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**Dietary Intakes, Use of Exclusion Diets and  
Supplements in Children aged 2 ½ - 8 years  
with Autism Spectrum Disorder in New Zealand**

**A thesis presented in partial fulfilment of the requirements  
for the degree of**

**Master of Science**

**in**

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## Abstract

**Background:** Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that affects 1 in 68 children. Children with ASD are thought to be a nutritionally vulnerable population due to a tendency to exhibit eating behaviours such as selective or picky eating. Gaining popularity among parents of children with ASD is the use of complementary and alternative medical (CAM) therapies such as exclusion diets and supplements. Little is known about the dietary intakes of this population and whether they are meeting nutrition guidelines. The aim of this study was to investigate the dietary intakes and use of exclusion diets/supplements in children with ASD in New Zealand.

**Methods:** Fifty children aged 2.5-8 years old with a medical diagnosis of ASD according to the DSM-V were recruited through Waitakere District Health Board (WDHB) and autism support groups. Parents were supplied with a 4-day food diary and dietary questionnaire which was used to collect information on dietary intakes, types of exclusion diets and supplements being used, reasons for use, perceived improvements, and where parents received information from. Dietary data from the 4-day food diaries was also used to conduct a food group analysis. The number of servings from each of the food groups was compared to the Ministry of Health Food and Nutrition Guidelines for Healthy Children and Young People (aged 2-18 years) recommended daily serves.

**Results:** Children in this study were found to have carbohydrate, protein and fat intakes within the acceptable range. Dietary fibre was found to be a nutrient of concern as 40% of children were not meeting the recommendation. There were a large proportion of children not meeting the Estimated Average Requirement (EAR) for calcium (26% of children). Children were not meeting the recommended number of daily serves of fruit, vegetables or dairy. Significant differences were found when looking at dietary intakes based on exclusion diet status, where children in the exclusion diet group have significantly lower calcium intakes than children in the non-

exclusion diet group ( $p=0.03$ ). This study also found that 31% of children were using exclusion diets and 55% were using supplements.

**Conclusion:** Results of this study suggest that children with ASD are not meeting the daily recommended servings of various food groups including fruits, vegetables and dairy. Although energy intakes were not impaired, certain nutrients in the diets of children with ASD in this study were below recommended daily intakes, specifically calcium, vitamin D and dietary fibre. Children with ASD may not receive a dietetic referral unless their growth is faltering and therefore nutritional deficiencies may go unnoticed. More research is needed to determine the impact of exclusion diet and supplement use on the nutritional intakes of children with ASD.

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## Abbreviations

### Abbreviation

AI	Adequate Intake
AMDR	Acceptable Macronutrient Distribution Range
ASD	Autism Spectrum Disorder
BMI	Body Mass Index
CAM	Complementary and Alternative Medical Therapies
CDC	Centre for Disease Control and Prevention
DHA	Docosahexaenoic Acid
DMG	Dimethylglycine
DRI	Dietary Reference Intakes
EAR	Estimated Average Requirement
EPA	Eicosapentaenoic Acid
FDA	Food and Drug Administration
FFQ	Food Frequency Questionnaire
GI	Gastrointestinal
GFCF	Gluten-free, Casein-free
GP	General Practitioner
IOTF	International Obesity Task Force
LBM	Lean Body Mass
RDI	Recommended Daily Intake
LRNI	Lower Reference Nutrient Intake
MOH	Ministry of Health
MUFA	Monounsaturated Fatty Acids
NCNS	National Children's Nutrition Survey
NHANES	National Health and Nutrition Examination Survey
NHI	National Health Index
PUFA	Polyunsaturated Fatty Acids
RDA	Recommended Dietary Allowance
RDI	Recommended Dietary Intake
SCD	Specific Carbohydrate Diet
SFA	Saturated Fatty Acids

TDC	Typically Developing Children
UL	Upper Level
WDHB	Waitamata District Health Board
WPPSI	Wechsler Preschool and Primary Scale of Intelligence

## **1.0 Introduction**

### **1.1 Background**

#### **1.1.1 Autism Spectrum Disorder**

Autism spectrum disorder (ASD) is a complex life-long neurodevelopmental disorder that is characterised by impairments in social and communication ability, as well as restrictive and repetitive patterns of behaviour (American Psychiatric Association, 2013). While these features are characteristic of people diagnosed with ASD, there are a range of symptoms and degrees of severity related to the condition. Given this heterogeneous nature of the condition, a diverse range of treatment options is required. Although no New Zealand specific data exist, the Centre for Disease Control and Prevention (CDC) reports the prevalence of ASD to be 1 in 68 children (CDC, 2014) in the United States. The latest guidelines from the Ministry of Health (MOH) estimate approximately 40,000 individuals in New Zealand have the condition, although many may not have received a formal diagnosis (Ministry of Health, 2008). There is no clear aetiology or known cure for ASD. However, the earlier diagnosis is made in childhood the more impact an intervention can have, potentially resulting in fewer challenging behaviours and better outcomes for families (Szatmari et al., 2003). Treatment with conventional methods usually includes intensive educational intervention, developmental therapies and behavioural treatment (Myers & Johnson, 2007).

Parents of children with ASD may face a unique set of challenges when it comes to ensuring their child is receiving adequate nutrition which is essential for good health, growth and development. Challenges that may impact the dietary intakes of children with ASD relate to the tendency of children with ASD to exhibit eating behaviours such as selective or picky eating and sensory sensitivity (Schreck et al., 2004). Although there is a wealth of information regarding diet and nutrition in typically developing children, there is limited evidence available in this area pertaining to children with ASD. From the few studies available which have compared dietary intakes of children with ASD to typically developing children, the majority have

examined variations in intake, unusual eating patterns (Marí-Bauset et al., 2013), gastrointestinal abnormalities (McElhanon et al., 2014) and high use of alternative therapies such as exclusion diets and supplements (Owen-Smith et al., 2015).

### **1.1.2 Symptoms Associated with ASD that may Impact on Dietary Intake**

#### *Selective eating*

Selective eating is characterised by a lack of dietary variety and inadequate consumption of nutritious foods such as fruits and vegetables, lean protein-rich foods, and wholegrain cereals providing dietary fibre and other nutrients (Dovey et al., 2008). Although this eating pattern is frequently seen in typically developing children, it appears to be more prevalent in children with ASD (Bandini et al., 2010; Cermak et al., 2010; Emond et al., 2010). Symptoms of ASD including a tendency to focus on detail, behavioural rigidity, sensory impairments and communication deficits have been postulated as the cause of selective eating in this population (Ahearn et al., 2001). Children with ASD who exhibit selective eating have been found to have an increased sensitivity to specific flavours, textures, colours and sounds (Cornish, 1998; Schreck et al., 2004) It is not uncommon for a child to fixate on a particular sensory attribute of a food and as a consequence demand to eat only that food (Cermak et al., 2010) or to exclude foods based on textures. As a consequence, children exhibiting these symptoms may have a restricted diet.

A child with selective eating may consume sufficient energy for growth but still be at risk of micronutrient deficiencies. If a child is not faltering in their growth trajectories then these micronutrient deficiencies may go undetected. In New Zealand children with ASD do not meet the criteria for referral to a dietitian unless there is an underlying clinical problem such as growth faltering or diagnosed micronutrient deficiency. As a result, they may not be referred to a dietitian or other health professional and any subtle nutritional deficiencies may go unnoticed (Emond et al., 2010).

#### *Gastrointestinal symptoms*

Children with ASD experience a range of gastrointestinal symptoms which include constipation, gaseousness, diarrhoea, indigestion, reflux and vomiting (Buie et al., 2010). Based on high numbers of children with ASD presenting with gastrointestinal

symptoms at clinics, it has been proposed that the prevalence of gastrointestinal disturbances among this population is high (Horvath et al., 1999). The reasons why gastrointestinal issues may occur more frequently in children with ASD is not well understood (NICE, 2011), however one theory is that children with ASD may be genetically predisposed to have defects in their gut mucosa causing abnormal intestinal permeability (Horvath & Perman, 2002). This condition has been referred to in the literature as leaky gut syndrome (White, 2003). Evidence of the increased gastrointestinal problems among children with ASD has been cited as a rationale for the use of exclusion diets and supplements which aim to ameliorate these symptoms (Jyonouchi et al., 2002; Whiteley et al., 2010).

### **1.1.3 Complementary and Alternative Medical Therapies**

The use of exclusion diets and supplements without a medically diagnosed allergy or nutrient deficiency is commonly referred to as complementary and alternative medical therapies (CAM). The use of CAM therapies are described by the American Academy of Paediatrics as “strategies that have not met the standards of clinical effectiveness, either through randomised controlled trials or through the consensus of the biomedical community“ (American Academy of Pediatrics, 2001). Given that conventional treatment is not a cure for ASD and does not always produce the desired effects, many parents turn to alternative therapies to reduce the symptoms of the disorder in their children (Levy & Hyman, 2005). A review of the literature or popular media reveals that there are a vast number of alternative treatments that propose to be effective for people with ASD. This abundance of treatment alternatives presents a challenge to parents in deciding on which one to use, and some families are adopting more than one alternative therapy (Levy & Hyman, 2015). Popular CAM therapies used by adults and children with ASD include exclusion diets such as the gluten and casein free diet (GFCF) and a variety of dietary and non-dietary supplements including multi-micronutrient supplements, melatonin and probiotics. Although these treatments have supporters who advocate their benefits, currently there is little scientific evidence to support their use (Green et al., 2006).

### *The gluten-free, casein-free diet*

The gluten-free, casein-free diet is one of the most commonly used exclusion diets. This diet involves eliminating all food items containing the proteins gluten (found in wheat, rye and barley) and casein (found in dairy products - milk, yoghurt, cheese, butter, cream and ice cream) from the diet. The “Opioid Excess Theory” is based on the hypothesis that some autistic symptoms may be a result of the opioid peptides crossing the intestinal mucosa intact (Reichelt, 1990; Shattock et al., 1990).

It is proposed that conditions such as the “leaky gut syndrome” allow these peptides to enter the blood stream and cross the blood-brain barrier which affects the endogenous opiate system that operates within the central nervous system leading to behavioural abnormalities (Panksepp, 1979; Shattock et al., 1990). Elimination of the proteins gluten and casein is said to result in an improvement in behaviours associated with ASD. Evidence to support the use of the GFCF is limited to case reports (Knivsberg et al., 1999; Whiteley et al., 1999) and small open label cohort studies (Knivsberg et al., 1990; Reichelt, 1990).

### *Impact of the gluten-free, casein-free diet*

Exclusion of foods from both the cereal/bread and dairy food groups has the potential to impact on the nutritional adequacy of the diet, particularly if this exclusion diet is not supervised by a dietitian. Children with ASD on a GFCF diet have been found to have lower plasma amino acid profiles which is suggestive of poor protein intake (Arnold et al., 2003). As casein free diets require the removal of all dairy products, which are an excellent source of calcium, they are associated with lower dietary calcium intakes (Knivsberg et al., 2001). From a paediatric perspective the use of the GFCF and the subsequent low intakes of dairy or fortified dairy alternatives may have a negative impact on bone development in young children (Hediger et al., 2008).

Parents have reported they feel they would rather explore all treatment avenues that seem to do no harm especially if the treatment is easy to adopt, requires little time and is widely accepted (Elder, 2008; Green et al., 2006). Food and nutrition guidelines for children in New Zealand promote eating a variety of foods to ensure that nutritional requirements are met (Ministry of Health, 2012). When exclusion diets



require food groups to be excluded such as dairy products and bread/cereal products, there is likely to be an impact on the intakes of key nutrients provided by these food groups. Although parents may perceive exclusion diets to be safe there is the potential for nutritional inadequacies and associated health outcomes if they are not supervised by a dietitian.

### *Use of supplements*

Vitamins and minerals are micronutrients that are required in small amounts for normal growth and development (Mahan et al., 2012). Consuming a healthy diet can usually supply the body with adequate amounts of vitamins and minerals with the exception of vitamin D, which for most children is obtained through exposure to sunlight. The Food and Nutrition Guideline statements for children aged 2-18 include the advice to “eat a variety of foods from each of the four major food groups each day” (Ministry of Health, 2012) which should provide a growing child with all the nutrients they require. Where this is not possible, for example when a child is on a restricted diet or has a food allergy, a supplement may be necessary (Ministry of Health, 2012). Recent studies show that 30% of parents who have children with ASD give their children extra vitamins, including vitamin C and B<sub>6</sub>, and over 25% are supplementing their children with essential fatty acids (such as DHA and EPA) and magnesium (Green et al., 2006). In the same study, more than 10% of parents report the use of vitamin A, mega-vitamin therapy, dimethylglycine (DMG) and L-glutamine. There is a possibility that parents may be choosing multiple supplements. A recent study showed children with ASD (n=288) who were on supplements, an average of two supplements were taken per day (range 0.3–13 supplements; median 1 supplement) (Stewart et al., 2015).

A major concern about supplement use without the supervision of a medical professional is that the child's intake may be exceeding the Upper Level (UL) for the supplemented nutrient - the highest recommended intake above which there is a risk of toxicity (National Health and Medical Research Council, 2006). Mega-dose vitamin therapy and the use of other nutritional supplements are common among children with ASD (Green et al., 2006). High doses of single fat-soluble vitamins such as vitamin A and D have the potential to cause toxicity. Hypervitaminosis, which primarily affects the fat soluble vitamins such as vitamins A and D, refers to the

condition where abnormally high levels of vitamins are stored in the body which can lead to toxic symptoms. The Food and Nutrition Guidelines for Healthy Children and Young People aged 2-18 years recommend if dietary supplementation is necessary it should be done so under medical supervision to avoid any adverse effects and should be based on the individuals requirements (Ministry of Health, 2012).

The use of multivitamin/mineral supplements may be reinforced by medical professionals if parents express concerns about eating behaviours which impact on dietary intake. Nearly 50% of medical professionals reported that they would recommend a multivitamin/mineral supplement to parents of children with autism if their child exhibited eating behaviours that could lead to nutrition deficiencies (Golnik & Ireland, 2009). The use of high dose nutritional supplements, beyond those prescribed for correcting nutritional deficiencies is not supported by any conclusive scientific evidence and further studies are required.

There are children with ASD who will have a diagnosed food allergy or nutritional deficiency that will require an exclusion diet or dietary supplement as medical treatment. However, for children that do not, it remains unproven whether CAM therapies such as the use of exclusion diets and supplements will be effective in ameliorating symptoms of ASD (Akins et al., 2010). It is apparent that parents are being left to navigate their way through vast amounts of information, controversial therapies and having to deal with a range of recommendations set out by popular media with little or no evidence to support their use.

## **1.2 Purpose of the Study**

It remains unknown whether children with ASD are a nutritionally vulnerable population (Ranjan & Nasser, 2015). This observational study will provide evidence on the dietary intakes, use of exclusion diets and supplements in a subgroup of children with ASD participating in a larger research study (The VIDOMA trial). To our knowledge there is no research pertaining to the dietary intakes of children with ASD living in New Zealand. There is also a gap in the research regarding the use of exclusion diets and supplement use of children with ASD in New Zealand.

This study will be the first to provide information regarding dietary intakes, exclusion diets and supplement use of children with ASD living in New Zealand. This thesis will also report on the types of exclusion diets/supplements being used and their perceived benefits alongside where parents are obtaining their information about exclusion diets and supplements. The findings from this study may highlight the need for dietitians and other health care professionals to engage with parents if they are considering the use of either exclusion diets or supplements for their child with ASD.

### **1.3 Aim and Objectives**

#### **1.3.1 Aim**

To investigate the dietary intakes and use of exclusion diets/supplements in children with ASD.

#### **1.3.2 Objectives**

- To assess dietary intakes of children with ASD aged 2 ½ to 8 years.
- To compare dietary intakes of children with ASD to current dietary recommendations.
- To determine use of exclusion diets and supplements in children with ASD.
- To determine reasons for use of exclusion diets and/or supplements.
- To investigate whether being on an exclusion diet adversely affects dietary intakes of children with ASD.

### **1.4 Hypothesis**

1. That children with ASD will not be meeting current dietary recommendations.
2. That children with ASD will be using a range of different types of exclusion diets and/or nutritional/non nutrition supplements.
3. That parents will use supplements and exclusion diets for their child to ameliorate symptoms of ASD.

4. That children on exclusion diets will not be meeting current dietary recommendations.

### **1.5 Structure of the Thesis**

This thesis is presented in six chapters. The first chapter introduces the topic and the importance of the research. The topics in this literature review include an overview of the research pertaining to potential causes and current treatments of ASD, an overview of the studies relating to nutritional intake of children with ASD, factors affecting the nutritional status of individuals with autism, and an overview of commonly used diets and supplements in children with autism. Chapter 3 will provide a detailed description and a justified description of the methods used in the study. Chapter 4 reports the results of the study which will then be interpreted and discussed in chapter 5. Conclusions will be made in Chapter 6, along with the strengths, limitations and future recommendations.

## 1.6 Researchers Contributions

**Table 1.1** *Researchers contribution to the current study*

<b>Author</b>	<b>Contribution</b>
Aimee Waring	Led the study, developed the 4-day food diary and dietary questionnaires, data entry of the 4-day food diary and questionnaires, data analysis, statistical analysis, interpretation of results and prepared the thesis manuscript
Dr Cath Conlon	Academic supervisor, assisted with the development of 4-day food diary and dietary questionnaire, revised and approved all chapters of this thesis including overseeing its preparation and editing
Dr Pamela Von Hurst	Academic supervisor, designed the research including the initial concept, applied for ethics, assisted with development of 4-day food diary and questionnaire, revision of literature review
Dr Cheryl Gammon	Assisted in statistical analysis and final editing of results section
Dr Louise Brough	Assisted in the final editing of all chapters
Micaela Makker	Assisted with 4-day food diary and dietary questionnaire design, assisted with data entry of 4-day food diary and questionnaires
Owen Mugridge	Research co-ordinator, data collection, main contact for parents
Hajar Mazahery	Data collection, reviewed methods section of thesis

## **2.0 Literature Review**

### **2.1 Introduction**

This chapter provides the background information to the study including a definition of Autism Spectrum Disorder and its diagnosis, potential causes of the condition and an overview of current treatment options. Currently available evidence on the dietary intakes and nutritional status of children with ASD is reviewed in detail. In addition studies which have investigated the use of exclusion diets and/or supplements are also reviewed.

The following online databases were systematically searched for relevant literature: PubMed, Google Scholar and Web of Science. The publication period ranged from 1979 to 2015. The key search terms relating to the population were autism, autism spectrum disorder, Asperger's disorder, and children. These key terms were used in combination with the two functions; 'AND' 'OR' which included: diet, exclusion diet, supplements, dietary intake, nutrient, feeding, eating and nutrition. Full text articles in English that matched search criteria were reviewed. A manual search was also undertaken using reference lists from relevant and recent articles, Ministry of Health Food and Nutrition Guidelines for Healthy Children and Young People (aged 2-18 years), and the Diagnostic and Statistical Manual of Mental Disorders (5<sup>th</sup> Edition) DSM-V.

### **2.2 Autism Spectrum Disorder (ASD)**

Autism spectrum disorder (ASD) is a complex life-long condition that affects an individual's social, communicative and cognitive development. The condition is characterised by two domains: 1) deficits in social communication and interaction and 2) restrictive, repetitive patterns of behaviour (American Psychiatric Association, 2013). Deficits in both domains are required for a diagnosis of ASD. Throughout the literature these characteristics are commonly referred to as the "core features" of ASD. Although these core features are characteristic among all people with ASD, there are varying degrees to which the disorder manifests. Where some individuals may be severely impaired, lacking any means of communication, others may be

considered high-functioning without language deficits, a term which has been used to describe people with ASD who have an IQ of greater than 70 (Sanders, 2009). This term can be misleading however, given that activities of daily life and social interactions may not be determined by intellectual functioning. Because the disorder presents itself differently within each individual, attempts at treatment can pose many challenges.

### **2.2.1 Onset**

The onset of ASD has been studied extensively and it is generally accepted that a clinical diagnosis may occur much later than the onset of symptoms (Ozonoff et al., 2008). The disorder usually begins in infancy, most commonly before three years of age and is usually brought to the attention of parents when their child cannot use words to communicate, although they can recite passages from videotapes or alphabets. At first the deficit in social development may not be obvious; however it may become more apparent as normally developing children advance and become more socially sophisticated. Young children with autism do not seek out contact with other children, do not call their parents by name and rarely point to objects of interest (Lord et al., 2000). Children with ASD, especially those with a mild form or limited speech delay, may not be diagnosed until a later stage when parents become concerned that their child is not reaching typical milestones of school age children (Johnson & Myers, 2008). Early detection of the condition has been acknowledged as a major advance in that it enables prompt intervention and may improve the prognosis in children with ASD (Goldstein, 2002).

### **2.2.2 Diagnosis**

The diagnostic assessment of ASD is made according to the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-V) and is presented in **Table 2.1** (American Psychiatric Association, 2013). The essential features that must be present are impairments in social communication and interaction (Criterion A) and restrictive, repetitive patterns of behaviour, interests or activities (Criterion B). The core features of ASD are present from early childhood and will exhibit effects on everyday functioning (Criterion C and D). The way each of these core features manifests has been described as autistic symptoms. For example, deficits in social communication and interaction will have associated symptoms such as reduced sharing of interests, non-verbal communicative behaviours, or in developing,

maintaining and understanding relationships. From the restricted, repetitive behaviours domain – stereotyped or repetitive motor movements, insistence on sameness and hyperactivity may be present. When diagnosing the condition, the medical professional will look for the core features of ASD, and then note any autistic symptoms as evidence for the disorder. Criterion E details that social communication deficits, which could also be accompanied by an intellectual disability, must not be better explained by the co-existing intellectual disability.

