionality to children's externalizing and internalizing problem behavior. *Child development,* 72(4), 1112-1134.

Epstein, R., Fonnesbeck, C., Williamson, E., Kuhn, T., Lindegren, M. L., Rizzone, K., & Wright, G. W. (2015). Psychosocial and Pharmacologic Interventions for Disruptive Behavior in Children and Adolescents. *Agency for Healthcare Research and Quality*, 15(16), EHC019-EF. Retrieved from: www.effectivehealthcare.ahrq.gov/reports/final.cfm.

Erskine, H. E., Ferrari, A. J., Polanczyk, G. V., Moffitt, T. E., Murray, C. J., Vos, T., & Scott, J. G. (2014). The global burden of conduct disorder and attention-deficit/hyperactivity disorder in 2010. *Journal of Child Psychology and Psychiatry*, 55(4), 328-336.

Farrington, D. P., & Loeber, R. (2000). Epidemiology of juvenile violence. *Child and Adolescent Psychiatric Clinics,* 9(4), 733-748.

Frensham, L. J., Bryan, J., & Parletta, N. (2012). Influences of micronutrient and omega-3 fatty acid supplementation on cognition, learning, and behavior: Methodological considerations and implications for children and adolescents in developed societies. *Nutrition reviews*, *70*(10), 594-610. https://doi.org/10.1111/j.1753-4887.2012.00516.x

Gajos, J. M., & Beaver, K. M. (2016). The effect of omega-3 fatty acids on aggression: A meta-analysis. *Neuroscience & Biobehavioral Reviews*, *69*, 147-158. doi:10.1016/j.neubiorev.2016.07.017

Gesch, C. B., Hammond, S. M., Hampson, S. E., Eves, A., & Crowder, M. J. (2002). Influence of supplementary vitamins, minerals and essential fatty acids on the antisocial behaviour of young adult prisoners: Randomised, placebo-controlled trial. *The British Journal of Psychiatry*, *181*(1), 22-28. https://doi.org/10.1192/bjp.181.1.22

Ghasemifard, S., Turchini, G. M., & Sinclair, A. J. (2014). Omega-3 long chain fatty acid "bioavailability": a review of evidence and methodological considerations. *Progress in lipid research*, *56*, 92-108. doi: 10.1016/j.plipres.2014.09.001.

Gillies, D., Sinn, J. K., Lad, S. S., Leach, M. J., & Ross, M. J. (2012). Polyunsaturated fatty acids (PUFA) for attention deficit hyperactivity disorder (ADHD) in children and

adolescents. *Cochrane Database of Systematic Reviews*, (7). doi: 10.1002/14651858.CD007986.pub2.

Goodman, R. (2001). Psychometric properties of the strengths and difficulties questionnaire. *Journal of the American Academy of Child & Adolescent Psychiatry*, *40*(11), 1337-1345.

Gould, J. F., Smithers, L. G., & Makrides, M. (2013). The effect of maternal omega-3 (n-3) LCPUFA supplementation during pregnancy on early childhood cognitive and visual development: a systematic review and meta-analysis of randomized controlled trials–. *The American Journal of Clinical Nutrition*, *97*(3), 531-544. doi: 10.3945/ajcn.112.045781.

Gow, R. V., & Hibbeln, J. R. (2014). Omega-3 fatty acid and nutrient deficits in adverse neurodevelopment and childhood behaviors. *Child and Adolescent Psychiatric Clinics,* 23(3), 555-590.

Gupta, S. K. (2011). Intention-to-treat concept: a review. *Perspectives in clinical research*, *2*(3), 109. doi: 10.4103/2229-3485.83221

Haidich, A. B. (2010). Meta-analysis in medical research. Hippokratia, 14, 29-37.