**Table 2.1** *Diagnostic criteria according to the DSM-V Manual*

Criterion	Examples
A. Persistent deficits in social communication and social interaction across multiple contexts	Reduced sharing of interests, emotions or failure to respond to social interactions
	Deficits in non-verbal communicative behaviours for social interaction including poorly integrated verbal and non-verbal communication
	Difficulty in developing and maintaining relationships
B. Restricted, repetitive patterns of behaviour	Stereotyped or repetitive motor movements, use of objects of speech
	Insistence on sameness, reliance on routines or ritualized patterns of verbal or non-verbal behaviour
	Highly restricted, fixated interests
	Hyper- or hyperactivity to sensory input or unusual interest in sensory aspects of the environment
C. Symptoms must be present in early developmental period, but may not manifest until later in life	
D. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functions	
E. These disturbances are not better explained by intellectual disability	

Source: (American Psychiatric Association, 2013)

### 2.2.3 Conventional Treatment Methods

The first step for treatment of ASD involves a comprehensive assessment (Volkmar et al., 2014). During this assessment information will be gathered on behavioural, emotional and mental health issues. Given that no one option works for all people with ASD, treatment is most appropriate when based on the comprehensive and on-going assessment of that individual (Volkmar et al., 2014). Conventional treatment of ASD involves a combination of educational therapies, medicines or both (Myers & Johnson, 2007).



Educational therapies focus on improving behaviour, communication, and social responsiveness and remain the most commonly used treatments for ASD. Facilitating learning and development, and educating and supporting families can help to achieve these goals (Myers & Johnson, 2007). Early behavioural therapy has been shown to improve learning, communication and social skills (Goldstein, 2002; Lorimer et al., 2002) and also reduce maladaptive behaviours (Campbell, 2003; Horner et al., 2002). The children require help to develop skills to interact with and understand others, as well as tolerate change.

Lovaas (1987) conducted a study with a group of 19 preschool-age children with autism and found that after an intensive behavioural intervention the children achieved normal intellectual and educational functioning and higher IQs than a group of 19 other children with autism by age seven. McEachin et al. (1993) followed up this study, where they assessed the same children at a mean age of 11.5 years. Results showed that the children receiving the behavioural intervention had maintained their advances achieved in the initial study. Thus, the authors concluded that intensive programmes that involve 40 hours of structured input to the child each week may produce long-lasting significant gains for children with autism. Although the study numbers were small, Lovaas and his colleagues were able to bring a new approach to treating autism and as a result prompted schools to develop specialised programmes that differ greatly from those designed for other developmental disorders (Rogers & Vismara, 2008).

Pharmacological approaches, rather than being a cure for ASD, act more to improve some of the symptoms that coincide with the disorder (Buitelaar & Willemsen-Swinkels, 2000). Although no medications are currently Food and Drug Administration (FDA) approved to treat the disorder itself, their use is largely aimed at reducing autistic symptoms such as hyperactivity, or self-injurious behaviour, rather than the core features of ASD. There are very few well-controlled studies which examine the effectiveness of medications in ASD, therefore many of the recommendations are based on expert opinions and short-term studies (Ministry of Health, 2008). Autistic symptoms such as hyperactivity and impulsiveness are often treated by stimulants such as methylphenidate (Ritalin).

Selective serotonin reuptake inhibitors (SSRIs) are prescribed for the treatment of conditions often comorbid with ASD such as depression, anxiety and obsessive-compulsive behaviours. A Cochrane Systematic review assessed nine randomised controlled trials evaluating various SSRI's (fluoxetine, fluvoxamine, fenfluramine and citalopram). Although one large, high-quality study in adults showed positive outcomes the efficacy of these treatments remains uncertain (Williams et al., 2013).

#### **2.2.4 Prevalence**

While there is no New Zealand specific data regarding the prevalence of ASD, the Centre for Disease Control and Prevention (CDC) reports a total prevalence of 1 in 68 children in the US (CDC, 2014). Boys are affected approximately four times more frequently than girls (Fombonne, 2003). A possible explanation for this gender imbalance could be that females with autism tend to be more likely to have an accompanying intellectual disability (Mandy et al., 2012). It has been suggested that girls who do not have intellectual disability or language delays may go unrecognised due to a subtler manifestation of the condition (Rivet & Matson, 2011).

Autism Spectrum Disorder cannot yet be tested for bio-medically, therefore there are questions around the accuracy of diagnosis, and subsequent prevalence rates (Matson & Kozlowski, 2011). According to data released from the CDC, there was an approximate four-fold increase in patient-reported ASD cases between the 1997-1999 and 2006-2008 surveillance period (Boyle et al., 2011). Further, the Autism and Developmental Disabilities Monitoring (ADDM) Network sites, reported a 78% increase in ASD between 2002 and 2008 (CDC, 2012). It is not clear whether this is due to an actual increase in incidence, increased awareness (Wing & Potter, 2002), inaccurate diagnosis (Barbaresi et al., 2009), or changes in the way the diagnostic criteria are interpreted (Matson & Kozlowski, 2011). Autism Spectrum Disorder is frequently discussed in the media, particularly with the unsubstantiated claims of vaccinations being a trigger to the disorder, and as a result parents are becoming more aware (Wing & Potter, 2002). Another potential cause of the increase in prevalence has been due to inaccurate diagnosis. Barbaresi et al. (2009) compared incidence of clinical diagnoses versus research-identified autism in a population of children and teenagers with autism. They reported a 22.1 fold increase in clinically-derived diagnoses from 1995 to 1997, yet only an 8.2 fold increase in research-identified autism cases.

Among one of the most commonly cited explanations for the large increase in diagnosed cases of ASD in recent years is the changing diagnostic criteria for ASD (Williams et al., 2005; Wing & Potter, 2002). Autism was not a named disorder until 1943 when Dr. Leo Kanner, a psychiatrist, defined the condition (Kanner, 1943). However, it was not until the Diagnostic and Statistical Manual of Mental Disorders, Third Edition (DSM-III) emerged in 1987 that formal diagnostic criteria became available (Matson & Kozlowski, 2011). As a result all diagnoses prior to this point were not assigned according to the same criteria. Each time the Diagnostic and Statistical Manual has been revised, more previously separate diagnostic categories have been added such as Asperger's disorder, Pervasive Developmental disorder and Not Otherwise Specified (PDD-NOS) thus increasing the possibility for an increase in the number of autism diagnoses due to misinterpretation (Williams et al., 2005; Wing & Potter, 2002).

### **2.2.5 Pathophysiology of ASD**

#### *Genetics*

Recent research suggests that there is a strong link between genetics and the causation of ASD. There have been a number of studies that have assessed the extent to which genetic factors influence the development of ASD (Bolton et al., 1994; Constantino et al., 2010; Sumi et al., 2006). Due to the large difference in concordance rates between monozygotic and dizygotic twins, autism is considered to be one of the most heritable of all neurodevelopmental disorders (Rosti et al., 2014; Sandin et al., 2014). Hallmayer et al. (2011) conducted a study to estimate the genetic heritability of autism and the effects of a shared environment. They assessed twin pairs with at least one twin with an ASD. From the results, they concluded susceptibility to ASD has moderate genetic heritability and a substantial shared twin environmental component. A later study by Sandin et al. (2014) involved a population-based cohort of Swedish children (n=2, 049, 973). The main outcome measure was the relative recurrence risk (RRR) that measures the risk of autism in a person with a sibling or cousin who has ASD compared with the risk of a person with no diagnosed family member. They found that for monozygotic twins the relative risk of autism was estimated to be 153.0; 8.2 for dizygotic twins and 10.3 for full siblings; 3.3 for maternal half siblings; 2.9 for paternal half siblings; and 2.0 for cousins. The authors conclude that among children born in Sweden, the risk of ASD increases

with genetic relatedness. Fragile X syndrome, a genetic disorder, is the most common single gene cause of autism (Hagerman et al., 2010). As many as 30-50% of individuals diagnosed with Fragile X syndrome will demonstrate some characteristics of ASD (Demark et al., 2003). Future directions for genetic studies and autism lie in identifying specific gene-environment interactions (Damiano et al., 2014).

### *Structural Abnormalities*

The use of magnetic resonance imaging (MRI) techniques has been used to identify structural abnormalities within the cerebral cortex, brainstem, cerebellum and many other parts of the limbic system to be identified in individuals with ASD (Amaral et al., 2008). Findings in neuroanatomical and neuroimaging tests, although they are not diagnostic, reveal an increased cerebral volume that affects both grey and white matter in the brain (Stanfield et al., 2008). Irregularities have been found in several regions within the brain of people with ASD (Amaral et al., 2008). Stanfield et al. (2008) conducted a systematic review on MRI studies of regional brain size in autism. The results showed that in people with ASD, total brain, cerebral hemispheres, cerebellum and caudate nucleus were increased in volume, whereas the corpus callosum area was reduced. They concluded that ASD may result from abnormalities in specific brain regions as a lack of integration due to brain enlargement. Increased total cerebral grey and matter in children with ASD was also found in later studies (Hazlett et al., 2011; Schumann et al., 2010). The cause of rapid brain growth in autism is unknown, however various mechanisms have been proposed including increased neurogenesis, decreased neuronal death or increased projection of non-neuronal brain tissue, which could include glia or blood vessels (Buitelaar & Willemsen-Swinkels, 2000).

### *Serotonin*

There is increasing evidence that people with autism may have serotonin levels outside of normal levels (Raznahan et al., 2009). Serotonin (5-HTP) is a neurotransmitter that serves numerous physiological and behavioural functions, modulating virtually all human biological processes such as cardiovascular function, bowel motility and bladder control (Berger et al., 2009). The behavioural and neuropsychological processes modulated by serotonin include appetite, memory, mood, perception, anger and reward to name a few. Biochemically, 5-HTP is derived

from tryptophan and found primarily in the gastrointestinal tract, blood platelets and central nervous system. A gene which makes one more susceptible for ASD has been studied and shown to be linked to GI problems. This gene encodes the integral membrane transporter to the neurotransmitter serotonin 5-HTP (Sutcliffe et al., 2005; Weiss et al., 2005). The coding variants within these genes, have been identified as risk factors for ASD as they result in an overstimulation of serotonin transporter activity leading to hyperserotonaemia, or increased blood serotonin. Hyperserotonaemia is seen in approximately 30% of individuals with ASD (Schain & Freedman, 1961). Autism spectrum disorder associated polymorphisms of this kind are likely to disrupt GI serotonin metabolism as the endocrine cells of the GI tract produce over 90% of the body's serotonin and enterocytes are known to express 5-HTP (Molloy et al., 2006). The consequence of these changes to normal function, would be a disruption of serotonin metabolism and subsequent serotonin-related processes within the GI tract such as regulation of the mucosal immune response, and the enteric reflex activations that are responsible for maintaining gut motility, secretion and sensation (Hsiao, 2014). Altered serotonin signalling has been associated with other conditions such as irritable bowel syndrome, irritable bowel disease and idiopathic constipation (Bakkaloglu et al., 2008; Gershon & Tack, 2007).

### *Environmental factors*

The number of ASD cases being diagnosed has increased in recent years (Autism and Developmental Disabilities Monitoring Network, 2012) which is thought to be primarily a result of changing diagnostic criteria and improvements to case ascertainment. However, according to Rutter (2005) and Weintraub (2011) environmental factors should not be ruled out as a potential cause to the increase in new cases. Kolevzon et al. (2007) identified prenatal and perinatal characteristics such as advanced maternal and paternal age, and maternal place of birth outside Europe or North America as risk factors for the increased occurrence of ASD. The researchers concluded that although not proven as independent risk factors, parental age and obstetric conditions are associated with an increased risk of autism.

## **2.3 Nutritional Intake and Status in Children with ASD**

Children with autism are potentially a nutritionally vulnerable population because of their tendency to be selective eaters (Lockner et al., 2008; Schreck et al., 2004) and

this may be further attenuated by the use of an exclusion diet which restricts certain food groups. A comprehensive nutritional assessment in any population requires anthropometric measures and dietary assessment. These procedures can be challenging in the paediatric ASD population and there is very limited evidence on the nutritional assessment of these children.

### **2.3.1 Anthropometric Measures**

Unusual eating behaviours and use of restrictive diets may imply risks of both excessive and insufficient intake, which could lead to anthropometric measurements outside of the normal range (Marí-Bauset et al., 2015). Hyman et al. (2012) conducted a large study in the USA assessing children with ASD (n=362) and compared with a matched National Health and Nutrition Examination Survey (NHANES) population by body mass index (BMI) category. Children with ASD in the 2-5 year old age group were more likely to be overweight ( $p<0.05$ ) or obese ( $p<0.001$ ) than the matched NHANES sample. For the age group 6-11 years, children with ASD were more likely to be underweight than the matched NHANES sample ( $p<0.05$ ). They also assessed use of restricted diets and found children on a restricted diet were more likely to be underweight than children not on a diet ( $p=0.02$ ).

### **2.3.2 Nutritional Intake**

Dietary diversity is important to ensure that children obtain all the nutrients they require from their diet but selective eating traits often seen in children with ASD may result in a compromised nutritional intake. Despite this concern, there is limited research investigating nutritional intake in typically developing children compared to children with ASD (**Table 2.2**). From the research which is available (**Table 2.2**) it is difficult to compare studies and results are often conflicting. In some studies comparisons are made to the Estimated Average Requirement (EAR) where others will use the Recommended Dietary Allowance (RDA) or Recommended Dietary Intake (RDI). This presents a challenge when attempting to interpret the outcomes of each study.

Some studies have reported no difference between the nutritional adequacies of children with ASD versus typically developing children. In a cross sectional

descriptive study by Lockner et al. (2008) nutrient intakes of children with ASD (n=20) were compared with a group of children without the disorder (n=20) using 3-day food record (**Table 2.2**). The results showed similar nutrient intake between the two groups. Vitamins A, E, dietary fibre and calcium when compared with EAR or AI were low, however they were low in both groups. The study did show children with ASD in their study were more likely to consume a vitamin mineral supplement which would suggest these children might have better intakes, however supplements were not included in the analysis. The authors conclude the results can be considered preliminary due to the small sample size, and therefore more research would be needed before generalised statements could be made about the nutritional intake of children with ASD.

Two older studies also report no difference in nutrient intake between children with ASD and without (Raiten & Massaro, 1986; Schreck et al., 2004; Shearer et al., 1982). The study by Raiten and Massaro (1986) (**Table 2.2**) involved using food records to compare the nutritional intake of children with ASD (n=40) and non-autistic children (n=34) over a 7 day period. Children with ASD had significantly greater intake of protein, carbohydrates, niacin, thiamin, riboflavin, calcium, phosphorus, and iron. The authors report there was no difference between the two groups in terms of nutritional adequacy. This study has various flaws including that the sample size was also too small (n=74) to make generalisations to the wider population. Furthermore, the ASD group had a greater mean age ( $10.6 \pm 4.3$  years) than the control group ( $8.8 \pm 4.8$ ), as well as having a greater proportion of males, 70% versus only 56% in the control group. These factors could have skewed the results to show greater nutrient intake in the ASD group. Not only that, it was noted that 38% of ASD subjects and 30% of the control subjects stated that they were regularly taking a multivitamin which would contribute to their nutrient intake, however this was not included in the analyses. They also did not compare these figures to any established reference standards rather solely to the control group, which could be considered another flaw in the study.

Ho et al. (1997) (**Table 2.2**) examined the nutrient intake of 54 school age Canadian children with ASD. Using 3-day food diaries to examine the intakes of energy, carbohydrate, vitamin and mineral content, comparisons were made between these nutrients and Canadian nutrition guidelines. In contrast to Raiten's findings where



children with ASD consumed adequate nutrients, only four children in this study met the recommended servings of each food group. All subjects had adequate protein consumption and on average consumed more carbohydrate foods than the typical Canadian child's diet. This was not the same for the fruit and vegetable consumption, where the subjects all consumed low amounts of these, indicating diets may be high in meats and carbohydrate foods, but low in fruit and vegetables. Another point of interest showed 61% of participants had mismatched supplement regimes – meaning that they were taking supplements even though these nutrients were being received in adequate amounts through diet alone. Similarly, where nutrients were not being consumed in adequate amounts via diet, no supplements were being taken to correct the shortfall.

Schreck et al. (2004) conducted a study where they asked parents of children with ASD (n=138) and parents of typically developing children (n=298) to complete a food preference inventory that indicated the number of foods eaten within each food group for both the child and the family (**Table 2.2**). These foods were broken down into dairy, fruits, vegetables, protein and starch. The autism group consumed the most foods from the starch group, followed by fruits and protein. Minimal foods were consumed from the dairy and vegetable groups. The control group consumed nearly double the amount of foods from all food groups when compared with the autism group. Interestingly, these food choices did not extend to the families of the children with ASD, where the families of the two groups had similar eating patterns. The shortfalls of this study are that they did not acquire food records therefore an analysis of macro and micro nutrients and nutritional adequacy could not be carried out.

In a study carried out by Schmitt et al. (2008) (**Table 2.2**) 3-day food records were used to compare nutrient intakes of boys with (n=20) and without autism (n=18). The study defined adequate intake as being greater than 67% of the Dietary Reference Intake (DRI). Both groups had adequate energy, carbohydrate, fat and protein intakes. However both groups also consumed below 67% of the DRI for fibre. Although no significant differences were found between groups, children with autism consumed below 67% of the reference values for vitamin E and K.



Johnson et al. (2008) (**Table 2.2**) found no differences for macro and most micronutrients, between children with ASD (n=19) and typically developing children (n=15). Vitamin K and vegetable intake were found to be significantly lower in children with ASD. Although these results indicate differences in the nutritional intake between children with ASD and typically developing children, the small sample size should be noted. Also, the study did not take into consideration the variability of intake across several days of the week given that only a food frequency questionnaire and 24-hour recall was used. Similarly, Whitney Evans et al. (2012) found children with ASD (n=53) consumed significantly fewer servings of vegetables than typically developing children (n=58). Children with ASD also consumed less fruit, and had significantly more servings of sweetened beverages.

A larger study carried out by (Hyman et al., 2012) examined 3-day food records for children aged 2-11 year with ASD (n=252) and compared data against the National Health and Nutrition Examination Survey (NHANES) where they matched for age, gender, family income and ethnicity. Children with ASD had lower intakes of energy, vitamin A and C, zinc and phosphorus when compared with the NHANES population ( $p < 0.05$ ). Mean macronutrient intakes of children with ASD were found to be within the acceptable macronutrient distribution range (AMDR) for children. Vitamin D intake was found to be insufficient for age group 1-3 years (86% not meeting EAR) and the 4-8 year olds (89% not meeting EAR). They were unable to compare these findings to NHANES data due to differences in analysis methods (NHANES used Average Intake (AI) as opposed to the Estimated Average Requirement (EAR)). Mean fibre intakes were low for each age group including 1-3 year olds (13.2g) and 4-8 year olds (11.8g). Potassium intakes were also low for 1-3 year olds (1.9mg) and 4-8 year olds (0.1mg). Low intakes for both fibre and potassium were consistent with NHANES data. Although this study was able to obtain larger participant numbers than other studies, there were limitations. The study asked for volunteers to participate, as a result parents who were more concerned about the nutritional intake of their child may have entered the study, thus presenting a potential bias.

**Table 2.2** Studies investigating nutritional intake in children with ASD

Author and year	Type of study	Children with ASD (n)	Control (n)	Dietary assessment method	Results
(Raiten & Massaro, 1986)	Observational Cross sectional	40	34	7-day food record	Children with ASD=significantly greater ( $p<0.02$ ) intake of protein, carbohydrate, niacin, thiamine, riboflavin, calcium, phosphorus, iron Fat, vitamin A, vitamin C did not differ between groups Children with autism=significantly greater caloric intake ( $p<0.02$ )
(Ho et al., 1997)	Observational Cross sectional	54	n/a	3-day food record	Adequate protein intake, but lower fat intake and higher carbohydrate intake than TDC 4 children (7.4%) met the recommended servings for each food group
(Schreck et al., 2004)	Observational Cross sectional	138	298	Food preference inventory	Children with ASD ate a significantly narrower range of foods than children without autism ( $p=0.00$ )
(Herndon et al., 2009)	Observational Case control	46	31	3-day food record	Majority consumed less than RDA in either group of: Fibre, calcium, iron and vitamin E and D intakes Children with ASD had significantly higher vitamin B6, Vitamin E, non-dairy protein servings, and less calcium intake and fewer dairy servings ( $p<0.05$ )
(Lockner et al., 2008)	Observational Case control	20	20	3-day food record	Majority of both groups consumed more than RDA for: Carbohydrate, protein, folate, iron, and vitamins B6 and C intakes Majority of both groups consumed less Fibre, calcium and vitamin E and A than the RDA
(Bandini et al., 2010)	Observational Case control	53	58	3-day food record, FFQ	Fibre intake inadequate in 97% of children with ASD

(Schmitt et al., 2008)	Observational Case control	20	18	3-day food record	Inadequate intake of vitamin D greater in children with ASD (79.2%) than TDC (55.4%) Adequate consumption defined as >67% of DRI Low fibre intakes (<67% for both groups), < 67% for vitamins E and A for children with autism
(Hyman et al., 2012)	Observational Cross-sectional	367 <sup>1</sup>	NHANES (2007-2008)	3-day food record	Energy, vitamins A and C, zinc, and phosphorus significantly lower for children with ASD ( $p<0.05$ )
(Xia et al., 2010)	Observational Cross-sectional	111	n/a	3-day dietary recall	Children met DRI for energy, protein, vitamin E, Niacin; Did not meet DRI for vitamin A, vitamin B1, B2, B6, vitamin C, calcium, iron, zinc and magnesium
(Bicer & Alsaffar, 2013)	Observational Cross-sectional	164 <sup>2</sup>	n/a	3-day food record	Children had inadequate intake for fibre, calcium, zinc, iron, vitamin A, vitamin B6; majority had sodium intakes above upper limit
(Johnson et al., 2008)	Observational Case control	19	15	FFQ, 24-hour recall	No significant differences between groups for macro or micro nutrients except vitamin K was lower for children with ASD ( $p=0.023$ ) Children with ASD consumed fewer vegetables than TDC ( $p=0.001$ ) Both groups had intakes low in fibre
(Whitney Evans et al., 2012)	Observational Case control	53	58	FFQ	Children with ASD consumed lower servings of fruit and vegetables than TDC ( $p=0.006$ ), and more sugar sweetened beverages ( $p=0.01$ )

ASD = Autism spectrum disorder; NHANES = National Health and Examination Survey; TDC = Typically developing children

<sup>1</sup>Food records were analysed for 252 children

<sup>2</sup>Food records were analysed for 115 children

## **2.4 Factors Affecting Nutritional Status of Children with ASD**

### **2.4.1 Gastrointestinal Problems**

Gastrointestinal (GI) problems may be more common among people with ASD than the general population (Nikolov et al., 2009). Parents of children with ASD have frequently reported cases of GI upset or digestion problems including stomach pains, diarrhoea, constipation, acid reflux, vomiting or bloating (Buie et al., 2010). It is estimated that one third of children with ASD have some kind of GI disorder (Santhanam & Kendler, 2012). Associations between GI symptoms and ASD were first studied in the early 1970's (Goodwin et al., 1971). Intestinal inflammation in children with autism is well described in the literature (Ashwood et al., 2004; Horvath et al., 1999) with duodenitis being exhibited in 66.7% of 36 patients with autism (Horvath et al., 1999). Ibrahim et al. (2009) conducted a longitudinal study where they followed children to 18 years of age in order to determine whether children with ASD (n=121) have a greater incidence of GI symptoms compared with typically developing children (n=242). The study showed children with autism have a greater incidence of constipation versus typically developing children (33.0% vs 17.6%).