Hamazaki, K., Syafruddin, D., Tunru, I. S., Azwir, M. F., Asih, P. B., Sawazaki, S., & Hamazaki, T. (2008). The effects of docosahexaenoic acid-rich fish oil on behavior, school attendance rate and malaria infection in school children-a double-blind, randomized, placebo-controlled trial in lampung, indonesia. Asia Pacific Journal of Clinical Nutrition, 17(2), 258-263. doi: 10.1016/j.jnutbio.2004.10.009*

Hernan, M. A., & Hernandez-Díaz, S. (2012). Beyond the intention-to-treat in comparative effectiveness research. *Clinical Trials*, *9*(1), 48-55. doi: 10.1177/1740774511420743

Higgins, J. P., & Green, S. (2011). Cochrane handbook for systematic reviews of interventions 5.1. 0. *The Cochrane Collaboration*, 33-49.. Available from www.cochrane-handbook.org.

Higgins, J. P., Thompson, S. G., Deeks, J. J., & Altman, D. G. (2003). Measuring inconsistency in meta-analyses. *BMJ: British Medical Journal*, *327*(7414), 557. doi: 10.1136/bmj.327.7414.557

Hooper, L., Thompson, R. L., Harrison, R. A., Summerbell, C. D., Ness, A. R., Moore, H. J., & Riemersma, R. A. (2006). Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. *British Medical Journal*, *332*(7544), 752-760. https://doi.org/10.1136/bmj.38755.366331.2F

Hopfer, C., Salomonsen-Sautel, S., Mikulich-Gilbertson, S., Min, S. J., McQueen, M., Crowley, T., ... & Hoffenberg, A. (2013). Conduct disorder and initiation of substance use: a prospective longitudinal study. *Journal of the American Academy of Child & Adolescent Psychiatry*, *52*(5), 511-518.

Itomura, M., Hamazaki, K., Sawazaki, S., Kobayashi, M., Terasawa, K., Watanabe, S., & Hamazaki, T. (2005). The effect of fish oil on physical aggression in schoolchildren—a randomized, double-blind, placebo-controlled trial. The Journal of Nutritional Biochemistry, 16(3), 163-171. https://doi.org/10.1016/j.jnutbio.2004.10.009*

Kim-Cohen, J., Arseneault, L., Newcombe, R., Adams, F., Bolton, H., Cant, L., ... & Matthews, C. (2009). Five-year predictive validity of DSM-IV conduct disorder research diagnosis in 4½–5-year-old children. *European child & adolescent psychiatry*, *18*(5), 284-291. doi: 10.1007/s00787-008-0729-1.

Kirby, A., Woodward, A., & Jackson, S. (2010). Benefits of omega-3 supplementation for schoolchildren: review of the current evidence. *British Educational Research Journal*, *36*(5), 699-732.

Kirby, A., Woodward, A., Jackson, S., Wang, Y., & Crawford, M. A. (2010). Childrens' learning and behaviour and the association with cheek cell polyunsaturated fatty acid levels. *Research in Developmental Disabilities*, *31*(3), 731-742.

Kirby, A., Woodward, A., Jackson, S., Wang, Y., & Crawford, M. A. (2010). A double-blind, placebo-controlled study investigating the effects of omega-3 supplementation in children aged 8–10 years from a mainstream school population. Research in Developmental Disabilities, 31(3), 718-730. doi: 10.1016/j.ridd.2010.01.014.*

Kuratko, C. N., Barrett, E. C., Nelson, E. B., & Salem, N. (2013). The relationship of docosahexaenoic acid (DHA) with learning and behavior in healthy children: a review. *Nutrients*, *5*(7), 2777-2810. doi: 10.3390/nu5072777

Lawrence, D., Johnson, S., Hafekost, J., Boterhoven de Haan, K., Sawyer, M., Ainley, J., & Zubrick, S. R. (2015). The mental health of children and adolescents: report on the second Australian child and adolescent survey of mental health and wellbeing.

Liu, J., Raine, A., Venables, P. H., & Mednick, S. A. (2004). Malnutrition at age 3 years and externalizing behavior problems at ages 8, 11, and 17 years. *American Journal of Psychiatry*, 161(11), 2005-2013.

Loeber, R., & Farrington, D. P. (2000). Young children who commit crime: Epidemiology, developmental origins, risk factors, early interventions, and policy implications. *Development and psychopathology*, *12*(4), 737-762.