The reasons why GI problems occur more frequently in people with ASD is not well understood (NICE, 2011). One theory is that people with ASD have defects in their gut mucosa. Gut epithelial cells are important for not only acting as an epithelial barrier; they also help with innate immunity, partly by the production of multiple mediators (Jyonouchi, 2009). Paneth cells produce antimicrobial peptides, which serve as a broad-spectrum antibiotic killing gram-positive and gram-negative bacteria (Porter et al., 2002). Dysfunction of the epithelial barrier in children with autism is referred to as the leaky gut hypothesis (White, 2003). The hypothesis postulates that impaired gut permeability permits the entry of macromolecules such as milk proteins into the blood stream, and as a result, the gut mucosal immune system is sensitised causing subsequent food allergy. This is based on findings from studies where they found increased urinary peptides (Reichelt et al., 1994) and abnormal intestinal permeability (Horvath & Perman, 2002). However these findings were not supported by other studies (Robertson et al., 2008; Williams & Marshall, 1992). It remains unknown whether impaired gut permeability is due to a defect of the intestinal barrier innately, or due to food allergy causing permeability (Jyonouchi,

2009). Dietary modification may be seen by parents as a strategy to combat GI symptoms or to address these proposed mechanisms causing gut permeability.

### *Food allergies*

Food allergy involves an adverse immunological response to food (Bruijnzeel-Koomen et al., 1995). Disorders relating to food allergy can be broadly categorised into those that are IgE-mediated and those that are not. IgE-mediated reactions usually result in an acute onset of symptoms which results in a sensitisation (Bruijnzeel-Koomen et al., 1995). When another exposure happens, the causal food protein binds to the IgE molecules specific for them and consequently triggers the release of mediators, such as histamine resulting in allergic symptoms (Bruijnzeel-Koomen et al., 1995).

Many children with ASD have been reported to exhibit “allergic symptomology”, even in the absence of positive skin or RAST tests (Angelidou et al., 2011). Gurney et al. (2006) reported on the findings of a the National Survey of Children’s Health which was conducted in the USA in partnership with the National Centre for Health Statistics, a division of the CDC, and found that children with autism had more allergic symptoms than children in the control group who did not have autism. As discussed previously, it has been postulated that children with autism may be sensitised to common food proteins because of an immature gut mucosal system. Due to the high number of GI issues reported in children with autism, it has been speculated that this is related to food allergies (Jyonouchi, 2009). One study which examined 30 children with autism who were age-matched to 39 children with a family history of allergic symptoms ( $p < 0.005$ ), reported a higher frequency of skin prick test reactivity. The children in the ASD group tested positive for at least one of the skin prick tests which were 12 common antigens (Bakkaloglu et al., 2008). Another study looked at the prevalence of atopy, asthma, food allergy in two subsets of children – children with ASD and without. Although parents of children with ASD had frequently reported “allergic symptoms”, there was no difference found to the control group (Jyonouchi et al., 2008). At this point it is still unknown whether prevalence of allergy is higher in children with ASD. Even in the absence of a diagnosed allergy, parents may choose an exclusion diet for their children with ASD.

### **2.4.2 Selective Eating**

Within the literature relating to the eating habits of children with autism the term “selective eating” has multiple meanings. These include concepts relating to food refusal, the limited range of accepted foods, and the tendency to consume a single food item frequently. This means the evaluation and comparison of studies can be difficult due to this lack of consensus. What is clear is that the problems related to food intake are variable, where at one end of the spectrum the effect of selective eating on intake is minimal to extreme cases where the child is either receiving excess intakes of certain nutrients or may need to be tube fed (Williams & Seiverling, 2010).

Early childhood is a time when children usually start to try new foods and experience new tastes and textures. Where a child repeatedly refuses to eat certain foods, parents often describe their child as a “picky eater” or “fussy”. In children who have ASD, restrictive eating can be even more extreme and may extend beyond early childhood (Tomchek & Dunn, 2007). Children with autism often resist the unknown, and will as a result, refrain from trying new foods. Many parents have described their children to be highly selective eaters and their food repertoire many be limited to as few as five foods (Cermak et al., 2010).

Various studies have shown children with autism to exhibit greater selectivity when compared with typically developing children (Ahearn et al., 2001; Buckley et al., 2005). Until recently, the majority of studies have looked at nutrition status among children with autism, rather than examining the nutritional status of selective eaters as a separate group. Zimmer et al. (2012) looked at food variety as a predictor of nutritional status among children with autism (n=22) compared with age matched typically developing children (n=22). The children with autism were split into groups depending on whether they were selective eaters. In this study, it was found that children with autism ate fewer foods on average than typically developing children (33.5 vs 54.5 foods, ( $p<.001$ )). In terms of nutrient intake, children with autism were found to have a higher intake of magnesium, and lower average intake of protein, calcium, vitamin B<sub>12</sub> and vitamin D when compared with controls. Selective eaters were significantly more likely than the typical controls to be at risk for at least one serious nutrient deficiency.



Feeding difficulties during meal times in children with autism are common problems faced by parents and caregivers. One study found that 69% of children with ASD were unwilling to try new foods and 46% had rituals when it came to eating times (Williams et al., 2000). Children have also been shown to be selective based on how the food is presented. Ahearn et al. (2001) conducted a study to examine the amounts of foods within each food group children with autism (n=30) typically consume. Approximately half of participants showed selectivity based on food category or food texture. Children with ASD were also shown to accept or reject food items consistently. However, it was concluded that the study's generalisability was limited due to the small sample size.

A larger study compared the eating habits of children with ASD to typically developing children (Schreck et al., 2004). The study compared food selectivity in children with ASD (n=128), and typically developing children (n=298). A food preference inventory was completed by parents to assess the extent to which children ate a variety of foods. Children with autism were found to eat fewer foods and exhibit greater food selectivity than children who did not have the disorder. In general, children with ASD ate approximately half the amounts of foods in each food group except from the starch food group, where they consumed approximately two thirds the amount of typically developing children. Children with ASD were also more likely to accept food if it was in a low-texture format such as pureed foods. The author concluded that children with ASD exhibited greater food selectivity than typically developing children. For all the food groups in the study, children with ASD ate fewer foods from each of these groups compared with other members of the family. However, individual food preference was found to be related to the family's food preferences as one would expect. These results were similar to a population-based study comparing children with autism with matched control subjects. Ibrahim et al. (2009) found greater food selectivity and feeding issues in children with autism when compared with typically developing children (24.5% vs 16.1%).

### **2.4.3 Family Factors**

Having a child with ASD has a substantial effect on the family. Parents and siblings of children with ASD are likely to experience depression, anxiety and stress (Bagenholm & Gillberg, 1991). Parental control of eating behaviours, parental stress, emotional responses and family food preferences are all factors that have been

proposed to play a role in the eating behaviours and subsequent nutritional status of an individual with autism (Collins et al., 2003; Martins et al., 2008). Lockner et al. (2008) reported that parents of children with autism view their child's eating habits more negatively and report more problems with eating when compared with typically developing children.

## **2.5 Complementary and Alternative Medicine Therapies**

Complementary and alternative medicine therapies (CAM) refer to therapies or treatments that are additional to traditionally prescribed interventions and sometimes used in place of mainstream treatments. Complementary and alternative medicine therapies are commonly used where mainstream treatments have been ineffective or unsatisfactory (Levy & Hyman, 2005). The use of these therapies is well documented however the safety and efficacy of these treatments in children is limited. Prevalence rates of CAM usage in children with ASD is variable with estimates ranging from 32 to 92% (Hanson et al., 2007; Harrington et al., 2006; Levy & Hyman, 2008). Children with ASD do not always progress on conventional treatment, therefore families explore alternative avenues either as a substitute for or in conjunction with accepted treatments in an attempt to ameliorate symptoms.

Complementary and alternative medical therapies can incur additional costs for families – both financially and the time invested. Christon et al. (2010) reported 47.9% of parents (n=73) interviewed regarding CAM therapy usage found it difficult to meet the costs of adopting an exclusion diet. Similarly, 50.7% of parents (n=67) found the costs involved with use of supplements were difficult to meet and 37% of respondents said they found undertaking a restrictive diet to be time consuming.

There are many CAM therapies reported for use by people with ASD, however for the purpose of this study, only CAM therapies involving the use of exclusion diets or supplements will be reviewed here.



### **2.5.1 Gluten Free- Casein Free Diet**

#### *Background*

Gaining popularity among parents of children with ASD is the use of the gluten-free casein-free diet (GFCF diet). Adherence to the GFCF diet involves removing all food items containing the proteins gluten (found most often in wheat, rye, barley, and commercially available oats) and casein (found most often in dairy products - milk, yoghurt, cheese, butter, cream and ice cream) from the diet. Most studies that have assessed the use of dietary restrictions in a group of children with ASD have reported usage to be around 30% (Herndon et al., 2009; Sharp et al., 2013; Wong & Smith, 2006).

The gluten-free diet is the prescribed medical treatment for individuals diagnosed with a gluten intolerance or coeliac disease. Coeliac disease is a chronic inflammatory disorder which is characterised by the flattening of villi on the small bowel mucosa, which is brought on in individuals with a genetic predisposition by the ingestion of proline-rich and glutamine rich proteins found in wheat, rye and barley (Di Sabatino & Corazza, 2009). The only proven treatment for coeliac disease is the strict adherence to a gluten-free diet (Di Sabatino & Corazza, 2009) Speculation around gluten as a proposed contributor to autism and other neuropsychiatric conditions was theorised not long after the original description of ASD by Dr Leo Kanner, a child psychiatrist (Kanner, 1943).

There is minimal research relating to the link between gluten and autism. A positive association between coeliac disease (CD) and ASD was suggested in a case report by Genuis and Bouchard (2010). Pavone et al. (1997) studied 11 children with autism and found no correlation between markers for coeliac disease and autism. Two larger studies present contradictory results both indicating there is no link between CD and ASD (Batista et al., 2012; Black et al., 2002). A more recent case-control study in Sweden examined 28 biopsy registries and collected data on 26,995 individuals with CD, 12,304 individuals with inflammation and 3,719 individuals with normal mucosa but a positive CD serology (Ludvigsson et al., 2013). In this study, a comparison was made against age and sex-matched controls. The results showed no association of ASD with CD or inflammation, however a markedly increased risk for individuals with ASD to have a normal mucosa yet a positive CD serology. It

remains unknown whether children with ASD present more frequently with coeliac disease.

#### *Proposed mechanism*

It has been suggested that peptides present in gluten and casein may play a role in exacerbating the symptoms of autism and that the physiology of autism may be linked to the “opioid-excess” linked to these peptides (Reichelt, 1990; Shattock et al., 1990). The rationale behind the use of the GFCF diet is its apparent positive affect on gastrointestinal (GI) problems and its relationship to the brain. Increased gut permeability is said to allow macromolecules to cross the intestinal membrane and enter the blood stream. Once in the blood stream these peptides are believed to cross the blood brain barrier and exert an “opioid like” effect on the central nervous system (White, 2003). By avoiding these peptides all together, the expected effect is alleviation of the behavioural outcomes produced by the proposed opioid effect.

#### *Effectiveness*

Studies have been conducted to assess whether the use of a GFCF diet can assist in the amelioration of ASD symptoms (**Table 2.3**) and (**Table 2.4**). Most of these studies are preliminary and with very small numbers of participants. Reichelt (1990) conducted an open label cohort study where 15 children with autism on a GFCF diet were followed up over 12 months. The results showed a statistically significant decrease in urinary peptides levels, improvements in antibodies and behaviour. The small sample size is of concern and in addition the authors have not attempted to control for possible alternative explanations.

Knivsberg et al. (1990) also reported improvement when they placed a group of children with autism (n=20) on a gluten-free diet. In the screening process, they identified children who had gliadorphin, a urinary peptide, in their urine. The authors suggested that the gluten-free diet prescribed would have potentially been helpful to a child who had coeliac disease, food allergy to gluten products, or maldigestion of these food products. They concluded that the use of a gluten-free diet could support the theory that at least a subgroup of children could benefit from the use of the gluten-free diet.

A study by Whiteley et al. (2010) was one of the first studies to involve a randomised, controlled, single-blind study design with long diet periods. The results

showed a significant improvement in autistic symptoms for the GFCF diet group (n=38) versus the control diet group (n=34) at 12 and 24 months. The authors concluded that there may be a possible diet-related phenotype associated with autism and that the use of GFCF diet may have a positive effect on the developmental outcomes of some children with ASD. Although the study design was an improvement on the other available studies, there were still areas for improvement due to high attrition rates reducing an already small sample size.

Similar to Whiteley's study, Knivsberg et al. (2002) reported improvements in children's development compared to the control group. The study involved 20 children with ASD who were randomly assigned to either the GFCF diet group (n=10) or the control group (n=10) who received a normal diet. Although the study duration was acceptable (12 month duration) a major limitation to the study was the very small sample size (n=10 in each arm), therefore it is difficult to make generalisations to the wider population. In both Whiteley's and Knivsberg's study, the children's adherence to diet was not measured. Secondly, most of the outcome measures were based on parental report and parents were aware of whether or not their child was on the GFCF diet.

Elder et al. (2006) conducted a 12-week, double-blind cross-over study in 15 children with autism (**Table 2.4**). They included a comparison diet group (n=15) who were placed on a matched diet included gluten and casein. The treatment group were only on the diet for six weeks. No statistical differences in developmental markers of behaviours were found. Johnson et al. (2011), in their randomised, parallel group study found similar findings when they assessed 22 preschool children with ASD (aged 3-5 years). Children were randomly assigned to either receive a GFCF diet (n=8) or a healthy control diet that was low in sugar (n=14) (**Table 2.4**). The authors report they did not have the resources to provide a GFCF and placebo diet to families and consequently parents were aware of which diet their child was on.

A limitation of studies using the GFCF diet is the duration which is often less than 12 weeks. Residues of gluten and its by-products are known to remain in the intestines of people with coeliac disease for 12 weeks after removing gluten-containing foods from the diet (Kumar et al., 1979). What should also be noted is the possible alternative explanation for outcomes exhibited in the studies.

**Table 2.3. Summary of studies assessing gluten and/or casein free dietary restriction: positive results**

Author and Year	Type and Duration	Participants	Type of intervention	Comparison diet	Outcome measures	Results	Comments
Lucarelli et al. (1995)	Open label before and after dietary restriction; Double blind placebo controlled cohort study for the casein load test Duration: 8 weeks	n= 36 30 males, 6 females 8-13 years old with autism	Cow's milk protein elimination diet	No comparison group	BSE Measurement of Ig levels	Specific Ig levels decreased after dietary restriction; improvements seen in behaviours; casein load test results were not conclusive	No treatment fidelity Has not attempted to control for possible alternative explanations (allergy present, medication, other therapies) No blinding
Knivsberg et al. (2002)	Randomised, single blind, placebo controlled trial Duration: 12 months	n=20 Gender not reported autism	GFCF diet N=10	Normal diet N=10	UPL, parent teacher behaviour ratings using DIPAB, LIPSA scale and Reynells spraktest for linguistic ability, movement assessment battery for children	Significant improvement in all areas for children on the GFCF diet	No double blinding Small sample size Has not attempted to control for possible alternative explanations (allergy present, medication, other therapies) No treatment fidelity Parents not blinded to the diet
Whiteley et al. (2010)	Randomised, single-blind, placebo controlled, two-stage study (at 12 months those not responding in the control group switch to diet group) Duration: 24 months	n=72 (gender not specified) age not specified autism	GFCF diet N= 38	Normal diet n = 34	ADOS, GARS, VABS, ADHD-IV, GARS	Significant improvement in the diet group at 12 and 24 months in the ADOS and repetitive categories, GARS social categories	Parents not blinded to the diet No power calculation provided High attrition rate Has not attempted to control for possible alternative explanations
Cade et al. (2000)	Prospective open label study Duration: 12 months	n= 150 128 males, 22 females Ages 3.5-16 years old autism	GFCF diet for 12 months	No comparison group	UPL, parent, guardian, teacher ratings regarding social isolation, eye contact, speech, learning	Significant improvement in autistic signs and symptoms	UPL analysis was conducted at baseline only No treatment fidelity No blinding Has not attempted to control for possible alternative explanations

Reichelt (1990)	Open label urinary peptides; GFCF cohort study Duration: 12 months	n = 15 10 males, 5 females 3-17 years old autism	Diets were prescribed based on participants specific UPL levels	No comparison group	UPL, behaviour questionnaire by parents and caregivers	Decrease in urinary peptides Improvement in some behaviours Decrease in epileptic seizures	No treatment fidelity Has not attempted to control for possible alternative explanations Small sample size
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skills, hyperactivity stereotypical activity, hygiene, panic attacks, self-mutilation; blood tests of antibodies to gluten and casein

Abbreviations: GFCF, gluten-free casein-free; UPL, urinary peptide level; DIPAB, A Danish assessment of autistic trait; CARS; Childhood Autism Rating Scale; ECO Ecological Communication Orientation Language Sampling Summary; BSE, Behaviour summarised evaluation

**Table 2.4. Summary of studies assessing gluten and/or casein free dietary restriction: no significant result**

Author and Year	Type and Duration	Participants	Type of intervention	Comparison diet	Outcome measures	Results	Comments
Elder et al. (2006)	Randomised, double-blind, repeated measures, crossover study Duration: 12 weeks	n=15 12 m, 3 f M age =7.4 years old	GFCF diet for 6 weeks	Matched diet but included gluten and casein	UPL, CARS, ECO	No statistically significant findings	Small sample size Study duration short
Johnson et al. (2011)	Randomised, parallel groups Duration: 3 months	n=22 18 m, 4 f Aged: 3-5 years	GFCF diet N= 8	Healthy Control Diet (Low sugar diet) N = 14	Mullen scales of early learning, Child behaviour checklist, direct behaviour observation measure	No statistically significant differences between the two groups	Small sample size GFCF diet = low diet adherence No attempt to control for possible alternative explanations
Irvin (2006)	Case report Duration: 4 days	n = 1 M age 12 years old Autism	GFCF	No comparison group	Direct observation of behaviours including self-injury, property destruction, and aggression	No behavioural change Increase in food refusal	Short study duration Has not attempted to control for possible alternative explanations Small sample size
Seung et al. (2007)	Retrospective study, randomised, double-blind, cross over study Duration: 3 months	n = 13 10 m, 3 f 2-16 years autism	GFCF diet for 6 weeks; Normal diet for 6 weeks	No comparison group	Assessment of verbal and nonverbal communication	No statistical significance	Short study duration Small sample size
(Hyman et al., 2015)	Randomised, controlled, double-blind cross-over	n = 14 3-5 years with autism	GFCF diet for 4-6 weeks	No comparison group	Physiological functioning Challenging behaviours Behaviours associated with ASD	No statistical significant	Small sample size Short study duration

Abbreviations: GFCF, gluten-free casein-free; UPL, urinary peptide level; DIPAB, A Danish assessment of autistic trait; CARS; Childhood Autism Rating Scale; ECO Ecological Communication Orientation Language Sampling Summary; BSE, Behaviour summarised evaluation

### *GFCF diet and nutritional adequacy*

Studies have also assessed the use of the GFCF diet and nutritional adequacy. Cornish (2002) conducted a retrospective case-control study on 8 children with ASD to assess whether children on a GFCF diet were at risk of nutrient deficiency. They compared food choices of children on a GFCF diet (n=8) versus children not on the diet (n=29). Using a 3-day food diary they examined nutrient intakes from each group. The lower reference nutrient intake (LRNI) value was used for comparison. They did not find any significant differences in energy, protein and micronutrients between children adopting the diet and children who were not. Twelve children who were not on the GFCF diet had nutrient intakes that fell below the LRNI for zinc, calcium, iron, vitamin A, vitamin B<sub>12</sub>, and riboflavin. Four children on the GFCF diet had intakes below the LRNI for zinc and calcium. Intakes of fruit and vegetables were higher, and cereal and potatoes were lower for children on a GFCF diet compared to the control group.

Graf-Myles et al. (2013) assessed dietary intakes of children with autism (n=69), and developmental delay (n=14) and compared with typically developing children (n=37) using three-day food records. Children with autism who were on a restrictive diet (n=23) were either on a gluten-free (n=3), casein free (n=5) or a combination of GFCF diet and soy-free diet (n=15). Their results showed children with ASD did not differ significantly to children with developmental delay for any dietary measures. Children with autism who were on a restrictive diet were found to have significantly lower intakes of calcium and serves of dairy than the typically developing children in this study. All groups had inadequate intakes of fibre, vitamin D, and vegetable intakes.

Overall, these studies suggest that the adoption of a GFCF diet may result in low intakes certain nutrients such as calcium due to dairy restriction, however the limited number of studies available are small and do not assess the effects of the GFCF diet specifically.