Matthys, W., & Lochman, J. E. (2017). *Oppositional defiant disorder and conduct disorder in childhood*. John Wiley & Sons.

McNamara, R. K., Vannest, J. J., & Valentine, C. J. (2015). Role of perinatal long-chain omega-3 fatty acids in cortical circuit maturation: mechanisms and implications for psychopathology. *World journal of psychiatry*, *5*(1), 15. doi:10.5498%2Fwjp.v5.i1.15

Meltzer, H., Ford, T., Goodman, R., & Vostanis, P. (2011). The burden of caring for children with emotional or conduct disorders. *International journal of family medicine*, *2011*. doi: 10.1155/2011/801203

Meyer, B. J. (2016). Australians are not meeting the recommended intakes for omega-3 long chain polyunsaturated fatty acids: results of an analysis from the 2011–2012 national nutrition and physical activity survey. *Nutrients*, *8*(3), 111. doi:10.3390/nu8030111.

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., ... & Stewart, L. A. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic reviews*, *4*(1), 1.

Montgomery, P., Spreckelsen, T. F., Burton, A., Burton, J. R., & Richardson, A. J. (2018). Docosahexaenoic acid for reading, working memory and behavior in UK children aged 7-9: A randomized controlled trial for replication (the DOLAB II study). Public Library of Science, 13(2), e0192909. https://doi.org/10.1371/journal.pone.0192909*

Oddy, W. H., Robinson, M., Ambrosini, G. L., Therese, A. O., de Klerk, N. H., Beilin, L. J., & Stanley, F. J. (2009). The association between dietary patterns and mental health in early adolescence. *Preventive medicine*, *49*(1), 39-44. doi:10.1016/j.ypmed.2009.05.009..

Prado, E. L., & Dewey, K. G. (2014). Nutrition and brain development in early life. Nutrition reviews, 72(4), 267-284. https://doi.org/10.1111/nure.12102

Pringsheim, T., Hirsch, L., Gardner, D., & Gorman, D. A. (2015). The pharmacological management of oppositional behaviour, conduct problems, and aggression in children and adolescents with attention-deficit hyperactivity disorder, oppositional defiant disorder, and conduct disorder: a systematic review and meta-analysis. Part 1: psychostimulants, alpha-2 agonists, and atomoxetine. *The Canadian Journal of Psychiatry*, *60*(2), 42-51. doi: 10.1177/070674371506000202

Pringsheim, T., Hirsch, L., Gardner, D., & Gorman, D. A. (2015). The pharmacological management of oppositional behaviour, conduct problems, and aggression in children and adolescents with attention-deficit hyperactivity disorder, oppositional defiant disorder, and conduct disorder: a systematic review and meta-analysis. Part 1: psychostimulants, alpha-2 agonists, and atomoxetine. *The Canadian Journal of Psychiatry*, *60*(2), 42-51.

Pringsheim, T., Lam, D., Ching, H., & Patten, S. (2011). Metabolic and neurological complications of second-generation antipsychotic use in children. *Drug Safety*, *34*(8), 651-668.

Raine, A., Cheney, R. A., Ho, R., Portnoy, J., Liu, J., Soyfer, L., ... & Richmond, T. S. (2016). Nutritional supplementation to reduce child aggression: a randomized, stratified,

single‐blind, factorial trial. Journal of Child Psychology and Psychiatry, 57(9), 1038-1046. doi: 10.1111/jcpp.12565.*

Raine, A., Portnoy, J., Liu, J., Mahoomed, T., & Hibbeln, J. R. (2015). Reduction in behavior problems with omega‐3 supplementation in children aged 8‐16 years: a randomized, double‐blind, placebo‐controlled, stratified, parallel‐group trial. Journal of Child Psychology and Psychiatry, 56(5), 509-520. doi: 10.1111/jcpp.12314*

Reiss, F. (2013). Socioeconomic inequalities and mental health problems in children and adolescents: a systematic review. *Social science & medicine*, *90*, 24-31. doi: 10.1016/j.socscimed.2013.04.026