### *Safety*

Inconclusive evidence exists as to the safety of GFCF diet (Mulloy et al., 2010). Arnold et al. (2003) used biochemical measures to assess the plasma amino acid



profiles in children with ASD. They reported a trend for children with autism who had adopted a restricted diet (n=10) to have an increased prevalence of essential amino acid deficiencies including lower plasma levels of tyrosine and tryptophan when compared with children with ASD on an unrestricted diet (n=26).

Removing sources of dairy from the diet without replacing with suitable calcium-rich alternatives may result in calcium deficiency (Konstantynowicz et al., 2007; Monti et al., 2007). Calcium is an essential for the growth and maintenance of healthy bones and teeth. Milk and some milk products are the best sources of calcium. In New Zealand, the Ministry of Health (MOH) states children require two to three servings of milk and milk products each day in order to meet their calcium requirements. The MOH also recommends children who are not consuming dairy products, calcium fortified and non-fortified alternatives are recommended. Non-dairy sources of calcium include canned fish with bones, leafy green vegetables, nuts and seeds and fortified breakfast cereals. Hediger et al. (2008) conducted an observational cross-sectional study and assessed the cortical bone density of a group of boys aged 4-8 years with ASD (n=75). Of these children, nine were on a casein-free diet. They found that cortical bone density was reduced in the nine children who were on a casein-free diet. The authors report that although the restriction of casein-containing foods and subsequent calcium and vitamin D intakes being low may have limited bone development in children on a casein restricted diet, it could have also been due to physical inactivity or lack of sunlight. Further, there were only nine children on a casein-free diet, a larger sample would be needed before any generalisations can be made.

## **2.5.2 Other Diets**

### *Specific Carbohydrate Diet*

The Specific Carbohydrate Diet (SCD) was designed by Elaine Gotschall to treat inflammatory bowel disease and other gastrointestinal disorders (Gotschall, 1994). The diet involves eliminating certain carbohydrates from the diet such as disaccharides and polysaccharides but allows monosaccharides. Common foods that appear regularly in many people's diets are eliminated such as cereal grains, potatoes and lactose-containing dairy products. In her book, the author suggested the SCD diet for use among individuals with autism as a means of treating GI



symptoms and behavioural issues. However, research to support the efficacy of the SCD in ameliorating symptoms of ASD is lacking and it may in fact pose a health risk due to reduced nutritional quality (Brown et al., 2011).

### *Ketogenic Diets*

The ketogenic diet was first used as a potential treatment method for reducing the intensity of epileptic seizures (Kang et al., 2007). The diet is based on obtaining the majority of daily energy needs from fat; protein recommendations are based on one's minimum daily requirements, and carbohydrates are restricted to a minimal level (Evangelidou et al., 2003; Kossoff et al., 2009). A lack of carbohydrate from diet forces the body to use fat as a fuel source. The rationale behind the use of such a diet in ASD is related to the hypothesis that autistic behaviour is associated with a disturbance in glucose metabolism. The hypothesis is that the disturbance in glucose metabolism affects mitochondrial energy production, which results in an excess of nicotinamide adenine dinucleotide (NADH) or lack of nicotinamide dinucleotide (NAD). The resulting hormonal changes include a reduction of circulating insulin therefore limiting glucose utilisation. The fuel that is subsequently produced is ketone bodies, which unlike fatty acids, can cross the blood-brain barrier and be used for fuel. The ketogenic diet is proposed to ameliorate this effect by improving mitochondrial function and in turn sparing NAD, which is consumed by the oxidation of glycolytic substrates (Evangelidou et al., 2003).

The use of the ketogenic diets in children with autism has not been studied in great detail. A pilot study examined the efficacy of the ketogenic diet in autism (Evangelidou et al., 2003). The study involved 30 children who were put on a ketogenic diet for 6 months. The results showed improvement in several parameters within the Childhood Autism Rating Scale, a behaviour rating scale that involves rating children on a scale from one to four for various criteria. The authors report that although this study is preliminary, there is some evidence to support the use of the ketogenic diet in reducing autistic symptoms in addition to conventional treatment or as an alternative therapy. Of the 30 children in the study, only 18 were able to carry out the diet to 6 months (Evangelidou et al., 2003). This highlights the difficulty of adhering to restrictive diets. More evidence is needed to understand the potential therapeutic use of the ketogenic diet in ASD.

### *The Feingold Diet (Additive/preservative free)*

The Feingold diet was developed in 1973 by Dr Benjamin Feingold, a paediatrician and allergist, who later went on to publish a book (Feingold, 1975). The diet involves eliminating artificial colours, flavours and food additives. Feingold proposed that salicylates and artificial colours and flavours were contributing to the cause of hyperactivity in children. The diet was proposed to assist children with ADHD, however according to the literature 40-80% of children with ASD also have ADHD as a co-morbid disorder (Leyfer et al., 2006; Matson & Nebel-Schwalm, 2007). Green et al. (2006) conducted an internet survey on parents of children with autism (n=522) and found that usage was 2.7%. This is notably less than the GFCF diet which was found to have usage rates of 20 to 70% throughout the literature. Most of the research pertaining to the use of an additive/preservative free diet to control symptoms of ASD remains controversial. Not only that, most of the research conducted is done on either children without ASD or ones with ADHD. A review study concluded that the use of the Feingold Diet in reducing hyperactivity in children is ineffective (Curtis & Patel, 2008). The studies they assessed had major flaws in study population size, lack of definitions in diet type and a lack of adequate placebo groups (Curtis & Patel, 2008). Even where a gold standard approach of study design was used, results to support the use of the Feingold diet were lacking (Conners et al., 1976).

### *Sugar Free diet*

The theory that sugar causes hyperactivity is a commonly held viewpoint among lay public and parents (Rojas & Chan, 2005). However, few studies have been able to provide evidence to support this link. In addition, most of the studies have focused on the effects of sugar in children with Attention Deficit Hyperactive Disorder (ADHD) rather than ASD.

Two observational studies have found that sugar ingestion was related to ADHD symptoms in children and young adults (Kim & Chang, 2011; Lien et al., 2006). For studies that used a randomised controlled trial study design results are variable. Shaywitz et al. (1994) assessed 15 children with ADHD, and found no significant difference between children receiving the sugar drink and children receiving aspartame based drink after 2 weeks. These results were similar to Milich and Pelham (1986) where they assessed 16 boys with ADHD and found no significant

difference between children taking a the sugar drink and children taking the placebo. Wender and Solanto (1991) assessed 17 children and found children taking the sugar drink had significantly increased inattention in a test after drinking compared with nine age-matched control subjects. To our knowledge, there are no studies examining the effects of a sugar-free diet on reducing symptoms of ASD.

### **2.5.3 Supplement Use in Children with ASD**

#### ***Introduction***

Vitamins and minerals are nutrients that are required in small amounts for normal growth and development (Mahan et al., 2012). For the most part, consuming a healthy diet can supply the body with the nutrients it needs to obtain adequate amounts of vitamins and minerals. Where these levels cannot be achieved by diet alone, a supplement may be necessary (Ministry of Health, 2012). Parents will often provide supplements to their children to compensate for what they believe to be nutritional deficiencies (Bailey et al., 2013).

Mega-dose vitamin therapy and the use of other nutritional supplements are common among the ASD community. Green et al. (2006) found that 30% of parents who have children with ASD give their children extra vitamins, including vitamin C and B6, and over 25% are supplementing their children with essential fatty acids and magnesium. In the same study, more than 10% of parents report the use of vitamin A, mega-vitamin therapy, DMG (dimethylglycine) and L-glutamine. A more recent cross-sectional study examined the use of dietary supplement use and micronutrient intake in 288 children with ASD (Stewart et al., 2015). They used three-day diet records to estimate usual intake of micronutrients from food and supplements and compared this with Dietary Reference Intakes (DRI). The study showed 56% of children with ASD were given supplements, particularly a multivitamin/mineral supplement. The study also found nutrient supplementation led to intakes above the UL for vitamin A, folate, and zinc across the whole sample. In children aged 2-3 years copper intake was elevated, and children aged 4-8 copper and manganese intakes were elevated.

The rationale for the use and expected benefits of these supplements is highly variable, as is the level of scientific evidence supporting their use. It is important to note however, that vitamin therapy and nutritional supplements beyond a multi-

vitamin to correct dietary shortfalls, is not yet fully supported by any conclusive scientific evidence and further studies are needed. An overview of the research concerning the use of supplements in children with autism will be outlined in the following section.

### ***Overview of commonly used supplements***

#### *Multivitamin/mineral supplement*

The use of a multivitamin/mineral supplement may be beneficial in ensuring adequate nutritional intake where intakes cannot be met by diet alone. Studies have attempted to show the benefit of a multivitamin supplement/mineral supplements in ASD. Adams and Holloway (2004) conducted a randomised double blind cross over study, where they assessed 20 children with ASD and the use of a multivitamin/mineral supplement. The supplement contained moderate levels of B vitamins, folic acid, calcium, zinc, selenium, and vitamins A, D and E. The results showed an improvement in sleep ( $p=0.03$ ) and GI symptoms ( $p=0.03$ ) when compared with a placebo and no adverse effects were observed.

#### *Vitamin C*

Vitamin C is a well-known as an anti-oxidant, which acts by donating electrons to free-radicals. Children with ASD have been shown to suffer from increased oxidative stress (Chauhan & Chauhan, 2006; James et al., 2004; McGinnis, 2004).

Based on this theory, various studies have looked at the effects of vitamin C supplementation on amelioration of autistic symptoms (Adams & Holloway, 2004; Dolske et al., 1993; McGinnis, 2004). A 30-week, double-blind, placebo-controlled study conducted by Dolske et al. (1993) found that autistic severity was reduced following supplementation with high-dose vitamin C (110mg/kg). The study numbers however were very small ( $n=18$ ).

Although there is some preliminary evidence to demonstrate a reduction in autistic symptoms following vitamin C supplementation, a vitamin C supplement is not currently recommended as part of conventional treatment (Levy & Hyman, 2015).

### *Vitamin A*

Vitamin A has also been shown to have high use among the ASD community (Green et al., 2006). Vitamin A is a fat-soluble vitamin therefore the body will store excess amounts, primarily in the liver. Concerns over toxicity is related to excess pre-formed vitamin A (known as hypervitaminosis A) than beta-carotene and other pro-vitamin A carotenoids (Grune et al., 2010). A recent Cochrane Review found that excessive vitamin A supplementation can increase mortality rate by 16%. Based on the available research, Levy and Hyman (2015) report there is not enough evidence to support the use of vitamin A supplementation as a potential treatment for ASD, and should be cautioned given its potential for toxicity.

### *Vitamin B<sub>6</sub>*

Vitamin B<sub>6</sub> (Pyridoxine) plays an important role in optimal neurological function and neurotransmitter synthesis (Lerner et al., 2002). In relation to autism, the theory is that children with autism are lacking in pyridoxine which is said to be secondary to an enzyme deficiency that converts the inactive form of pyridoxine to the active form pyridoxal-5-phosphate (P5P) (Adams et al., 2006; Adams & Holloway, 2004). There is no evidence to support this theory.

A number of studies have been conducted in an attempt to assess the effects of vitamin B<sub>6</sub>. A Cochrane Review was carried out to determine the efficacy of vitamin B<sub>6</sub> and magnesium for treating social, communication and behavioural response of children and adults with autism. Only three studies were able to be included in the review and therefore due to such a small number, no recommendation could be made regarding the use of B<sub>6</sub> and magnesium as a treatment for autism (Nye & Brice, 2005).

### *Folic acid and Vitamin B<sub>12</sub> supplementation*

Vitamin B<sub>12</sub> is a co-factor which is vital in the regeneration of methionine from homocysteine by providing methyl groups which are used in the transmethylation metabolic pathway (Mattson & Shea, 2003). Recent studies have suggested that children with autism have abnormal methylation cycles, and as a result are predisposed to oxidative stress. A deficiency in levels of folic acid and vitamin B<sub>12</sub> has been put forward as a potential cause (James et al., 2009). An open-label study

examining the effects of vitamin B<sub>12</sub> and folic acid (an active form of folate) on 40 children with autism found that oxidative stress was reduced after twice a day supplementation for 3 months (James et al., 2009). In addition, there were significant increases found in cysteine, cysteinylglycine, and glutathione concentrations, all of which are important factors involved in the proper functioning of metabolic pathways. Although the study found that supplementation in children with ASD with B<sub>12</sub> and folic acid resulted in normalising the metabolic imbalance, they did not report on actual intake before supplementation giving no interpretation of the true intakes of these nutrients in the children's diets.

Researchers have questioned whether a B<sub>12</sub> deficiency causes behavioural deficits in children with ASD (Bertoglio et al., 2010). A double-blind placebo controlled cross over study involving 30 children with autism, were given injectable methyl B<sub>12</sub>. The authors observed no significant differences in behavioural assessments between the treatment group and placebo (Bertoglio et al., 2010).

Vitamin B<sub>12</sub> and/or folic acid supplementation is not supported by current research at this time as a treatment method for autism and further studies would be required before any recommendations could be made.

### *Dimethylglycine*

Dimethylglycine (DMG), a derivative of the amino acid glycine, is a substance that is found in small amounts in cereal grains, and liver (Balch & Balch, 1997). There is very little research pertaining to the use of DMG for reducing the effects of ASD and most of the research is preliminary with very numbers of participants. A double-blind, placebo-controlled crossover pilot study by Bolman and Richmond (1999) found no significant benefit in behavioural outcome measures due to DMG when they placed eight children with autism on a DMG supplement for 1 month. Despite the lack of evidence to support the safety and efficacy of DMG, the supplement remains popular. Green et al. (2006) reports 14% of parents (who had a child with ASD) surveyed were currently using DMG and 27.4% had used it in the past.

### *Omega-3 fatty acids*

The use of omega-3 fatty acid supplements is popular among the ASD community. Green et al. (2006) reports 1 in 4 parents give their children omega-3 fatty acid

supplements. A survey carried out by Hanson et al. (2007) reported that 23% of 112 families reported use of food supplements which included omega-3 fatty acids.

Omega-3 fatty acids, are polyunsaturated fatty acids which are important for brain development and cannot be produced in the human body. Omega-3 fatty acids, specifically the long chain (LC) omega-3 polyunsaturated fatty acids (PUFA) derived from fish and seafood, are also of great importance to neural function and has been shown to be used in the development of synapses and neural membranes (Horrocks & Farooqui, 2004; Moriguchi et al., 2000). Docosahexaenoic acid (DHA) is the most abundant omega-3 fatty acid in the brain (Innis, 2007).

Recent investigations have shown a link between neurodevelopmental disorders, including autism spectrum disorder and deficiencies of omega-3 fatty acids. Amminger et al. (2007) conducted a randomised, double-blind, placebo-controlled 6-week pilot trial investigating the effects of 1.5g/day of omega-3 fatty acids (0.84g/day eicosapentaenoic acid (EPA) and 0.7g/day DHA) on children with ASD. They found that children in the intervention group had reduced hyperactivity and stereotypy when compared with placebo group. The study was small (n=12), yet the authors report it provides preliminary evidence that omega-3 may be a potential treatment to reduce autistic symptoms.

A Cochrane review assessed the current evidence of the effectiveness of omega-3 supplementation in children with ASD and a reduction of associated symptoms. Only two small studies were able to be included in the review. They concluded that there is insufficient evidence that omega-3 fatty acid supplementation is an effective treatment for ASD and that a more high quality large randomised controlled trials of omega-3 fatty acid supplementation are needed before any clear conclusions can be made about this potential treatment method (James et al., 2011).

### *Melatonin*

Many children with autism have problems sleeping with a reported prevalence of 44-88% (Richdale, 1999). Melatonin, an endogenous pineal hormone, is related to sleeping patterns, and if there are insufficient levels within the body, problems with falling and staying asleep may ensue. Children with autism have been shown to be more likely to have a mutation/polymorphism in their acetyl serotonin O-methyl-



transferase gene, which leads to problems with the synthesis of melatonin within the body (Melke et al., 2008). Rossignol (2009) conducted a systematic review which examined emerging treatments for autism spectrum disorder. In this study melatonin was the only dietary supplement to be assigned the highest grade (A) as its efficacy had been supported by at least 2 prospective clinical trials.

Giannotti et al. (2006) concluded long-term effectiveness of melatonin supplementation may provide an effective and well tolerated treatment for children with sleeping difficulties. The ASD guideline by the Ministry of Health recommends caution be taken with the dosage given, that melatonin is not registered for use in New Zealand and can only be purchased at retail pharmacies on presentation of a prescription and dosages are not standardised (Ministry of Health, 2008).

### *Probiotics*

Probiotics are another treatment gaining popularity for children with ASD. An internet survey conducted on 522 parents regarding the use of treatments used by parents of children with autism reported use of probiotics by 20.5% of the study population (Green et al., 2006). The theory behind the use of probiotics is the proposed beneficial effect a probiotic supplement has on gastrointestinal issues commonly experienced in ASD. Although no ASD specific research has been conducted, there are studies to show therapeutic benefits for certain probiotic strains such as *Lactobacillus reuteri*, *Lactobacillus GG* and *Saccharomyces boulardii*. These strains have been shown to restore gut microflora in order to treat certain conditions such as necrotising enterocolitis (NEC), acute diarrhoea and inflammatory bowel disease (Alfaleh et al., 2011; Goossens et al., 2003; Szajewska et al., 2006). The use of probiotics is not currently regulated by the FDA, however they are generally considered safe for use unless someone has a compromised immune system (Ishibashi & Yamazaki, 2001). Although research indicates positive results in gastrointestinal conditions, ASD specific research is needed before recommendations can be made regarding probiotics as a potential treatment for ASD.

### *Digestive enzymes*

Different enzymes are needed to break down protein, carbohydrate and fat in the digestive system (Mahan et al., 2012). Carbohydrate malabsorption may result in



flatulence, abdominal pain, and loose stools (Horvath et al., 1999). Children with autism have been found to have lower levels of certain enzymes, or less active enzymes (Horvath et al., 1999; Williams et al., 2000). This is more commonly found in children who have GI issues such as constipation or diarrhoea. Horvath (1999) evaluated disaccharidase activity from endoscopic biopsies in 90 children with autism and reported 49% to be deficient in at least one enzyme. Lactase and maltase deficiencies were the most frequent, followed by low activity of sucrase, palatinase and glucoamylase. The study showed that 58% of children in the study had disaccharidase/glucoamylase enzyme activity below normal ranges. Children who had low enzyme activity also had loose stools and problems with flatulence. Similarly, a large study by Kushak et al. (2011) involving biopsy samples of 199 children and adults with autism found that many had deficiencies in disaccharidase – ranging between 58-65% across the age groups studied. Of the sample, 62% had deficiencies in lactase, 16% were deficient in sucrase and 10% were deficient in maltase. The enzyme deficiencies were as common in adults as they were in children suggesting that these problems are life-long.

## **2.6 Summary**

There are many factors that may be attributing to the nutritional status of a child with autism spectrum disorder including selective eating behaviours, gastrointestinal upsets, and the use of exclusion diets and supplements. A wealth of information on alternatives treatments such as exclusion diets and supplements is easily available to parents, most of which is anecdotal, and many of these treatments have little scientific evidence to support their use. There is currently no New Zealand specific data regarding the use and effect that exclusion diets and supplements have on the ability for children with ASD to meet the Food and Nutrition Guidelines and NRVs. This study aims to address the gap in current research by examining the dietary intakes, use of exclusion diets and supplements in a group of children with ASD in New Zealand.

## **3.0 Methods**

### **3.1 Study Design**

This study is an observational study of children with autism spectrum disorder (ASD), aged 2.5–8 years living in New Zealand. This study was undertaken as part of the larger VIDOMA trial. The VIDOMA trial is a randomised placebo controlled double blind trial of vitamin D and omega-3 fatty acids in children with ASD. This observational study reports on the dietary intakes, use of exclusion diets/supplements and reasons for their use from participants who enrolled in the VIDOMA study between December 2014 and late October 2015.

### **3.2 Ethical Approval and Funding**

Ethical approval for the study was obtained from the Health and Disability Committee (HDEC), NZ Reference No. 14/NTA/113. Each parent gave written informed consent for their child to participate in the study. The VIDOMA study has been registered with the Australian New Zealand Clinical Trial Registry, ACTRN12615000144516.

Funding: This study was supported by a post graduate funding award (\$3000) from the School of Food & Nutrition at Massey University. No conflicts of interest were associated with this source of funding.

### **3.3 Setting**

This study was conducted at the Human Nutrition Research Unit at Massey University in Auckland, New Zealand. Children and their parents were initially recruited to the study from the Auckland region, but interested parents from other regions were also recruited if they expressed their interest and were willing to cover the travel cost to Auckland. Auckland is the largest urban centre in New Zealand and families who have a child with ASD have access to a number of different health and educational services in the region.

### **3.4 Study Population**

Children aged 2.5 to 8 years, with a medical diagnosis of autism spectrum disorder by a paediatrician according to the Diagnostic and Statistical Manual of Mental Disorders – Fourth or Fifth Edition (DSM-IV or DSM-V) (American Psychiatric Association, 2013) and an onset of symptoms of ASD after 18 months of age were recruited for the VIDOMA study. This age group was chosen based on the requirements for certain assessment tools used in the VIDOMA study. The family was required to be proficient in the English language for this reason also.

Exclusion criteria for the VIDOMA study included if there were developmental delays since birth and any child who was currently taking omega-3 supplements. For children who had been taking omega-3 supplements, there was a washout period before they could be eligible to take part in the VIDOMA study.

For this study participants were those recruited for the VIDOMA study between December 2014 and October 2015, and an additional inclusion criteria for this study was that parents had to have completed a 4-day food diary for their child.

### **3.5 Recruitment of Participants**

Participants were recruited using a number of different recruitment strategies. Initially participants were recruited via the Waitamata District Health Board (WDHB) ASD co-ordinators and paediatricians, followed by advertising through autism support groups and organisations such as Autism New Zealand. The study was also advertised through social media including Facebook and a study website.