Richardson, A. J., Burton, J. R., Sewell, R. P., Spreckelsen, T. F., & Montgomery, P. (2012). Docosahexaenoic acid for reading, cognition and behavior in children aged 7–9 years: a randomized, controlled trial (the DOLAB Study). Public Library of Science, 7(9), e43909. doi: 10.1371/journal.pone.0043909.*

Richardson, A.J. and Montgomery, P., 2005. The Oxford-Durham study: a randomized, controlled trial of dietary supplementation with fatty acids in children with developmental coordination disorder. Pediatrics, 115(5), pp.1360-1366.*

Ryan, A. S., & Nelson, E. B. (2008). Assessing the effect of docosahexaenoic acid on cognitive functions in healthy, preschool children: a randomized, placebo-controlled, double-blind study. *Clinical pediatrics*, *47*(4), 355-362. https://doi.org/10.1177%2F0009922807311730

Sampaio, F. (2016). *Prevention and Treatment of Externalizing Behaviour Problems in Children through Parenting Interventions: An Application of Health Economic Methods* (Doctoral dissertation, Acta Universitatis Upsaliensis).

Schuchardt, J. P., Huss, M., Stauss-Grabo, M., & Hahn, A. (2010). Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children. *European Journal of Pediatrics,* 169(2), 149-164. doi: 10.1007/s00431-009-1035-8

Scott, S., Knapp, M., Henderson, J., & Maughan, B. (2001). Financial cost of social exclusion: follow up study of antisocial children into adulthood. *British Medical Journal*, 323(7306), 191.

Shier, A. C., Reichenbacher, T., Ghuman, H. S., & Ghuman, J. K. (2013). Pharmacological treatment of attention deficit hyperactivity disorder in children and adolescents: clinical strategies. *Journal of central nervous system disease*, *5*, JCNSD-S6691. doi:10.4137/JCNSD.S6691

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & pharmacotherapy*, *56*(8), 365-379.

Simopoulos, A. P. (2016). An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients,* 8(3), 128. doi: 10.3390/nu8030128.

Smuts, C. M., Greeff, J., Kvalsvig, J., Zimmermann, M. B., & Baumgartner, J. (2015). Long-chain n-3 PUFA supplementation decreases physical activity during class time in iron-deficient South African school children. British Journal of Nutrition, 113(2), 212-224. https://doi.org/10.1017/S0007114514003493*

Sourander, A., Aromaa, M., Pihlakoski, L., Haavisto, A., Rautava, P., Helenius, H., & Sillanpää, M. (2006). Early predictors of deliberate self-harm among adolescents. A prospective follow-up study from age 3 to age 15. *Journal of affective disorders*, *93*(1-3), 87-96.

Sourander, A., Elonheimo, H., Niemela, S., Nuutila, A. M., Helenius, H., Sillanmaki, L., & Almqvist, F. (2006). Childhood predictors of male criminality: a prospective population-based follow-up study from age 8 to late adolescence. Journal of the American Academy of Child & Adolescent Psychiatry, 45(5), 578-586

Tammam, J. D., Steinsaltz, D., Bester, D. W., Semb-Andenaes, T., & Stein, J. F. (2016). A randomised double-blind placebo-controlled trial investigating the behavioural effects of vitamin, mineral and n-3 fatty acid supplementation in typically developing adolescent schoolchildren. British Journal of Nutrition, 115(2), 361-373. doi: 10.1017/S0007114*

The Australian Bureau of Statistics. Foods and Nutrients, 2011-12. *Canberra, Australia: The Australian Bureau of Statistics*.

Thijssen, J. (2016). Children with externalizing behavior problems: risk factors and preventive efforts.

Tully, L. A., & Hunt, C. (2016). Brief parenting interventions for children at risk of externalizing behavior problems: a systematic review. Journal of Child and Family Studies, 25(3), 705-719. https://doi.org/10.1007/s10826-015-0284-6

Voigt, R. G., Llorente, A. M., Jensen, C. L., Fraley, J. K., Berretta, M. C., & Heird, W. C. (2001). A randomized, double-blind, placebo-controlled trial of docosahexaenoic acid supplementation in children with attention-deficit/hyperactivity disorder. *The Journal of pediatrics*, *139*(2), 189-196.

Von Schacky, C., & Harris, W. S. (2007). Cardiovascular benefits of omega-3 fatty acids. *Cardiovascular research*, *73*(2), 310-315. https://doi.org/10.1016/j.cardiores.2006.08.019

Von, C. S. (2010). Omega-3 fatty acids vs. cardiac disease--the contribution of the omega-3 index. *Cellular and molecular biology (Noisy-le-Grand, France*), 56(1), 93-101.

Waylen, A., Ford, T., Goodman, R., Samara, M., & Wolke, D. (2009). Can early intake of dietary omega-3 predict childhood externalizing behaviour? Acta paediatrica, 98(11), 1805-1808.

Appendix A

Final search terms

PUBMED			
Omega 3	Behaviour	RCT	**Child**
"fish oils" [mh] OR "fatty acids, unsaturated"[mh:noexp] OR "fatty acids, omega-3"[mh] OR fish oil*[tiab] OR omega-3*[tiab] OR cod liver oil*[tiab] OR marine oil*[tiab] OR algal oil*[tiab] OR algae oil*[tiab] OR long chain polyunsaturated[tiab] OR pufa[tiab] OR eicosapentaenoic acid*[tiab] OR epa[tiab] OR docosahexaenoic acid*[tiab] OR docosahexenoic acid*[tiab] OR **Docosahexanoate*[tiab]** OR **Docosahexaenoate*[tiab]** OR dha[tiab]	"behavior and behavior mechanisms" [mh:noexp] OR "behavior"[mh:noexp] OR "Child behavior"[mh] OR "adolescent behavior"[mh:noexp] OR behaviour*[tiab] OR behavior*[tiab] OR oppositional behav*[tiab] OR externalizing*[tiab] OR conduct*[tiab] OR disruptive behav*[tiab] OR defiant[tiab] OR anti-social behav*[tiab] OR aggressive behav*[tiab] OR aggression[tiab]	"randomised controlled trial*"[mh;noexp] OR randomized controlled trial*[tiab] OR randomised controlled trial*[tiab] OR "controlled trial"[mh] OR rct[tiab] OR rcts[tiab] OR placebo*[tiab] OR clinical trial*[tiab] OR randomise*[tiab] OR randomized*[tiab OR double blind*[tiab] OR supplement*[tiab] OR	"child"[mh] OR "adolescent"[mh] OR child*[tiab] OR adoles*[tiab] OR teen*[tiab] OR paediatric[tiab] OR Pediatric[tiab]

or fatty acid*[tiab] OR N-3[tiab]			

PsycINFO			
Fish Oil	**Behaviour**	**RCT**	**Child**
fatty acids.sh OR fish oil*.tw OR omega-3.ti,ab OR cod liver oil*.ti,ab OR marine oil*.ti,ab OR algal oil*.ti,ab OR PUFA.ti,ab OR long chain polyunsaturated*.ti,ab OR Eicosapentaenoic Acid*ti,ab OR EPA.ti,ab OR docosahexaenoic acid*.ti,ab OR docosahexenoic acid*.ti,ab OR Docosahexanoate*.ti,ab OR Docosahexaenoate*.ti,ab OR DHA.ti,ab OR Fatty acid*.ti,ab OR N-3	behavior.sh OR behavior problems.sh OR behavior disorders.sh OR behaviour*.ti,ab OR Behavior*.ti,ab OR oppositional*.ti,ab OR externalizing*.ti,ab OR conduct*.ti,ab OR disruptive*.ti,ab OR Defiant*.ti,ab OR Anti-social*.ti,ab OR aggressive*.ti,ab OR Aggression*.ti,ab	randomised controlled trial*.ti.ab OR randomized controlled trial*.ti.ab OR placebo.sh OR Controlled trial*.ti,ab OR RCT*.ti,ab OR Placebo*ti,ab + mesh OR Clinical trial*.ti,ab + PT OR Randomise*.ti,ab OR Randomize*.ti,ab OR Double blind*.ti,ab	Child*.ti,ab OR Adolescent*.ti,ab OR Teen*.ti,ab OR Paediatric.ti,ab OR Pediatric.ti.ab OR Child.ag OR