Consultation with health professionals and organisations that support families with a child who has ASD were an important part of the recruitment strategy. Local ASD co-ordinators were fully informed about the study and agreed to make the first approach to any families, whose child met the age and diagnosis criteria, under their care to take part in the study. Parents were provided with a flier about the study and any initial questions were answered by the co-ordinators. Parents were then directed to the study website for more information and to express their interest in taking part in the study.

Posters and fliers containing information and contact details about the study were sent to local paediatricians, early childhood education centres, and primary schools. Advertisements for participants were also made via newsletters released by autism support groups and organisations, which encouraged parents to register interest on the study website or contact the study manager for more information.

### **3.6 Study Procedures**

Parents who expressed an interest in taking part in VIDOMA study were contacted by the study manager who determined the eligibility of the child. During this telephone interview, verbal consent was obtained and the study manager then asked a range of demographic and medical questions (Appendix D). If eligible, contact details (Appendix C) were collected and parents and their child were enrolled into the study. Information on the diagnosis of ASD was also collected from parents during this call. If the child had been recruited through the WDHB paediatrician (who was part of the research team) this was considered as a confirmed diagnosis, if they had been recruited through other avenues a secondary method of confirmation was used such as cross-referencing the child's National Health Index (NHI) number with the WDHB data base or letter from a paediatrician.

A "social story" was created to provide children with a short description of Massey University and what to expect during their visit, a method created by Gray and Garand (1993) to help teach social skills to people with autism. Parents of eligible children were then sent a pack which contained an information sheet outlining the procedures for the VIDOMA study. In this pack there was also the social story and a consent form which needed to be signed. Additionally, parents received a questionnaire and a 4-day food diary and instructions for completion. The questionnaire was designed to gather data on outcome variables used in this study such as use of exclusion diets and supplements. A self-addressed envelope to Human Nutrition Research Unit at Massey University was also supplied.

On receipt of the consent form, the study office contacted the parent to arrange an appointment at the Human Nutrition Research Unit at Massey University. The participants were randomly assigned into the VIDOMA trial.

Participants and their parents came into the Human Nutrition Research Unit for a baseline visit. During this visit the study psychologist conducted a psychological assessment which indicated the severity of ASD present according to the Wechsler Preschool and Primary Scale of Intelligence test (WPPSI-IV). A trained researcher also took the child's anthropometric measurements and checked the completeness of the questionnaires and food diary with the parents.

Parents/caregivers who had not completed the 4-day food diary and/or questionnaire were asked again if they could complete a food diary and were given a stamped addressed envelope if they answered in the affirmative.

### **3.7 Data Collection and Questionnaire**

#### *Participant characteristics*

The personal details questionnaire included age, gender, full contact details and name of their general practitioner, while the demographic questionnaire included questions about ethnicity, household income, number of children in the household and if there were any other children in the family with an ASD diagnosis (Appendix D).

#### *Medical history*

The medical history questionnaire consisted of questions relating to current health conditions, medications, blood loss in the last 6 months, history of low iron, and breastfeeding practices of the mothers. These questions were asked in relation to the VIDOMA study.

#### *Use of exclusion diets and supplements, nutrition knowledge and sources of information*

The use of exclusion diets and supplement questionnaire was developed to determine if the children were on any type of exclusion diet, reasons for adopting an exclusion diet, perceived changes parents observed in their child following the use of the exclusion diet, where the parents received their information about the diet from, whether the child had any allergy testing done and if so where the testing was conducted (Appendix B). Types of diets included as options in the questionnaire

were ones that had been identified in the literature as having been used to improve symptoms: gluten-free, casein-free diet, dairy-free diet, gluten-free diet, specific carbohydrate diet (SCD), sugar-free diet, Feingold diet (additive/preservative free diet), egg free diet, low glycaemic index (GI) diet, yeast free diet and caffeine free diet.

Questions were asked to determine if the children were on any nutritional or non-nutritional supplements. If they were on any supplements, questions were asked to determine, what they were, where the parents received information from regarding supplement use, what their reasons were for using supplements were and if there were any perceived changes to their children in terms of behaviour and health after they can commenced the supplements. Again the types of supplements included as options were ones that had been identified in the literature proposed to have beneficial effect. If children were found to be on supplements, dose and frequency of the supplements were confirmed by the study manager at their first appointment to the VIDOMA study. There were also additional questions in the questionnaire that were for use by another researcher.

#### *Four day food diary*

A 4-day food diary which has previously been used to assess dietary intakes in adolescents by the research team was adapted by two dietetic students at Massey University prior to recruitment. The 4-day food diary which was designed to assess dietary intake of macro- and micro-nutrients of the children (Appendix A). The adaptations made included the use of appropriate foods for the age group of the children in this study and appropriate serving sizes outlined in the Food and Nutrition Guidelines for Healthy Children and Young People (aged 2-18 years) (Ministry of Health, 2012), brands such as Milo, Weetbix and Meadow Fresh and types of foods such as snack size packets of chips were given as examples. Parents were asked to record detailed information regarding the amount and types of all foods and beverages their child consumed for four consecutive days, one of which was a weekend day as dietary intake has been shown to change depending on whether it was a weekday or not (Willett et al., 1985). Detailed instructions were provided in the front of the diary and contact details for the study manager provided if parents needed advice on completing the diary. Parents were advised to weigh foods where

possible. An example of a day's intake was provided to ensure parents understood how to complete the food diary in the correct format. Parents were asked to provide cooking methods, brands and submit homemade recipes where possible. Parents were also asked to record the actual amount of the food or beverage item consumed or an estimation if this was not possible with the use of household measures to estimate portion size.

Once complete, parents were asked to mail back the 4-day food diary to Massey University or bring the diary to their appointment for the VIDOMA study (if it was within a week of completing the food diary). All returned food diaries were reviewed for completion by a member of the VIDOMA research team. If there was missing information or any details were unclear then parents were contacted via the telephone or email and any omissions clarified.

The 4-day food records were analysed by entering in Foodworks Professional diet analysis programme version 7 (Xyris Software (Australia) Pty Ltd, 2012) by two dietetic students from Massey University. If specific food items were not included in the Foodworks database, a food with a similar nutritional profile was chosen and the nutritional profile was adjusted to match the food. For homemade recipes, individual ingredients were entered as a 'recipe' and the number of serves the recipe provided. Both students entered the same food diary into Foodworks 7 and macro and micro nutrient intakes from the two records were compared. This was repeated with two other 4-day food diaries. When a substitute brand was used within the Foodworks database, this was recorded on an assumption sheet detailing the substitute food used. Food diary entries were checked by one of the dietetic students to enhance accuracy and consistency.

### **3.8 Anthropometric Measurements**

Anthropometric measurements were conducted by the study manager or study coordinator using the International Society for the Advancement of Kinanthropometry (ISAK) anthropometry methods.

As it was considered to not practical to obtain two measurements, as this population group tends to be non-compliant, only one measurement of weight and height was obtained and recorded.

### *Weight*

Children were weighed wearing light indoor clothing and barefoot. Weight was recorded to the nearest 0.1kg using an electronic scale Tanita THD646 electronic digital scale. If the child was not compliant and did not accept to stand on the scale, the parent's weight was measured and recorded. Then, the parent's weight while holding the child was measured on the same scale and recorded. The child's weight was determined by subtraction.

### *Height*

The children's height (cm) was recorded to the nearest 0.1 cm using a portable standard stadiometer (Holtain Ltd., England) with no shoes or hat. The child was asked to look at a picture hanging on the front wall to keep him/her standing still.

Body mass index was then calculated and participants group according to the revised International Obesity Task Force (IOTF) BMI cut-offs points (**Appendix E**) for children aged 2-14 years to classify children into thin (<18.50), normal (18.50-24.99), overweight (25.00-29.99) and obese ( $\geq 30$ ) categories (Cole & Lobstein, 2012). The IOTF cut-off points have been designed to coincide with the WHO BMI cut-off points for adults. One of the category name differs between adults and children. The underweight category is referred to as "thinness" which specifies that the children in that category have a low BMI for their age.

## **3.9 Data Handling**

### *Dietary analysis*

After the dietary data was entered in Foodworks Professional (version 7), the macronutrients examined in this study were: total energy (KJ), total fat (g), saturated (g), monounsaturated fat (g), polyunsaturated fat (g), total sugars (g) and dietary fibre (g). Further, the percentage energy contribution from macronutrients was also examined: (carbohydrate, fat, saturated fat and protein).



The following micronutrients were investigated: vitamin A equivalents ( $\mu\text{g}$ ), thiamin (mg), riboflavin (mg), niacin equivalents (mg), vitamin B<sub>6</sub> (mg), vitamin B<sub>12</sub> ( $\mu\text{g}$ ), vitamin C (mg), vitamin D ( $\mu\text{g}$ ), vitamin E (mg), sodium (mg), potassium (mg), magnesium (mg) calcium (mg), phosphorus (mg), iron (mg), manganese ( $\mu\text{g}$ ), copper (mg), selenium ( $\mu\text{g}$ ), and zinc (mg). Nutrient intakes were compared to Nutrient Reference Values for Australia and New Zealand based on age range (National Health and Medical Research Council, 2006). For children under 4 years, recommendations for 1-3 year olds was used. For children over 4 years the recommendations for 4-8 year olds were used.

For energy percentage contribution from macronutrients, an Acceptable Macronutrient Distribution Range (AMDR) was used (IOM, 2002). The Food and Nutrition Guidelines for Healthy Children and Young People (Aged 2-18 years) state AMDR's are for use only in those 14 years or older, or are suitable for use in a healthy population of children provided they are growing normally (Ministry of Health, 2012). The Institute of Medicine has set AMDR's specifically for use in children and for this reason, results from this study will be compared based on age group recommendations. These recommendations differ for age groups 1-3 years and 4-8 years.

Where possible a comparison to the estimated average requirement (EAR) was made. This approach was used because the EAR is the best estimate of an individual's requirement (Murphy & Poos, 2002). Where no EAR was available an Adequate Intake (AI) was used. The Upper Level (UL) was used to assess whether any children were exceeding nutrient recommendations. The UL is used to assess whether an individual's usual intake is so high that they may be at risk of adverse health effects (National Health and Medical Research Council, 2006).

Using information gained from the 4-day food diary, intakes were also converted into servings of the following food groups: breads and cereals; lean meat, poultry, fish, shellfish, eggs legumes nuts and seeds; fruit; vegetables; and dairy and dairy alternatives. For each individual, the number of serves of each food group was totalled for each day and then a four-day average was determined. Once 4-day food diaries had been converted into food group servings, these were then compared to the Food and Nutrition Guidelines for Healthy Children and Young People (Aged 2-

18 years) recommended daily servings (Ministry of Health, 2012). The age groups were categorised based on the age ranges outlined in the guidelines which are for children aged 2<5 and 5-8 years.

A further analysis was made where participants were categorised into one of two groups - those on an exclusion diet and those who were not. To be categorised in the exclusion diet group, children needed to be on one or more of the following: gluten free, dairy free, gluten-free and casein free, additive/preservative free, egg-free, additive/preservative free, sugar-free and other. If a parent selected any of the exclusion diets listed, the child was considered to be on an exclusion diet. Supplement data were not included for analysis within the food records and therefore nutrient analysis was based solely on food intake alone in order to better compare the nutritional adequacy of diets between groups.

### **3.10 Data Analysis**

All data were checked and coded prior to being entered into SPSS (IBM SPSS Statistics Version 21.0) for analysis. The variables were tested for normality using the Kolmogorov-Smirnov, Shapiro-Wilk tests and normality plots. Non-normally distributed data was transformed into approximate normal distributions by logarithmic transformations. Descriptive statistics were carried out and mean plus standard deviation (or geometric mean plus 95% confidence interval if the data had been log transformed) or median and 25<sup>th</sup> and 75<sup>th</sup> percentile (IQR) were reported.

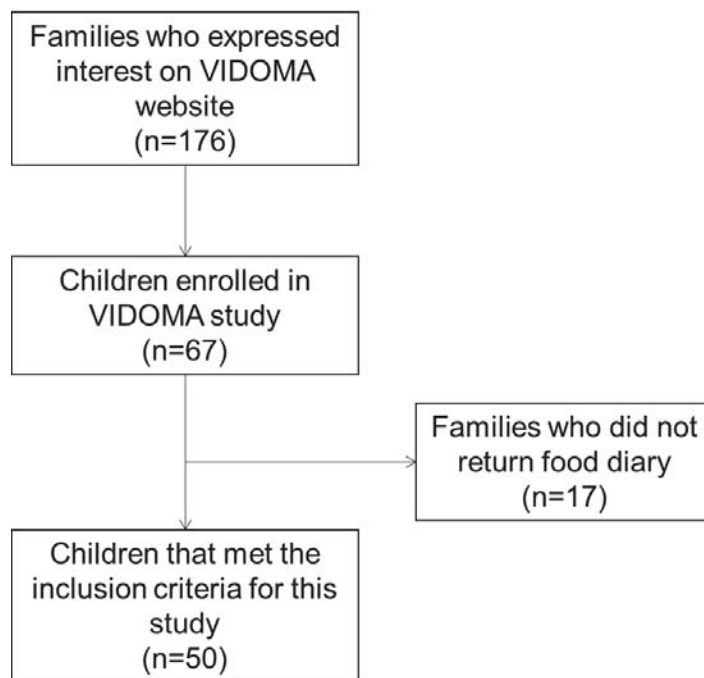
Descriptive statistics were used to describe the characteristics of participants. For the purpose of this study, groups were made according to the use of exclusion diet – those on an exclusion diet were compared to those not on an exclusion diet. Data was tested for homogeneity using the Levene's test. Tests to compare the two groups were performed depending on the nature of distribution. If the data was parametric, independent t-tests were used. For non-parametric data, the Mann-Whitney test was used. To test for relationships between categorical variables a Pearson's chi-square ( $\chi^2$ ) test was used. Where group sizes were too small, (<5) the assumptions for the  $\chi^2$  were not met, the Fisher's exact test was used. A *p*-value of less than 0.05 was considered to be significant.

## 4.0 Results

### 4.1 Study Participants

#### 4.1.1 Participant Numbers

A flow diagram detailing the final participant numbers analysed in this study is presented in **Figure 4.1**.



**Figure 4.1** Flow diagram of final participant numbers

Between December 2014 and October 2015, there were 176 families who expressed an interest in the VIDOMA study (**Figure 4.1**). Of these families, 67 children were enrolled in the VIDOMA study. Completion of a 4-day food diary and dietary questionnaire was required in order to be included in this study. Of the 67 children enrolled in the VIDOMA study, 17 parents did not return a 4-day food diary. Therefore, fifty children who were enrolled in the VIDOMA study fulfilled the inclusion criteria for this study.

#### 4.1.2 Description of Participants

The characteristics of the participants included in this study are presented in **Table 4.1**. The mean  $\pm$  SD age of the participants was  $5.20 \pm 1.3$  years. There were more male participants than female (43 vs 7 respectively). The majority of children were identified as being New Zealand European (n=36, 72%). There were no Māori participants. Those who identified as 'other' were Russian (n=1), South African (n=1) and Latin American (n=1). The mean  $\pm$ SD for BMI was  $16.8 \pm 2.0\text{kg/m}^2$ .

Using International Obesity Task Force (IOTF) BMI cut-offs, for the majority of children (n=34, 68%), their weight in relation to height fell within a normal range, four (8%) were classified as thin, and 12 (24%) as overweight or obese. Nearly one third of participants were on an exclusion diet (n=15, 31%) and 26 children (55%) were using supplements. The majority of parents (n=23, 56%) had an average annual household income within the \$60,000 to \$140,000 bracket (**Table 4.1**).

**Table 4.1** Participant characteristics

Characteristics (n=50)	n	%
Age (years) (Mean $\pm$ SD), range	5.20 $\pm$ 1.3	2.5-8
Height (cm) (Mean $\pm$ SD), range	112.8 $\pm$ 9.4	95-135
Weight (kg) Geometric mean (95% CI), range	21.1 (19.8, 22.4)	15-38.5
BMI (kg/m <sup>2</sup> ) <sup>a</sup>		
Thinness	4	8
Normal	34	68
Overweight	7	14
Obese	5	10
Sex		
Male	43	86
Female	7	14
Ethnicity		
New Zealand European	36	72
Pacific	4	8
Asian	7	14
Other	3	6
ASD severity rating <sup>b</sup>		
Mild	18	36
Mild/moderate	5	10
Moderate	17	34
Moderate/severe	2	4
Severe	8	16
Exclusion diet		
Yes	15	31
No	33	69
Missing data	2	
Supplement		
Yes	26	55
No	21	45
Missing data	4	
Income		
<\$60,000	12	29
\$60,000-140,000	23	56
>140,000	6	15
Missing data	9	
Parents have more than one child with ASD		
Yes	3	7
No	43	93
Missing data	4	

ASD = Autism Spectrum Disorder; BMI = body mass index

<sup>a</sup>International Task Force on Obesity cut-offs for children aged 2-18 years (Cole & Lobstein, 2012)

<sup>b</sup>Wechsler Preschool and Primary Scale of Intelligence (WPPSI) Test

## 4.2 Dietary Intakes

### 4.2.1 Macronutrient Intakes

Macronutrient intakes analysed from the 4-day food diaries for the total study population are presented in **Table 4.2**. Dietary intakes were compared against the Food and Nutrition Guidelines for Healthy Children and Young People (aged 2-18 years) which is broken down by age groups 1-3 years, and 4-8 years (Ministry of Health, 2012). There was a larger proportion of 4–8 year olds (86%) in the exclusion diet group compared with the non-exclusion diet group (78%). Median energy intakes for children aged 2.5-3 years and 4-8 year were 5618 KJ and 7261 KJ respectively. The median energy contributions to daily energy intake from carbohydrate, protein and total fat were within the Acceptable Macronutrient Distribution Ranges (AMDR) for children. Although the majority of children (n=35, 70%) had energy contribution from fat within the AMDR, 13 children (26%) exceeded the AMDR. Seven children across both age groups had intakes below the AMDR for fat (14%). For energy contribution from carbohydrate, 14 children (28%) had intakes below the AMDR. Two children had intakes below the AMDR for protein, both of which were in the 4-8 year age group. No children in either age group were below the EAR for protein (g). Although the median intakes of dietary fibre were above the adequate intake (AI) recommendations for children across the two age ranges, 40% of children from the total study population were not meeting the AI (**Table 4.2**).

**Table 4.2 Daily macronutrient intakes from dietary sources only for children aged 2.5-3 years (n=9) and 4-8 years (n=41) compared with Nutrient Reference Values<sup>a</sup> and AMDR<sup>b</sup>**

	Children aged 2.5-3 years				Children aged 4-8 years			
	Median intake (IQR)	<AMDR n (%)	>AMDR n (%)	Range	Median intake (IQR)	<AMDR n (%)	>AMDR n (%)	Range
Total energy (KJ)	5618 (5527, 6197)			3225-8019	7261 (6266, 7844)			4482-10981
% energy from protein	14.8 (13.0, 18.0)	0	1 (11)	10.9-22.2	14.7 (13.4, 16.7)	2 (5)	0	8.8-22.9
% energy from carbohydrate	51.8 (45.2, 55.9)	2 (22)	0	34.4-65.0	48.5 (44.0, 53.8)	12 (30)	1 (2)	34.7-66.1
% energy from fat	31.7 (28.5, 38.7)	4 (44)	1 (11)	18.9-42.9	31.8 (29.6, 39.2)	3 (7)	12 (29)	19.1-48.4
% SFA	9.1 (8.7, 11.9)			5.6-16.2	12.9 (11.2, 15.4)			4.8-22.7
Total fat (g)	54.6 (46.3, 59.4)			33.1-92.4	64.4 (50.1, 73.9)			32.3-141.2
SFA (g)	13.9 (12.5, 19.4)			9.4-34.9	26.9 (20.5, 31.2)			7.1-48.2
MUFA (g)	15.7 (12.6, 16.0)			10.9-32.9	21.9 (19.2, 27.3)			11.5-69.2
PUFA (g)	6.9 (5.7, 7.1)			5.2-16.7	8.1 (6.54, 10.9)			4.1-28.6
Protein (g)	54.6 (46.3, 59.3)	0 <sup>c</sup>		42.1-68.1	61.9 (54.7, 67.6)	0 <sup>c</sup>		39.3-105.1
Total sugars (g)	90.9 (62.1, 115.4)			39.1-214.3	83.3 (57.3, 108.6)			34.6-250.6
Dietary fibre (g)	17.9 (9.1, 18.9)	4 (44) <sup>d</sup>		7.6-29.5	21.4 (16.1, 36.7)	16 (39) <sup>d</sup>		6.6-35.5

AMDR = Acceptable Macronutrient Distribution Range; SFA = Saturated fatty acid; MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid

<sup>a</sup>Nutrient Reference Values for Australia and New Zealand based on children aged 1-3 years and 4-8 years

<sup>b</sup>Institute of Medicine AMDR recommendations for children (IOM, 2002)

1-3 years: AMDR for protein = 5-20%; AMDR for carbohydrate = 45-65%; AMDR for fat = 30-40%

4-8 years: AMDR for protein = 10-30%; AMDR for carbohydrate = 45-65%; AMDR for fat = 25-35%

<sup>c</sup>n (%) < Estimated Average Requirement for protein; 12g (1-3 years) and 16g (4-8 years)

<sup>d</sup>n (%) < Adequate Intake for fibre; 14g (1-3 years) and 18g (4-8 years)

#### 4.2.2 Micronutrient Intakes

Micronutrient intakes are presented in **Table 4.3**. The median intakes for children were above the EAR or AI for thiamin, riboflavin, niacin equivalents, vitamin B<sub>6</sub>, magnesium, calcium, phosphorus, iron and zinc across both age groups. A third of children in the 2.5-3 year age group were not meeting the EAR for calcium (n=3, 33%). In the 4-8 year age group, ten children (25%) were not meeting the EAR for calcium. Six children (12%) exceeded the upper level for vitamin A equivalents. The majority of children (n=48, 96%) exceeded the upper level for sodium. Three children had intakes below the EAR for vitamin B<sub>12</sub>, all of whom were in the 4-8 year age group. Ten children were not meeting the EAR for vitamin C. The majority of children (n=42, 84%) had vitamin D intakes below the AI. Over a third of children were receiving intakes below the EAR for vitamin E (n=18, 36%). Median intakes for potassium were above the EAR, however the majority of children (n=27, 54%) had intakes below the EAR. A large proportion of children (n=21, 42%) had copper intakes below the AI.