EMBASE			
Fish Oil	Behaviour	RCT	Child
"fish oil"/de OR "unsaturated fatty acid"/de OR "omega 3 Fatty acid"/de OR "docosapentaenoic acid"/de OR "docosahexaenoic acid"/de OR "fish oil*":ti,ab OR "omega 3*":ti,ab OR "cod liver oil*":ti,ab OR "marine oil*":ti,ab OR "algal oil*":ti,ab OR "algae oil*":ti,ab OR "long chain polyunsaturated*":ti,ab OR PUFA:ti,ab OR "eicosapentaenoic Acid*":ti,ab OR epa:ti,ab OR "docosahexaenoic acid*":ti,ab OR "docosahexenoic acid*":ti,ab OR "docosahexanoate":ti,ab OR "docosahexaenoate":ti,ab OR dha:ti,ab OR "fatty acid*":ti,ab OR n-3:ti,ab	"behavior"/de OR "child behavior"/exp OR "adolescent behavior"/de OR behaviour*:ti,ab OR behavior*:ti,ab OR oppositional*:ti,ab OR externalizing*:ti,ab OR conduct*:ti,ab OR disruptive:ti,ab OR defiant*ti,ab OR "anti-social*":ti,ab OR aggressive*:ti,ab OR Aggression:ti,ab	"randomized controlled trial"/de OR "randomized controlled":ti,ab OR "randomised controlled":ti,ab OR RCT:ti,ab OR RCTs:ti,ab OR "controlled trial":ti,ab OR placebo*:ti,ab OR "clinical trial":ti,ab OR randomise*:ti,ab OR randomize*;ti,ab OR "double blind*"ti,ab	child/de OR "school child"/de OR adolescent/de OR child*:ti,ab or adoles*:ti,ab OR teen*:ti,ab OR paediatric:ti,ab OR pediatric:ti,ab

Appendix B

Example Data extraction sheet

THE COCHRANE COLLABORATION®

Data Extraction Form

Review ID:	**Study ID**: Montgomery 2018	**Reference ID:** Montgomery 2018
Person extracting data (data should be extracted independently by at least 2 people**):**	**Date of date extraction:** 01/08/2018	**Year of study publication:** 2018
Title: Docosahexaenoic acid for reading, working memory and behavior in UK children aged 7-9: A randomized controlled trial for replication (the DOLAB II study).		
Author: (Montgomery, Spreckelsen, Burton, Burton, & Richardson)		
Reference: Montgomery, P., Spreckelsen, T. F., Burton, A., Burton, J. R., & Richardson, A. J. (2018). Docosahexaenoic acid for reading, working memory and behavior in UK children aged 7-9: A randomized controlled trial for replication (the DOLAB II study). PloS one, 13(2), e0192909.		
Other publications from same study (additional reports of the same study should be grouped under the same study identifier see "Organising studies and references", p 35 RevMan User Guide**):**		

Study design

Type of study design (e.g. parallel; cluster; cross-over trial)

Parallel, randomized, double-blind placebo-controlled trial

Participants and setting

Describe setting: Schools provided with a 16 week supply of capsules (labelled with each participating child's name) to dispense 3 capsules to all participating children once a day at lunch time during school terms. Parents were also given a 16-week supply of capsules dispense to their children at weekends, school holidays and at any other time when their children were not in school. Primary outcomes were assessed at baseline for all children, and again at 16-week follow-up.