**Table 4.3** Daily micronutrient intake from dietary sources only for children aged 2.5-3 years (n=9) and 4-8 years (n=41) compared with Nutrient Reference Values<sup>a</sup>

	Children aged 2.5-3 years					Children aged 4-8 years				
	Median intake (IQR)	EAR	RDI	<EAR n (%)	Range	Median intake (IQR)	EAR	RDI	<EAR n (%)	Range
Vitamin A equivalents (µg)	456.9 (201.4, 425.8)	210	300	3 (33)	102.2-1452.1	425.5 (298.4, 715.5)	275	400	9 (22)	50.1-1342.3
Thiamin (mg)	1.3 (0.9, 1.7)	0.4	0.5	0	0.7-3.6	1.5 (1.2, 1.9)	0.5	0.6	0	0.6-5.1
Riboflavin (mg)	1.3 (1.0, 2.0)	0.4	0.5	0	0.6-2.4	1.8 (1.3, 2.2)	0.5	0.6	0	0.6-3.5
Niacin Equivs (mg)	21.4 (18.2, 24.5)	5	6	0	9.0-28.5	25.1 (22.5, 27.4)	6	8	0	15.0-53.0
Vitamin B <sub>6</sub>	1.6 (1.2, 1.9)	0.4	0.5	1 (11)	0.5-2.2	1.5 (1.2, 2.0)	0.5	0.6	0	0.6-2.7
Vitamin B <sub>12</sub> (µg)	2.4 (1.7, 2.7)	0.7	0.9	0	1.1-4.2	2.7 (1.7, 3.9)	1.0	1.2	3 (7.5)	0.0-267.9
Vitamin C (mg)	60.0 (25.2, 96.2)	25	35	2 (22)	17.2-245.9	72.4 (33.4, 92.6)	25	35	8 (20)	14.2-296.1
Vitamin D (µg)	1.7 (0.1, 1.9)	5 <sup>b</sup>		8 (89) <sup>b</sup>	0.07-10.8	1.6 (0.9, 2.9)	5 <sup>b</sup>		34 (85) <sup>b</sup>	0.0-9.2
Vitamin E (mg)	5.3 (4.6, 5.9)	5 <sup>b</sup>		3 (33) <sup>b</sup>	3.8-15.3	6.9 (5.1, 9.2)	6 <sup>b</sup>		15 (37) <sup>b</sup>	2.6-18.9
Sodium (mg)	1688 (1493, 1962)	200-400 <sup>b</sup>	1000 <sup>c</sup>	8 (89) <sup>c</sup>	796.2-3020.7	2198 (1999, 3071)	300-600 <sup>c</sup>	1400 <sup>c</sup>	40 (97) <sup>c</sup>	1316-4261
Potassium (mg)	2043 (2004, 2394)	2000 <sup>b</sup>		5 (55) <sup>d</sup>	1126-3250	2201 (1783, 2702)	2300 <sup>b</sup>		22 (55) <sup>d</sup>	1118-3852
Magnesium (mg)	199.1 (182.6, 233.0)	65	80	0	92.3-265.3	238.8 (200.0, 286.1)	110	130	0	125.5-415.6
Calcium (mg)	614.4 (260.7, 849.5)	360	500	3 (33)	225.0-1401.4	616.7 (515.5, 808.3)	520	700	10 (25)	125.5-415.6
Phosphorus (mg)	863 (704.1, 1156.5)	380	460	0	493.1-1254.8	1052 (944, 1178)	405	500	0	195.5-1636.1
Iron (mg)	10.7 (9.2, 11.5)	4	9	0	4.9-20.9	11.7 (8.7, 13.5)	4	10	0	598.8-1725.7
Zinc (mg)	7 (4.7, 8.2)	2.5	3	0	3.7-9.3	8.2 (6.8, 9.8)	3.0	4	0	3.0-20.6
Manganese (µg)	1840 (1339, 3414)	2000 <sup>b</sup>		5 (55) <sup>b</sup>	647.4-4974.5	3473 (2906, 4103)	2500 <sup>b</sup>		7 (17) <sup>d</sup>	582.6-6416.0
Copper (mg)	0.8 (0.7, 1.0)	0.7 <sup>b</sup>		2 (22) <sup>b</sup>	0.3-35.8	1.0 (0.7, 1.2)	1.0 <sup>b</sup>		19 (46) <sup>d</sup>	0.5-2.2
Selenium (µg)	25.8 (16.7, 31.1)	20	25	3 (33)	10.8-47.1	33.3 (26.7, 43.9)	25	30	6 (15)	11.5-90.5

EAR = Estimated Average Requirement; RDI = Recommended Dietary Intake

<sup>a</sup>Nutrient Reference Values for Australia and New Zealand based children aged 1-3 years and 4-8 years

<sup>b</sup>Adequate Intake;

<sup>c</sup>n (%)>Upper level; <sup>d</sup>n (%)<AI

### 4.2.3 Food Group Analysis

The number of servings from each of the food groups was calculated from the 4-day food diaries and compared to the Ministry of Health Food and Nutrition Guidelines for Healthy Children and Young People (aged 2-18 years) recommended daily serves (**Table 4.4**). The number of servings of dairy foods was found to be below the recommended number of servings for the majority of children. Only two children were receiving the recommended number of serves of dairy foods. The majority of children were consuming the recommended number of servings of breads and cereals (n=32, 64%). Most of the children (n=44, 88%) were not consuming the recommended number of servings of fruit per day. None of the children consumed the recommended servings of vegetables per day, and the majority were consuming less than 1 serve per day (n=31, 62%). The majority of children were receiving adequate serves of meat, fish, eggs and pulses per day (n=34, 68%).

**Table 4.4** Food groups servings for children aged 2<5 years (n=20) and 5-8 years (n=30) compared to the Food and Nutrition Guidelines<sup>a</sup>

Food group	Recommended number of servings <sup>a</sup>		Number of servings	No of children meeting guidelines by age group n (%)		No of children meeting guidelines n (%)
	2<5 years	5-8 years		2<5 years (n=20)	5-8 years (n=30)	
						Total group (n=50)
Breads, cereals	4+	5+	<2 2<4 4-<5 5+	3 (15) 4 (20) 3 (15) 10 (50)	1 (3) 5 (17) 5 (17) 19 (63)	32 (64)
Fruit	2+	2+	<1 1<2 2+	11 (55) 7 (35) 2 (10)	15 (50) 11 (37) 4 (13)	6 (12)
Vegetables	2+	3+	<1 1<2 2<3 3+	14 (70) 5 (25) 1 (5) 0	17 (57) 9 (30) 4 (13) 0	1 (2)
Meat, fish, eggs, pulses	1-2	1-2	<1 1<2 2+	5 (25) 12 (60) 3 (15)	11 (37) 15 (50) 4 (13)	34 (68)
Dairy and dairy alternatives	2-3	3+	<1 1<2 2<3 3+	9 (45) 9 (45) 1 (5) 1 (5)	15 (50) 13 (43) 2 (7) 0	2 (4)

<sup>a</sup>Food and Nutrition Guidelines for Healthy Children and Young People (Aged 2-18 years)

### 4.3 Use of Exclusion Diets

#### 4.3.1 Types of Exclusion Diets

Children were classified by whether they were on an exclusion diet or not (**Table 4.5**). Fifteen of the children (31%) were reported to be on an exclusion diet and of these eleven were on more than one type of exclusion diet. One child was on four different types of diet. The most popular combination of the exclusion diets was the gluten free and dairy free diet (n=6, 40%). Of the responses classified as 'other' there was a range of different types of exclusion diets including a low-oxalate diet, a nut-free diet, and a cheese and tomato sauce exclusion.

The majority of children on an exclusion diet were on a dairy-free diet (n=9, 60%). Of these parents who reported their child was on a dairy-free diet, according to 4-day diet records, five were using a dairy-free calcium fortified alternative.

**Table 4.5** *Types of exclusion diets*

Exclusion diet	% (n)
Dairy free	9 (60)
Gluten free	8 (53)
Additive/preservative free	6 (40)
Sugar free	4 (27)
Other diet e.g. low oxalate diet or nut free diet	7 (47)

\*Parents could report more than one type of exclusion diet

### 4.3.2 Macro and Micronutrient intakes

#### *Macronutrients*

Macronutrient intakes according to exclusion diet status are presented in **Table 4.6**. There were no differences found between those on exclusion diets and those who were not for energy contribution from carbohydrate ( $p=0.91$ ) protein ( $p=0.14$ ) or fat ( $p=0.55$ ). Although there was no significant difference found between the mean fibre intakes between the two groups, a greater proportion of those adhering to exclusion diet had intakes below the AI (47% vs 39%).

No children in the exclusion diet were below the AMDR for protein. Two children were below the AMDR for protein in the non-exclusion diet group, both of which were in the 4-8 year age group. No children in either diet group were below the EAR for protein. A greater proportion of children from the non-exclusion diet group were below the AMDR for carbohydrate when compared with the exclusion diet group (33% vs 21%).

#### *Micronutrients*

Micronutrient intakes based on the use of exclusion diet are presented in **Table 4.7**. Of the micronutrients analysed from the 4-day food diaries, calcium was the only micronutrient found to be significantly different between the two groups. Calcium intakes were found to be significantly lower for those children on an exclusion diet than those who were not ( $538.4 \pm 361.6$  vs  $737.4 \pm 242.6$ ;  $p=0.03$ ). A greater proportion of those adhering to an exclusion diet (47%) did not meet the EAR for calcium when compared with those not on a diet (15%).

Vitamin D intakes were lower for the children on an exclusion diet compared with children not on an exclusion diet (1.2 [0.1, 2.2]  $\mu\text{g}$  vs 1.8 (1.2, 3.7]  $\mu\text{g}$ ) this was approaching significance ( $p=0.08$ ). When examining intakes below the AI for vitamin D, a greater proportion of those in the exclusion diet group ( $n=14$ , 93%) were receiving intakes below the AI when compared with the non-exclusion diet group ( $n=26$ , 79%).

**Table 4.6** Daily macronutrients from dietary sources only for children on exclusion diet (n=15) compared with children not on exclusion diet (n=33)

	Exclusion diet group			Non-exclusion diet group			p-value	
	Mean $\pm$ SD intake	n (%) < AMDR	n (%) > AMDR	Range	Mean intake	n (%) < AMDR		n (%) > AMDR
Total energy (KJ)	6442 $\pm$ 1138			4887-7726	7212 $\pm$ 1499		3225-10981	0.08
% energy from protein	15.6 $\pm$ 2.0	0	0		14.7 $\pm$ 2.9	2 (6)	1 (3)	0.14
% energy from carbohydrate	49.6 $\pm$ 5.2	3 (20)	0	43.0-58.9	46.0	11 (33)	1 (3)	0.91
% energy from fat	32.1 $\pm$ 6.7	4 (27)	4 (27)	21.9-42.2	14.7 $\pm$ 2.9	3 (9)	9 (27)	0.55
% saturated fat	11.5 $\pm$ 4.6			4.5-18.4	13.4 $\pm$ 3.6			0.13
Total fat (g)	55.8 $\pm$ 19.3			32.3-86.6	65.6 $\pm$ 23.5			0.22
Saturated fat (g)	20.1 $\pm$ 10.9			7.1-38.3	26.2 $\pm$ 9.1			0.79
Monounsaturated fat (g) [Geometric mean – 95% CI]	20.5 (16.6, 25.1)			11.5-38.5	23.1 (19.9, 27.2)			0.20
Polyunsaturated fat (g) [Median IQR]	10.5 (7.2, 11.5)			4.8-16.2	7.2 (6.3, 9.3)			0.20
Protein (g)	59.8 (51.9, 69.8)	0 <sup>c</sup>		47.9-76.1	61.9(54.6,67.0)	0 <sup>c</sup>		
Total sugars (g) [Geometric mean – 95% CI]	72.9 (16.6, 25.0)			42.4-144.4	86.5 (19.7, 27.2)			0.23
Dietary Fibre (g)	22.6 $\pm$ 6.9	7 (47) <sup>d</sup>		14.1-35.5	20.1 $\pm$ 7.8	13 (39) <sup>d</sup>		0.76

<sup>a</sup> Nutrient Reference Values for Australia and New Zealand based children aged 1-3 years and 4-8 years  
<sup>b</sup> Institute of Medicine AMDR recommendations for children (IOM, 2002) AMDR = Acceptable Macronutrient Distribution Range  
1-3 years: AMDR for protein = 5-20%; AMDR for carbohydrate = 45-65%; AMDR for fat = 30-40%  
4-8 years: AMDR for protein = 10-30%; AMDR for carbohydrate = 45-65%; AMDR for fat = 25-35%  
<sup>c</sup> n (%) < Estimated Average Requirement for protein; 12g 1-3 years and 16 g 4-8 years  
<sup>d</sup> n (%) < Adequate Intake for fibre; 14g (1-3 years) and 18 g (4-8 years)

**Table 4.7** Daily micronutrients from dietary sources only for children on an exclusion diet (n=15) and children not on an exclusion diet (n=33) compared with Nutrient Reference Values<sup>a</sup>

	Exclusion diet group			Non-exclusion diet group			p-value
	Mean intake ± SD	n (%) < EAR	Range	Mean intake ± SD	n (%) < EAR	Range	
Vitamin A equivalents (µg)	519.6 ± 317.7	2 (13)	50.5-1342.3	534.0 ± 345.6	9 (27)	102.2-1452.1	0.73
Thiamin (mg) [median, IQR]	1.5 (1.2, 1.8)	0	0.6-3.0	1.7 (1.4, 2.1)	0	0.6-5.1	0.24
Riboflavin (mg)	1.5 ± 0.6	0	0.7-2.2	1.78 ± 0.7	0	0.6-3.5	0.11
Niacin Equivs (mg) [median, IQR]	24.7 (21.4, 27.2)	0	16.9-37.9	24.7 (21.7, 27.4)	0	16.5-53.0	0.92
Vitamin B6	1.6 ± 0.5	0	0.7-2.3	1.6 ± 0.6	1 (3)	0.6-2.7	0.79
Vitamin B12 (µg) [median, IQR]	3.3 (1.9, 3.7)	1 (6)	0.0-267.9	2.6 (1.8, 3.8)	1 (3)	1.0-40.2	0.41
Vitamin C (mg) [Geometric mean – 95% CI]	60.3 (41.7, 88.2)	1 (7)	17.7-188.9	61.5 (45.1, 83.9)	8 (24)	14.2-345.9	0.14
Vitamin D (µg)	1.2 (0.1, 2.2)	14 (93) <sup>b</sup>	0.0-3.8	1.8 (1.2, 3.7)	26 (79) <sup>b</sup>	0.1-9.2	0.08
Vitamin E (mg)	7.5 ± 2.8	4 (27) <sup>b</sup>	4.5-13.9	7.5 ± 3.8	13 (39) <sup>b</sup>	2.6-18.9	0.99
Sodium (mg)	2070	14 (93) <sup>c</sup>	1316-4184	2197 <sup>c</sup>	32 (97) <sup>c</sup>	796-4261	0.95
Potassium (mg)	2280.9 ± 446.3	8 (53)	2004-2983	2220.3 ± 628.6	18 (55)	1126-2852	0.10
Magnesium (mg)	246.3 ± 64.9	0	191.0-415.6	227.6 ± 63.5	0	92.3-333.5	0.36
Calcium (mg)	538.4 ± 361.6	7 (47)	230.1-795.6	737.4 ± 242.6	5 (15)	225.1-119.4	0.03*
Phosphorus (mg)	1012.0 ± 266.1	0	843.2-1224.4	1069.7 ± 228.7	0	493.1-1558.2	0.45
Iron (mg)	10.4 ± 2.4	0	7.8-15.4	11.8 ± 4.2	0	3.1-18.1	0.33
Zinc (mg)	8.7 (7.0, 9.5)	0	5.1-9.9	7.5 (6.5, 9.8)	0	4.8-15.1	0.84
Manganese (µg)	3336.7 ± 951.2	3 (20) <sup>b</sup>	1983.0-5390.3	3256.6 ± 1442.1	9 (27) <sup>b</sup>	582.6-6416	0.85
Copper (mg)	1.0 (0.8, 1.0)	5 (33) <sup>b</sup>	0.7-1.9	0.9 (7, 1.2)	15 (46) <sup>b</sup>	0.5-35.8	0.42
Selenium (µg)	37.6 ± 21.6	2 (13)	10.8-90.5	33.9 ± 12.9	7 (21)	14.4-74.3	0.47

EAR = Estimated Average Requirement; RDI = Recommended Dietary Intake

<sup>a</sup>Nutrient Reference Values for Australia and New Zealand based children aged 1-3 years and 4-8 years

<sup>b</sup>Adequate Intake

<sup>c</sup>n (%) < AI

\*Significant difference between groups (p<0.05) (independent t-test)

### 4.3.3 Food Group Analysis

The number of servings from each food group for children based exclusion diet status are presented in **Table 4.8**. The majority of children in the exclusion diet group (n=10, 66%) and non-exclusion diet group (n=20, 60%) were meeting the guidelines for breads and cereals. A larger proportion of children in the exclusion diet group met the guidelines for servings of fruit when compared with the non-exclusion diet group (13% vs 6%). None of the children in the exclusion diet group met the recommended number of servings of vegetables in either age group, and only one child met the guidelines in the non-exclusion diet group. The majority of children in both exclusion diet and non-exclusion diet groups were receiving adequate servings of meat, fish, eggs and pulses (80% and 64% respectively). Within the exclusion diet group, no children in the 5-8 year old age group were meeting the guidelines for serves of dairy or dairy alternatives, and one child was meeting guideline for 2<5 year olds. One child in the non-exclusion diet group was meeting the guideline and was in the 2-5 year age group.



**Table 4.8** Food group servings for children on exclusion diet (n=15) and not on exclusion diet (n=33) compared to the Food and Nutrition Guidelines recommended daily servings<sup>a</sup>

Food group	Recommended number of servings <sup>a</sup>		Number of servings	Exclusion diet group (n=15)			Non-exclusion diet group (n=33)		
	Age 2<5 years	Age 5-8 years		Age 2<5 years n=7 n (%)	Age 5-8 years n=8 n (%)	No. of children meeting guidelines <sup>a</sup> n (%)	Age 2<5 years n=12 n (%)	Age 5-8 years n=21 n (%)	No. of children meeting guidelines <sup>a</sup> n (%)
Breads, cereals	4+	5+	<2	0	10 (66)	2 (16)	1 (5)	20 (60)	
			2<4	1 (13)		2 (16)	4 (19)		
			4<5	1 (13)		2 (16)	4 (19)		
			5+	6 (75)		6 (50)	12 (57)		
Fruit	2+	2+	<1	6 (75)	2 (13)	9 (75)	9 (43)	2 (6)	
			1<2	2 (25)		3 (25)	10 (47)		
			2+	0		0	2 (10)		
Vegetables	2+	3+	<1	3 (38)	0	9 (75)	13 (62)	1 (3)	
			1<2	4 (50)		2 (17)	5 (24)		
			2<3	1 (13)		1 (8)	3 (14)		
			3+	0		0	0		
Meat, fish, eggs, pulses	1-2	1-2	<1	2 (25)	12 (80)	3 (25)	9 (42)	21 (64)	
			1<2	4 (50)		8 (67)	10 (48)		
			2+	2 (25)		1 (8)	2 (10)		
Dairy and dairy alternatives	2-3	3+	<1	6 (75)	1 (6)	4 (33)	8 (38)	1 (3)	
			1<2	2 (25)		7 (58)	11 (52)		
			2<3	0		0	2 (10)		
		3+	0			1 (8)	0		

<sup>a</sup> Food and Nutrition Guidelines for Healthy Children and Young People (aged 2-18 years)

#### 4.3.4 Reasons for Adopting Exclusion Diets

Parents who had children on an exclusion diet (n=15) were asked to provide their reasons for adopting an exclusion diet for their child (**Table 4.9**). The most commonly selected reason was to improve behaviour (n=10, 67%). Eight parents (53%) reported they adopted an exclusion diet after being recommended to do so by a health professional. Five parents, who had children on a gluten-free, casein-free diet, reported they had received a health professional's recommendation to do so. All five of these parents reported an improvement in behaviour.

**Table 4.9** Parental report of reasons to adopt the use of exclusion diets

Reason	n (%)
To improve behaviour	10 (67)
Due to health professional recommendation	8 (53)
To improve developmental levels	6 (40)
To improve health	4 (27)
Other e.g. allergy diagnosis	3 (20)

\* Parents could report more than one reason

#### 4.3.5 Parental Report of Perceived Improvements due to Exclusion Diets

Parents whose children were on an exclusion diet (n=15), were asked if they had observed any changes in their child since commencing the exclusion diet (**Table 4.10**). The majority of parents (n=10, 67%) reported that they noticed improved behaviour. Eight parents who reported an improvement in behaviour had children on a gluten-free diet. Of the 15 parents, 27% reported no improvement following the use of an exclusion diet.

**Table 4.10** Parental report of perceived improvements due to exclusion diet

Perceived improvement	n (%)
Improved behaviour	10 (67)
Improved communication	6 (43)
Improved sleep pattern	3 (20)
Improved social interaction	3 (20)
No improvement	4 (27)
Other e.g. reduced asthma and eczema symptoms	4 (27)

\* Parents could select more than one improvement

### 4.3.6 Information Source

Parental report of their information source regarding the use of exclusion diets is presented in **Table 4.11**. Advice and information on the use of exclusion diets was obtained from a variety of sources; however the most commonly reported were naturopath, General Practitioner (GP) and a consultant paediatrician who specialises in biomedical treatments (n=4, 27%).