Inclusion criteria: Healthy Children aged 7-9 underperforming in reading – 20th percentile

Exclusion criteria: Children with medical disorders, learning difficulties, medications which can effect learning and behaviour, eating fish 2 times per week regularly

Intervention

Experimental intervention: Fixed dose of 600 mg DHA (from algal oil), three 500mg caps per day(200 mg) each
Total number randomised: n=187

Comparison

Control/Comparison intervention: Placebo – three, taste-and colour-matched 500 mg capsules per day containing corn/soybean oil
Total number randomised: n=189

Outcomes:

Outcomes:
Behaviour (Oppositional & hyperactivity) - Conners' Rating Scale (CPRS-L) – Parent
Behaviour (Oppositional & hyperactivity) - Conners' Rating Scale (CTRS-L)- Teacher

Risk of Bias assessment
See Chapter 8 of the Cochrane Handbook. Additional domains may be added for non-randomised studies.

Domain	Risk of bias Low High Unclear	Support for judgement *(include direct quotes where available with explanatory comments)*	Location in text or source *(pg & ¶/fig/table/other)*
Random sequence generation *(selection bias)*	☒ ☐ ☐	Randomization was Independently performed by a statistician at Sealed Envelope Ltd with minimization via a 1:1 allocation ratio. "Algorithim ensured balanced allocation of participants between the treatment groups for each school (to allow for any sociodemographic/school differences)	Page 7

		and sex of the child but also included a 30% random allocation element".	
Allocation concealment *(selection bias)*	☒ ☐ ☐	Use of sealed envelopes used for allocation	Page 7
Blinding of participants and personnel *(performance bias)*	☒ ☐ ☐	Active treatment and placebo were matched for taste and appearance. Investigators, participants and those assessing outcomes were all blind to treatment allocation. Blinding was assessed post intervention for teachers and parents	Page 7 & 11
Blinding of outcome assessment *(detection bias)*	☒ ☐ ☐	Outcome group: Parent/teacher rated behaviour – Both parents and teachers were blinded to allocation "double blind"	Page 7
Incomplete outcome data *(attrition bias)*	☒ ☐ ☐	Outcome group: Parent rated behaviour(oppositional)- Post intervention 46/147 missing from intervention group, 45/156 missing from placebo. Intention to treat analysis used and analysis of attrition bias determined.	Page 16 & 8
(if separate judgement by outcome(s) required)	☒ ☐ ☐	Outcome group: Teacher rated behaviour(oppositional)- Post intervention 6/136 missing from intervention group, 6/133 missing from placebo Intention to treat analysis used and analysis of attrition bias determined.	Page 19
Selective outcome reporting? *(reporting bias)*	☒ ☐ ☐	All outcomes described in method are reported in results	Page 10-20
Other bias	☒ ☐ ☐		
Notes:			

Additional information requested

Information requested: From: Date:

Response:

Outcomes for main analysis

	Outcome Measures (Continuous)	Total number of participants in study =					
		Intervention group Total no. in group =			**Control group** Total no. in group =		
		mean	SD	total	mean	SD	total
	Primary						
1	Teacher rated Oppositional behaviour CRS	54.2	10.9	187	53.8	10.1	189
2	Parent rated Oppositional behaviour CRS	53.6	8.8	187	53.3	8.7	189
	Secondary						
4	Teacher ADHD	54.1	8.5	187	52.8	8.6	189
5	Parent ADHD	54.3	8.9	187	53.1	9.2	189

Outcomes for sub-group analyses

	Outcome Measures (Dichotomou	Total number of participants in study =			
		Intervention group Total no. in group =		**Control group** Total no. in group =	
		events	total	events	total
1	Total – sex 376	M= 235	62.5%	F = 141	37.5%
2	Male	120	64.2	115	63.2
3	Female	67	35.8	74	40.7

	Outcome Measures (Continuous)	Total number of participants in study =376					
		Intervention group Total no. in study =			**Control group** Total no. in study =		
		mean	SD	total	mean	SD	total
	Primary						
1	Age = 105.5 (10.1)	105.6	10.2	187	105.3	10.1	189
2	Free school meals = 78 (20.7)	33	17.6	187	45	24.7	189
3							

General conclusions

Very brief summary of study authors main findings/conclusions:

Replication study of DOLAB 1. Significant differences were not found for behaviour outcomes and therefore did not replicate results of original study.

This form was adapted from "Good practice templates" developed by the Cochrane Editorial Resources Committee
http://training.cochrane.org/authors/presentations/collecting-data