**Table 4.11** Parental report of information source regarding use of exclusion diet

Source	% (n)
Naturopath	4 (27)
GP	4 (27)
Paediatrician specialising in biomedical treatments	4 (27)
Friends and family	3 (20)
Website	3 (20)
Parent support group	3 (20)
Dietitian	2 (13)
Magazine	2 (13)
Coeliac Society	1 (7)

\*Parents could select more than one information source

## 4.4 Use of Supplements

### 4.4.1 Types of Supplements

Parents were asked to provide information on all supplements their children were taking (**Table 4.12**). Nutritional supplement use was recorded by 26 (55%) of parents. The most commonly used supplements were probiotics (n=13) and melatonin (n=12). This was followed by general multivitamin (n=10) and omega-3 fatty acid supplement (n=9). Single micronutrient supplementation use was greatest for vitamin C (n=7) and magnesium (n=7).

When looking at whether the children were also on an exclusion diet, in the non-exclusion diet group (n=31) diet there was almost an equal split between those taking and those not taking supplements (48% vs 52%), whereas in the exclusion diet group (n=15) 68% took supplements compared to 33% not taking them.

However, the difference between the two groups and whether they were taking a supplement, was not significant  $p=0.24$ .

By age group, of the two children in the 1-3 age group and also on an exclusion diet, only 1 child took supplements, while 4 out of the 11 children in this age group and not on an exclusion diet took supplements.

The median (IQR) number of supplements taken by each child was 2.00 (1.00, 4.50) supplements. When comparing by exclusion diet group, children on the exclusion diet group took 5.50 (3.75, 8.00) supplements each, compared to 1.00 (1.00, 2.00) supplements in the non-exclusion diet group. The highest number of supplements taken was 10, this was by a child in the exclusion diet group.

Grouping subjects by whether they took more or less than the median number of supplements ( $\leq 2$  or  $> 2$ ), 90% of those in the exclusion diet group were taking more than 2 supplements compared with only 13% in the non-exclusion diet group,  $p < 0.001$ .

The most commonly taken supplements in the exclusion diet group were multivitamins, probiotics and omega-3. The two children taking calcium supplements were also on a dairy free diet. Whereas in the non-exclusion diet group the most commonly taken supplement was melatonin.

**Table 4.12** Supplement use by parental report <sup>a</sup>

	Total Group n=26 (n)	Exclusion diet group n=10 (n)	Non-exclusion diet group n=15 (n)
<b>Supplement</b>			
Vitamins and minerals			
Multivitamin	10	7	3
Vitamin B <sub>6</sub>	2	2	0
Vitamin B <sub>12</sub>	6	5	1
Vitamin C	7	5	2
Vitamin D	6	6	0
Calcium	2	2	0
Iron	2	1	0
Magnesium	7	5	2
Selenium	1	1	0
LC PUFA			
Omega 3 fatty acids	9	7	2
Other supplements			
Echinacea	1	1	0
Melatonin	12	4	7
Probiotics	13	9	4
Other	8	6	2

LC PUFA = Long chain polyunsaturated fatty acids

<sup>a</sup>Total group includes one participant whom did not respond to the exclusion diet questions

<sup>b</sup>Other includes supplements such as dimethylglycine (DMG), 5-MTHF, and grapefruit extract

#### 4.4.2 Information Source

Parental report of the information source regarding the use of supplements is presented in **Table 4.13**. The most common source of information was from the parents GP (n=9, 35%), followed by websites (n=8, 31%). The two respondents who selected 'other' reported they received their information from a paediatrician.

**Table 4.13** *Information source regarding supplement use by parental report*

<b>Source</b>	<b>n (%)</b>
General Practitioner	9 (35)
Website	8 (31)
Friends and family	7 (27)
Autism New Zealand	6 (23)
Paediatrician specialising in biomedical treatments	4 (15)
Naturopath	3 (12)
Parent support group	3 (12)
Dietitian	2 (8)
Coeliac Society	1 (4)
Magazine	1 (4)
Other e.g. paediatrician	2 (8)

\* Parents could select more than one source of information

#### **4.4.3 Reasons for Using Supplements**

Parents were asked to report their reasons for adopting the use of supplements (**Table 4.14**). The main reasons parents report to use supplements for their children is to attempt to improve their behaviour (n=11, 42%) and prevent nutritional deficiency (n=11, 42%). Of the parents who reported multivitamin use, 63% were using supplements to prevent nutritional deficiency. Eight parents who reported supplement use to improve behaviour were giving their child a probiotic. Three parents who selected 'other' reported the reason they chose to use supplements was to assist with sleep.

**Table 4.14** *Parental report of reasons to adopt the use of supplements*

<b>Reason</b>	<b>n (%)</b>
To improve behaviour	11 (42)
To prevent nutritional deficiencies	11 (42)
To improve developmental levels	9 (35)
Due to health professional recommendation	9 (35)
To improve health	6 (23)
Other e.g. assist with sleep	8 (31)

\*parents could select more than one reason to adopt a supplement

#### 4.4.4 Parental Report of Perceived Improvements Due to Supplements

Improvement in children's behaviour due to supplements as perceived by parents is presented in **Table 4.15**. Parents most commonly reported improved sleep patterns (n=12, 46%) and behaviour improvements (n=11, 42%) in their children. Six parents (24%) report no improvement in their children following the use of supplements. Nine out of ten parents who had children on melatonin supplements reported an improvement to sleep patterns. Of those who reported communication improvements, six were taking a general multivitamin and omega-3 supplement, and eight were also taking a probiotic. All seven of the parents who reported an improvement in social interaction were taking a probiotic.

**Table 4.15** *Parental report of perceived improvements due to supplement*

<b>Perceived improvement</b>	<b>n (%)</b>
Improved sleep pattern	12 (46)
Improved behaviour	11 (42)
Improved communication	10 (39)
Improved social interaction	7 (27)
No improvement	6 (24)
Other e.g. improved health over winter	3 (12)

\*parents could select more than one perceived improvement

## 5.0 Discussion

The aim of this cross-sectional observational study was to investigate dietary intakes, use of exclusion diets and supplements in children with ASD from the VIDOMA study. This included an analysis of macronutrients and micronutrients intakes, and food groups against current dietary recommendations. Secondly, the study reported on types of exclusion diets and supplements being used, reasons for use, perceived improvements, and where parents received information from. Although there have been studies to assess the dietary intakes and use of complementary and alternative medical therapies among this population including exclusion diet and supplements, this is the first study in New Zealand that has been conducted in this area.

### 5.1 Description of Participants

The study population was recruited through the Waitemata District Health Board (WDHB), paediatricians, and advertising through autism support groups and organisations. The initial number of families interested in the VIDOMA study were 176 yet only 67 enrolled. The reason for this was partly due to the blood test required for any child taking part in the trial. Parents were concerned their child would not be able to carry out the blood test due to behavioural aspects relating to ASD. The uneven gender distribution of males and females in this study (86% of participants were male) reflects prevalence data for ASD from overseas (CDC, 2012). The mean  $\pm$  SD age of children in this study was  $5.20 \pm 1.3$  ranging from 2.5-8 years of age. The majority of the children in this study were in the normal BMI category. The number of obese children (10%) in this study were similar to figures reported in the latest data released from the Ministry of Health which found 11% of children aged 2-14 years to be obese (Ministry of Health, 2015). However, our study population was not representative of New Zealand given there were no Māori participants taking part in this study. The latest data from Statistics New Zealand report 15% of the population are Māori (Statistics New Zealand, 2013). Low numbers of Māori receiving ASD-related advice, treatment and information has been discussed in the Ministry of Health New Zealand ASD Guideline (Ministry of Health, 2008). They



report reasons for this including geographic isolation, insufficient knowledge and awareness of ASD and reluctance to seek treatment. Perhaps one or more of these reasons could be a contributing factor to the lack of Māori representation in this study. The majority of parents reported their annual household income to be within the \$60,000 to \$140,000 bracket. Data from Statistics New Zealand reported that the average annual income for year ended 2015 was \$93,880 which falls within the \$60,000 to \$140,000 bracket.

## **5.2 Dietary Intakes**

### **5.2.1 Macronutrient Intakes**

Median energy intakes for children aged 4-8 years (7261 KJ) were similar to the children in the National Children's Nutrition Survey (NCNS) from the 5-6 year age group (7573 KJ for males, 6703 KJ for females)(Ministry of Health, 2003). Energy intake does not appear to be compromised in children with ASD according to other research (Herndon et al., 2009; Hyman et al., 2012; Schmitt et al., 2008).

The Institute of Medicine recommends energy contribution from carbohydrate should be between 45-65% of total energy. Median energy contribution from carbohydrate was within the AMDR (51.8% for 2.5-3 year olds; 48.5% for 4-8 year olds). When comparing with median intakes for 4-8 year old children in this study (48.5% energy contribution from carbohydrate) with 5-6 year old children from the NCNS (53-54% energy contribution from carbohydrate) the children in this study had lower energy contribution from carbohydrate. The majority of children in this study were found to be within the AMDR for mean energy contribution from carbohydrate. Although 14 children (28%) were below the AMDR for carbohydrate, other studies have found children with ASD have a preference for high carbohydrate foods such as cake, French fries, pasta, pizza and ice cream which would suggest this population might have higher intakes of carbohydrate than a typically developing population (Ahearn et al., 2001; Schreck et al., 2004).

Protein intakes did not appear to be of concern as none of the children were below the EAR in either the 2.5-3 year age group or the 4-8 year age group. Other studies that have assessed dietary intakes in children with ASD found no difference in

protein intakes between children with ASD and typically developing children (Emond et al., 2010; Herndon et al., 2009; Hyman et al., 2012; Schmitt et al., 2008).

Although the majority of children in this study (70%) had fat intakes within the AMDR, 11% of children in the 2.5-3 year age group and 29% in the 4-8 year age group exceeded the AMDR for fat. Median energy contribution for saturated fat of children in the 4-8 year age group (12.9% energy contribution) were lower than the findings of the NCNS which showed median energy contribution from saturated fat to be higher for the females and males in the 5-6 year age group (14.5% and 14.4% energy contribution respectively).

Median dietary fibre intakes were above the AI for both age groups. However 44% of children in the 2.5-3 year age group and 40% of children in the 4-8 year age group were not meeting the AI for dietary fibre. When compared to the NCNS which reported median intakes for the children in the 5-6 year age group were 17g for males and 15g for females, median intakes from the children in this study were higher (21.4g for children aged 4-8 years). The higher intakes of children in the 4-8 year old age group could be explained by the fact that the NCNS group did not include 6-8 year olds who would potentially be consuming more dietary fibre. The low intakes of vegetables and fruit by children in this study could explain why children were not meeting the AI for dietary fibre. Dietary fibre is important for bowel health, as well as reducing the risk of cardiovascular disease and diabetes by improving blood lipid profiles and glucose levels (American Dietetic Association, 2008). Dietary fibre might be even more important in children with ASD due to an increased prevalence of GI symptoms such as constipation (Wang et al., 2011).

### **5.2.2 Micronutrient Intakes**

Median calcium intakes for children in this study were found to be above the EAR for both age groups. However, 33% of children in the 2.5-3 year age group, and 25% of children in the 4-8 year age group were not meeting the EAR for calcium. Herndon et al. (2009) found that children with ASD consume significantly less calcium than typically developing children (746mg vs 894mg). The differences in intake were reportedly due to parental dietary restrictions, primarily the gluten-free, casein-free diet. A direct comparison to this study cannot be made because the authors did not report data where they adjusted for age and therefore only reported findings for the

total group which included 2.75-8 year olds. Low calcium intakes in children with ASD have also been found in other studies. Shearer et al. (1982) reported lower intake of calcium compared with typically developing children, however the study numbers were extremely small with 12 children in each group. In contrast to the findings in the current study, Hyman et al. (2012) found no difference in calcium intakes between children with ASD (n=252) and typically developing children (n=252).

Median vitamin D intakes were below the AI for both age groups in this study. It should be noted that the recommended dietary intakes assumes little to no sun exposure, therefore the requirement may be reduced (Ministry of Health, 2012). This study found that 89% of children in the 2.5-3 year age group, and 85% of children in the 4-8 year age group were below the AI for vitamin D. These findings were similar to those reported by Hyman et al. (2012) where 86% of children in the 1-3 age group and 89% in the 4-8 year age group had intakes below the EAR for vitamin D. Currently in Australia it is a mandatory requirement for edible oils, spreads and margarines to contain no less than 55 µg/kg<sup>6</sup> of vitamin D. This mandatory requirement does not yet apply to New Zealand. However vitamin D may be added to cheese, dairy desserts, yoghurts, butter, and edible oils and spreads and sold in New Zealand. Risk of vitamin D deficiency is greatest for people with dark skin (e.g. Middle Eastern, Indian, African peoples), and those with limited sun exposure. Although the results from this study indicate that the majority of children were receiving intakes of vitamin D below the AI, the New Zealand food composition database does not always reflect true vitamin D intakes from foods. The Foodworks database is only as good as the New Zealand composition database which may be lacking up-to-date nutrient information. Furthermore, if nutritional information panels were not supplied by the parents, a substitute brand was used from the Foodworks database. This food substitute may not have been fortified.

Potassium was another nutrient of concern, with over half of children in each age group having inadequate intakes. This is not surprising given the low fruit and vegetable intakes among children in this study.

The majority of children in both the 2.5-3 years and 4-8 year age group exceeded the UL for sodium (89% and 97% respectively). This is of concern given that it has been

suggested that sodium intake in infancy and childhood may carry over to adolescence and beyond (Geleijnse, 1997; Hofman et al., 1983). There is also evidence that early exposure to high sodium intakes increases a child's preference for salty foods as they get older (Mennella, 2014). High sodium intakes increase blood pressure which in turn is a major risk factor for developing cardiovascular disease. Emerging evidence suggests that several chronic conditions including hypertension are more prevalent in adults with ASD compared to adults without ASD (Croen et al., 2015).

However, the assessment of sodium intake by way of food records has methodological flaws given the variability of sodium content in processed foods and the difficulty quantifying total intakes. Although the gold-standard would be to use 24-hour urinary sodium excretion (McLean, 2014) it was not conducted in this study because of the participant burden of carrying out this task. Furthermore, it might be difficult to achieve in some children due to the nature of ASD.

### **5.2.3 Food Group Analysis**

The food and nutrition guidelines recommend 2+ servings of fruit per day and 3+ servings of vegetables. Fruits and vegetables provide a range of vitamins and minerals necessary for health and wellbeing, cancer prevention, bowel health and perform many functions in the body. This study found that consumption of fruit and vegetables among this group of children with ASD to be considerably lower than the recommended daily serves set by the Ministry of Health (Ministry of Health, 2012). Only 14% of children were consuming the recommended number of servings of fruit per day, and no children consumed the number of vegetable serves recommended. These intakes are much lower than the findings reported in the NCNS (Ministry of Health, 2003). Currently, many children in New Zealand do not meet the recommended servings of fruit and vegetable servings. The NCNS found that 43% of children aged 5 to 14 years ate two servings of fruit per day, and 57% ate three or more serves of vegetable per day demonstrating that perhaps children with ASD consume fewer fruits and vegetables than typically developing children.

Adequacy of food group intake has been researched in children with ASD compared with typically developing children. Children with ASD have been found to consume less fruit and/or vegetable servings than children without ASD (Emond et al., 2010;

Johnson et al., 2008; Whitney Evans et al., 2012). Whitney Evans et al. (2012) reported children with ASD (n=53) consumed significantly fewer serves of vegetables (1.3 serves) than 58 typically developing children (2.1 serves). Emond et al. (2010), using a food frequency questionnaire also showed children with ASD consumed few vegetables than typically developing children.

Although children with ASD may be receiving enough energy for growth (and therefore their growth may not be faltering), it is concerning that without adequate servings of fruits and vegetables they may not be receiving adequate vitamins, minerals and dietary fibre necessary for good health and disease prevention.

The majority of children in this study did not meet the recommended serves of dairy foods. Only two children in the 2<5 year age group and no children in the 5-8 year age group met the recommendations outlined by the Food and Nutrition Guidelines for Healthy Children and Young People (aged 2-18 years). If dairy and/or calcium fortified milk alternatives are not part of a child's diet, meeting calcium requirements can be difficult, hence the findings that up to a third of this study population across both age groups were not meeting the EAR for calcium.

## **5.3 Use of Exclusion Diets**

### **5.3.1 Types of Exclusion Diets**

This study found 31% of children to be on an exclusion diet. These findings are similar to other studies. Christon et al. (2010) found 35% of children with ASD to be on an exclusion diet when they surveyed 248 parents of children with ASD. The most commonly used exclusion diet in the current study was the dairy-free diet. Furthermore, many children in our study were on more than one type of diet. The most popular diet combination was gluten-free and casein-free diet. With nearly a third of children with ASD in our study being placed on an exclusion diet, findings suggest that more referrals to a dietitian may be necessary given the challenges faced when excluding one or more food groups.

### 5.3.2 Dietary Intakes of Children according to Exclusion Diet Status

#### *Macronutrient intakes*

Energy and protein intakes did not differ between exclusion diet and non-exclusion diet groups. This was similar to findings found elsewhere (Herndon et al., 2009). A greater proportion of children who were on an exclusion diet (47%) were not meeting the AI for dietary fibre than children who were not on a non-exclusion diet (39%). This study found that 53% of the children on an exclusion diet were on a gluten-free diet. Gluten containing foods such as wholegrain breads and cereals are a good source of dietary fibre in the diet. This study did not determine if high-fibre gluten-free alternatives were used which could be considered a limitation of this study.

#### *Micronutrients*

Calcium intakes were found to be significantly lower for children on an exclusion diet versus the non-exclusion diet group ( $p=0.03$ ). In the comparison of calcium intakes between exclusion diet and non-exclusion diet users, there was a larger proportion of 4–8 year olds (86%) in the exclusion diet group compared with the non-exclusion diet group (78%). The 2.5–3 year old age group proportions were similar between the two groups (22% vs 21%). One would expect the calcium intakes of the exclusion diet group to be higher given the larger proportion of older children, however this was not the case, suggesting the differences may have been even larger if we had been able to stratify based on age groups. Due to the small study numbers it was not possible to split the data by exclusion diet status and age category. Nearly half (47%) of children on an exclusion diet were not meeting the EAR for calcium compared with 15% of those in the non-exclusion diet group. These findings are similar to another study that found intentional diet restriction accounted for the lower intake of calcium in ASD compared to control subjects (Herndon et al., 2009). Dairy products and calcium-fortified alternatives are a good source of calcium (Ministry of Health, 2012). Low calcium intakes throughout childhood and adolescence can lead to low bone mineral density and result in an increased risk of fracture.

Most of the studies assessing dietary intakes have assessed children with ASD versus typically developing children. Herndon et al. (2009) found lower calcium intakes for children with ASD ( $n=46$ ) when compared to typically developing children

(n=31). When they controlled for those on exclusion diets, the low calcium intakes persisted.

There are a number of reasons why calcium intakes could be lower in the exclusion diet group. Firstly, restricting dairy products and not replacing with a suitable calcium fortified alternative could lead to lower intakes. In this study, of the nine children on a dairy-free diet, 4-day food records showed five were receiving a dairy-free calcium fortified alternative such as soy or almond milk. A limitation of this study was that identification of whether parents used a calcium-fortified alternative was achieved by reviewing the 4-day food diary. It would have been more beneficial to add a question in the dietary questionnaire to determine whether dairy-free diet users replaced dairy sources with a calcium-fortified alternative. The significance of these findings are that parents, although they are adopting a diet which they perceive to be doing no harm, are unknowingly restricting their child's calcium intakes.

### **5.3.3 Food Group Analysis**

The majority of children in both the exclusion diet and non-exclusion diet group were found to be meeting the guidelines for breads and cereals. This was not surprising given the literature states that children with autism have a tendency to prefer carbohydrate foods such as bread, rice crackers and snack foods (Ahearn et al., 2001; Williams et al., 2000). Breads and cereals contain grains which are high in B vitamins which would also explain why both groups are meeting their B-vitamin requirements. The proportion of children on an exclusion diet who were on gluten-free alternatives was not determined which could be considered a limitation of this study.

Of parents who reported their child to be a gluten-free, casein-free diet, 2 out of 5 respondents showed gluten-containing foods in their 4-day food records. This indicates that although parents may identify with a certain diet, they may not necessarily comply with it. This could either be knowingly, or they simply do not realise gluten may be in these food products.

Fruit and vegetable consumption was low in both groups, with neither group meeting the recommendations for fruit or vegetable serves. As previously mentioned, fruit and vegetables are a good source of dietary fibre. The lack of fruit and vegetables in



the diets of children with ASD in this study could be contributing to the low dietary fibre intakes.

Neither group met the recommendation for 2-3 serves of dairy per day across both age groups. This is of concern nutritionally, as serves of dairy are important for bone health (Ministry of Health, 2012). Herndon et al. (2009) found that overall children with ASD consumed significantly more non-dairy protein, and significantly fewer servings of dairy than the group who did not have ASD. They reported that when findings were adjusted for parental dietary restrictions, this did not have a substantial effect on the significant  $\pm$  differences for servings of non-dairy protein, and servings of dairy. In contrast to the findings outlined in the present study, Herndon's findings would suggest dietary restriction does not affect the amount of dairy or dairy alternatives being consumed by children with ASD.

#### **5.3.4 Reasons for Adopting Exclusion Diets**

The majority of parents (67%) reported the reason to use an exclusion diet for their child was in order to improve their behaviour and 53% reported it was because of a health professional's recommendation. In other research 32% of parents received medical advice to use an exclusion diet (Christon et al., 2010). A possible explanation for the higher proportion of parents receiving advice from a health profession to use an exclusion in this study, could be due to the fact that the term health professional was not defined in the questionnaire. A health professional is a broad term and unless clearly defined a parents interpretation of defining a health professional may be subjective. Further, it is hard to know whether the physicians specifically encouraged the use of exclusion diet, or did not advise against it when they were asked about its use.

#### **5.3.5 Perceived Improvements from Parents**

Parents reported on their perceived improvements in their child based on the use of an exclusion diet. The majority (67%) of parents reported the main improvement to be the behaviour of their children. Although there is no evidence to support the use of the GFCF diet in improving behavioural symptoms in children with ASD it is interesting that parents perceive an improvement from the use of the diet. Sandler and Bodfish (2000) propose reasons for the placebo effect. Firstly, there may be a placebo effect when parents perceive the treatment to be doing no harm. Secondly,



anecdotal reports of success about the diet from other parents may influence their perception of diet efficacy. Thirdly, a placebo effect may be observed due to the amount of effort required in terms of time and money invested by parents in order to implement the diet. A limitation of this study was that it did not assess the amount of time and effort required from parents to implement an exclusion diet. This could have provided valuable insight. Furthermore, there was no option for parents to report if the diet resulted in an improvement in GI symptoms such as constipation, bloating, or gut irritability. Given that many of the exclusion diets are based on the underlying theory that they reduce GI symptoms, this might have provided further insight presenting another limitation of the study.

### **5.3.6 Information Source for Exclusion Diet Use**

This study showed the most common source of information for exclusion diet use was from a naturopath (27%), GP (27%) and paediatrician specialising in biomedical treatments (27%). These findings are in contrast to studies in other countries. A study conducted in the United States by Smith and Antolovich (2000) reported parents of children with ASD are much more likely to receive their information from other parents and the media when it comes to the use of CAM treatments. Interestingly, a study conducted in the United States found that 53% of parents reported they would like to discuss alternative therapies such as exclusion diets and supplement use with their paediatrician, however only 36% of these parents had discussed with their paediatrician (Sibinga, 2004). The proliferation of internet based communities discussing and promoting the use of treatments not supported by any concrete evidence is of concern. Furthermore, 75% of health professionals had been asked for advice about the GFCF diet (Winburn et al., 2014) indicating a need for health professionals to be prepared for questions from parents regarding exclusion diets.

## **5.4 Use of Supplements**

### **5.4.1 Types of Supplements**

Findings from the current study suggest that supplement use among this study population is high, specifically non-nutritional supplementation. When compared to the NCNS where 5% of children were identified as consuming supplements, 26

children (55%) from the current study were taking supplements. However, supplementation practices of the general population may have changed since its publication date in 2002. The most commonly used supplements were probiotics (n=13) and melatonin (n=11). In another study, these supplements were reported to be the most helpful according to parents (Owen-Smith et al., 2015). Older research reports probiotic use among this population to be 20.5% (Green et al., 2006). The higher percentage of probiotic users for the current study along with other more recent studies, is not surprising given in recent years, the prevalence of use of probiotics among the general population has increased dramatically (Clarke et al., 2015).

In this study, multivitamin use was recorded by 20% of parents. This is lower than the numbers recorded in a recent study by Owen-Smith et al. (2015) where they found 43.7% of parents with a child who has ASD to be using a multivitamin. While a multivitamin may be necessary for those children who exhibit selective eating and do not have a varied diet, the results from this study indicate the only nutrients of concern were calcium intakes, exacerbated by the use of an exclusion diet such as the casein-free diet.

The effect of supplement intake on meeting/exceeding dietary requirements was not examined in the current study due to the limitation of low study numbers. With larger study numbers, it would be possible to examine under and over consumption of individual nutrients. The effect of supplement intake has been examined previously in other studies, up until recently only in the general paediatric population (Bailey et al., 2013; Dwyer et al., 2013). These studies showed that supplement use does not correct micronutrient deficiencies and can lead to excessive intake for certain nutrients. A study conducted by Stewart et al. (2015) was the first study to assess micronutrient intake from both food and supplements in children with ASD and showed similar results to Bailey et al. (2013) and Dwyer et al. (2013). Similarly to the current study, Hyman et al. (2012) also found that children with ASD are given supplements more frequently when compared with the general population.

#### **5.4.2 Information Source**

Parents most commonly reported the GP as their information source regarding supplementation (n=9, 35%). This was greater than the number of parents receiving

information from GP regarding the use of exclusion diet. Of concern is that 31% of parents received information regarding supplements from the internet. It was unclear whether children in this study were supplementing their children under the supervision of a medical professional which may have been useful to know.

#### **5.4.3 Supplement Use by Exclusion Diet Status**

Supplementation was common among the exclusion diet group (68%) which was greater than the proportion of children in the non-exclusion diet group taking supplements (52%), although this difference was not significant ( $p=0.24$ ). The majority of children in the exclusion diet group (90%) were found to take more than 2 supplements compared with only 13% in the non-exclusion diet group ( $p=0.001$ ). Further, the most commonly taken supplement for those on an exclusion diet was a multivitamin supplement ( $n=7$ , 70%) where only 3 (20%) parents used a multivitamin for their children in the non-exclusion diet group. Parents of children on an exclusion diet may be more concerned about nutritional deficiencies than those who were in the non-exclusion diet group given the more frequent use of a multivitamin supplement. However only 4 out of 7 parents in the exclusion diet group (57%) using a multivitamin reported they used supplements to prevent nutritional deficiencies.

Parental report of perceived improvements due to supplementation and exclusion diet was varied. It was difficult to know how parents decided whether the exclusion diet or supplement had led to the improvement. This study did not find out if parents were undecided as to which treatment had provided benefit if two treatments had been introduced at the same time which could be considered another limitation of this study.

## 6.0 Conclusion

### 6.1 Summary of the Study

This study was designed to investigate the dietary intakes and use of exclusion diets/supplements in children with ASD. Fifty children with a clinical diagnosis of ASD were included in this study. Four-day food diaries and dietary questionnaires were used to collect information regarding diet and supplement use. Statistical analysis using independent t-tests, Mann-Whitney tests and Fishers Exact tests were used. A  $p$ -value of  $<0.05$  were considered significant.

The primary objectives of this study were to assess the dietary intakes of children with ASD and compare intakes against current dietary recommendations. Results of this study found that there are nutrients of concern in the diets of children with ASD. These nutrients include vitamin A, C, D, E, potassium, and calcium. Children were not meeting the AI recommendation for dietary fibre. Sodium was found to be consumed in excess of the upper level recommendation. Children in this study were not meeting the guidelines for recommended serves of fruit, vegetables and dairy. Only one child met the guidelines for servings of vegetables, six children for fruit, and two children for dairy or dairy alternatives. Therefore, the alternative hypothesis ( $H_1$ ) that children with ASD will not be meeting current dietary recommendations can be accepted.

The third objective was to determine the use of exclusion diets and supplements in children with ASD. Exclusion diets were used by 31% of children, the most popular was the dairy-free diet, followed by the gluten-free diet. This study found that 55% of children were using supplements. When compared with typically developing children from the National Children's Nutrition Survey only 5% were taking supplements. This demonstrates supplement use in the children with ASD in this study is high. The median (IQR) number of supplements taken by children in this study was 2.0 (1.0, 4.5) supplements, with the highest number of supplements (10 supplements) was taken by a child in the exclusion diet group. When comparing supplement use by exclusion diet status, children on an exclusion diet took 5.5 (3.7, 8.0) supplements each which was higher than those not on an exclusion diet who took 1.0 (1.0, 2.0) supplements. When examining then number of children taking more or less than the

median number of supplements (<2 or >2), it was found that 90% of children in the exclusion diet group took more than two supplements compared with only 13% in the non-exclusion diet group ( $p<0.001$ ). The most commonly used supplements for the total group were non-nutritional supplements: probiotics (26%) and melatonin (24%). The alternative hypothesis that children will be using a range of different types of exclusion diets and/or nutritional/non-nutritional supplements can be accepted.

The fourth objective was to determine reasons for use of exclusion diets and/or supplements. The majority of parents (67%) reported they were using exclusion diet to improve their child's behaviour. Further, 42% of parents reported they were using supplements to improve their child's behaviour. The alternative hypothesis that parents will use exclusion diets and supplements for their child to ameliorate symptoms of ASD can be accepted

The fifth objective was to investigate the impact of exclusion diets on dietary intakes in children with ASD. Calcium intakes were found to be significantly lower for those in the exclusion diet group than those in the non-exclusion diet group ( $p=0.03$ ). A greater proportion of children in the exclusion diet group ( $n=7$ , 47%) were not meeting the EAR for calcium than children in the non-exclusion diet group ( $n=5$ , 15%). Vitamin D intakes were found to be low in both the exclusion diet and non-exclusion diet groups. The vitamin D intakes were lower for children in the exclusion diet group. The difference between groups was approaching significance ( $p=0.08$ ). The alternative hypothesis that children on exclusion diets will not be meeting current dietary recommendations can be accepted.

## **6.2 Strengths and Limitations**

### *Study population*

To our knowledge this is the first known study in New Zealand that has assessed the dietary intakes and exclusion diet and supplement use in a group of children with ASD. This study adds to the paucity of research pertaining to the dietary intakes in children with ASD.

This study was part of the larger VIDOMA trial. Therefore, only attracting families who wanted in take part in the VIDOMA study which involved supplementing children

with omega-3 fatty acids and vitamin D. Given one of the objectives of this study was to look at supplement use; this could have caused some bias.

The small sample size and no representation of Māori in this study make it difficult to generalise to children with ASD living in New Zealand. Furthermore, due to small study numbers, any child who was on some kind of restriction diet was grouped together. Had there been larger numbers, it would have been valuable to stratify those on particular diet such as the gluten-free, casein-free diet in order to examine the effects of individual diets on dietary intakes. A larger study would also enable an examination of dietary intakes by sex.

Although there were limitations regarding the study population for this study, findings do highlight the need to undertake further studies to identify the dietary intakes and use of exclusion diets/supplements in a group of children with ASD that are representative of this population in New Zealand.

#### *Comparison to the National Children's Nutrition Survey*

A strength of this study was that a comparison where possible was made between the findings from this study against the median dietary intakes reported in the NCNS. This enabled a comparison to be made between children with ASD in this study to a population of typically developing children. A limitation however is that although the NCNS is the most current data available in New Zealand, it was published in 2002, so there may have been a shift in dietary intakes. Furthermore, it was difficult to make a direct comparison because the NCNS reports their findings for 5-6 year age groups, where the groupings in this study were based on the nutrient reference value age groups which are 1-3 years and 4-8 years. This could have resulted in higher intakes for the 4-8 year old versus the 5-6 year olds from the NCNS due to the broader age range.

#### *Use of nutrient reference values*

A strength of this study is that nutrient intakes were compared to the Estimated Average Requirement (EAR) where possible. This has been documented as the best estimate of an individual's requirement (Murphy & Poos, 2002). Despite this recommendation, other studies have compared intakes to the Recommended Dietary Intake (RDI). This study also reported on proportions of the group which had

inadequate intakes which provides more insight than looking at mean intakes versus recommendations in isolation.

#### *Use of BMI*

This study used BMI to determine body size of children with ASD in this study. In order to compare to the NCNS, this study also used International Obesity Task Force (IOTM) BMI cut-off points (Cole, 2007; Cole et al., 2000). This enabled a comparison to be made between children with ASD in this study to a population of typically developing children from the NCNS. However, there are limitations of using BMI for assessing body fatness. Firstly, it does not distinguish between fat and lean body mass (LBM). Individuals with the same BMI could have different proportions of fat and LBM (World Health Organisation, 2000). Furthermore, there may be ethnic differences in the ratio of body fat to LBM. For example, Pacific populations have been found to have a higher proportion of LBM (Swinburn et al., 1999).

#### *Foodworks database*

When determining nutrient intake for those on exclusion diets using Foodworks, the use of gluten-free food products and other special foods are not routinely indexed in the available database. Using a food product with similar nutritional profile was the standard procedure. For example, if a gluten-free cereal was not available, a cereal with a similar nutrition profile was chosen. Many breakfast cereals available today (and within the Foodworks database) are fortified. Where possible, non-fortified alternatives were used. Substituting a conventional fortified food product when conducting the nutrient analysis may have resulted in an overestimation of certain nutrients. Therefore, the study may not have identified true nutrient deficits of the exclusion diet group.

As mentioned previously, the New Zealand food composition database which was used in Foodworks does not always reflect true vitamin intakes from foods. The Foodworks database is only as good as the New Zealand composition database which may be lacking up-to-date nutrient information.



### *The use parental report*

Another limitation in data collection is the use of parental report to obtain information. Caution should be used in interpreting parents ideas of efficacy of treatments (Christon et al., 2010; Goin-Kochel et al., 2009). When asking a parent whether they think their child made improvements based on adopting an exclusion diet or supplement, leaves room for subjective opinion. Further to this, the questionnaire did not have a rating to the extent to which they believed their child had improved/not improved. This means some parents might have noticed only a small change, but was not able to quantify that change in this study. Furthermore, relying on parental report for indication of diet use may not be the best method. As noted previously, some parents reported their child to be undertaking a particular exclusion diet yet their 4-day food diary revealed otherwise. The only way to confirm strict adherence to a particular diet would be to use blood tests (e.g. serum IgA levels) in addition to the food diaries.

### *Non response*

Despite efforts to obtain responses for missing data, in some cases this was not possible following multiple phone and email contacts with parents. As mentioned previously, the burden of caring for a child with ASD has a substantial effect on the family. Parents and siblings of children with ASD are likely to experience depression, anxiety and stress (Bagenholm & Gillberg, 1991). This could be the reason why so many families showed an interested in taking part in the study, however logistics of day to day life and fulfilling commitments to research were potentially difficult for families. This could also explain why only 50 parents returned the completed food diary.

### *Dietary questionnaire*

Although the questionnaire was piloted on two mothers, they did not have a child or children with ASD. It may have been more beneficial to pilot the questionnaire on a group of parents who had children with ASD.

The questionnaire did not define terms for the parents. For example, "health professional" is a broad term and unless clearly defined a parents interpretation of defining a health professional may be subjective. Furthermore, there was no option



for parents to report if the diet resulted in an improvement in GI symptoms such as constipation, bloating, or gut irritability. Given the research that has been conducted in this area, it would have been useful to know.

There were many instances where parents did not complete the questionnaire correctly. For example, some parents responded that they did not use supplements yet responded to questions relating to information sources of supplements. Furthermore, some parents only completed half the questionnaire before returning it for analysis. The use of an online survey programme such as SurveyMonkey as opposed to a paper copy would ensure complete and correct data capture.

#### *Use of four-day food diaries*

A strength of this study was the use of four recording days for the food diaries. The use of four to five days in recording dietary intakes has been found to be the ideal duration (Stram et al., 1995). Increasing the number of recording days results in an increased participant burden and less accurate data (Stram et al., 1995; Willet, 1998). This could be even more relevant to the current study population in that the parents may already be time-poor and stressed in caring for their child with ASD. Therefore, limiting the number of days required for recording could have helped with response rates.

Although there were strengths of using this method, limitations can also be noted. Parents were asked to weigh food where possible; however an estimation could also be made using household measures. Furthermore, some parents who had children at kindergarten or school could only report intakes based on foods left over at the end of the school day. This could have meant an under or overestimation of foods consumed resulting in inaccurate dietary data.

#### *Dietary assessment of certain nutrients*

This study identified a large proportion of children were not meeting the Average Intake (AI) recommendations. Assessing vitamin D status through dietary assessment has its limitations. As previously mentioned, food composition data does not always reflect true vitamin D content in foods. Furthermore, vitamin D status is greatly influenced by sun exposure (Haddad & Hahn, 1973). The best indicator of

vitamin D status is the concentration of serum 25-hydroxyvitamin D which would require a blood test to be undertaken.

### **6.3 Recommendations for Future Studies**

A larger study population would mean that a more detailed analysis could be carried out. Although information regarding the ASD severity of children in the study was available, there were not enough study numbers to stratify the data this way. Further, stratifying by diet use could also be helpful to assess the impact of individual diets.

While examining the 4-day food diaries for the present study, a reoccurring theme was a high proportion of children's diets contained high energy snack food such as potato crisps, baked snacks, fruit roll-ups and popcorn. While this was beyond the scope of this thesis, an examination into the effect of selective eating on a population of children with ASD in New Zealand would be valuable.

It might have been helpful to know how much money parents are spending on treatments and exclusion diets. Parents of children with ASD who use exclusion diets and/or supplements have been reported elsewhere to be spending greater amounts of time and money on adopting these treatments than if they were to go with mainstream dietary advice (Goin-Kochel et al., 2009).

This study assessed dietary intakes from food sources only. A study which assesses dietary intakes including supplements could be beneficial to determine if children with ASD are receiving nutrients in excess due to supplement regimes.

Furthermore, an understanding of health professionals knowledge of exclusion diets could be a helpful insight. This could identify areas where more tools and supports are needed by health professionals which would enable them to guide and inform families of children with ASD who wish to use an exclusion diet.

### **6.4 Conclusion**

Results of this study suggest that children with ASD are not meeting the daily recommended servings for various food groups including fruits, vegetables and dairy and dairy alternatives. Although energy intakes were not impaired, certain nutrients

in the diets of children with ASD in this study were lacking, specifically calcium and vitamin D. As a consequence children with ASD may not receive a dietetic referral unless their growth is faltering and nutritional deficiencies may go unnoticed.

In addition, the use of exclusion diets in this population was high and results showed their use may attenuate nutrient intakes specifically calcium intakes. This suggests a need for dietetic referrals for any child with ASD on an exclusion diet, not just children who have had a positive coeliac test.

Furthermore, the use of supplements in this population was high. Although this study did not assess the impact of supplementation on dietary intakes, results indicated that although the majority received information regarding supplements from their GP, nearly a third received information from websites. This indicates a need for routine assessment from GP's to assess if any supplements are being taken and if so, a referral to the dietetic service may be required to assess the supplement intakes in the context of the child's diet.

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## Appendices

### *Appendix A: Four-day Food Diary*

## The VIDOMA Study



### **4 Day Food Record**

***Thank you very much for taking part in this study. We are extremely grateful for your time, effort and commitment***

*If you have any questions, please contact Owen Mugridge on 09 213 6650*

*All information in this diary will be treated with the strictest confidence. No one outside the study will have access to this.*

*Please bring the food diary with you when you bring your child in for assessment at Massey University.*

## **4 day food diary - what to do?**

- Record all of the food that your child eats and drinks on the following dates.
- **Please complete the diary on consecutive days for 1 weekend day and 3 week days. For example, Sunday, Monday, Tuesday and Wednesday OR Wednesday, Thursday, Friday and Saturday.**
- If possible record food at the time of eating or just after – try to avoid doing it from memory at the end of the day.
- Include all meals, snacks, and drinks, even tap water.
- Include anything you have added to foods such as sauces, gravies, spreads, dressings, etc.
- Write down any information that might indicate size or weight of the food to identify the portion size eaten.
- Use a new line for each food and drink. You can use more than one line for a food or drink. See the examples given.
- Use as many pages of the booklet as you need.
- You can also save any packets such as muesli bar wrappers and bring them in with your child's food diary
- Please answer the short questionnaire at the back of this booklet regarding your child's diet

### **Describing Food and Drink**

- Provide as much detail as possible about the type of food eaten. For example **brand names and varieties / types** of food.

General description	Food record description
---------------------	-------------------------

Breakfast example – cereal, milk, sugar	2 Weetbix (Homebrand) 1 cup Pam's whole milk 1 tsp Chelsea white sugar
Lunch – Ham sandwich	2 slices of wholegrain bread (Vogels) 1 slice ham 2 slices edam cheese 2 tsp flora margarine Water 1 cup to drink
Dinner – Spaghetti Bolognese	½ cup mince sauce (see attached recipe) 1 cup spaghetti pasta (Homebrand) Milk 1 cup Pam's whole milk
Snacks	Flemmings apricot chocolate chip muesli bar (35g) 1 small banana 2 Salada crackers with 1 tsp peanut butter Small packet of Bluebird salt and vinegar chips

- Give details of all the **cooking methods** used. For example, fried, grilled, baked, poached, boiled...

General description	Food record description
2 eggs	2 size 7 eggs fried in 2tsp canola oil 2 size 6 eggs (soft boiled)
Fish	100g white fish pan-fried

- When using foods that are cooked (eg. pasta, rice, meat, vegetables, etc), please record the **cooked portion** of food.

General description	Food record description
Rice	1 cup cooked Jasmine rice (cooked on stove top)
Meat	½ cup of casserole beef or 5 chicken nibbles in honey soy marinade
Vegetables	½ cup cooked mixed vegetables (Wattie's peas, corn, carrots)

- Please specify the **actual amount of food eaten** (eg. for leftovers, foods where there is waste)

General description	Food record description
Apple	1 x 120g Granny Smith Apple (peeled, core not eaten – core equated to ¼ of the apple)
Fried chicken drumstick	100g chicken drumstick (100g includes skin and bone); fried in 3 Tbsp Fern leaf semi-soft butter

General description	Food record description
Milo	1 x cup Milo made with Milo powder and 150mls Calci-trim milk, 100 ml hot water. No sugar

- **Record recipes** of home prepared dishes where possible and the proportion of the dish your child ate. There are blank pages for you to add recipes or additional information.



## Recording the amounts of food your child eats

It is important to also record the quantity of each food and drink consumed. This can be done in several ways.

- By using household measures – for example, cups, teaspoons and tablespoons. Eg. 1 cup frozen peas, 1 heaped teaspoon of sugar.
- By weight marked on the packages – e.g. a 425g tin of baked beans, a 32g cereal bar,
- Weighing the food – this is an ideal way to get an accurate idea of the quantity of food eaten, in particular for foods such as meat, fruits, vegetables and cheese.
- For bread – describe the size of the slices of bread (e.g. sandwich, medium, toast) – also include brand and variety.
- Using comparisons – e.g. Meat equal to the size of a pack of cards, a scoop of ice cream equal to the size of a hen's egg.
- Use the food record instructions provided to help describe portion sizes.

General description	Food record description
Cheese	1 heaped tablespoon of grated edam cheese 1 slice cheese edam (8.5 x 2.5 x 2mm) 1 cube edam cheese, match box size

- If you go out for meals, describe the food eaten in as much detail as possible.
- ***Please try to have your child eat as normally as possible – ie. Don't adjust what he/she normally eats just because you are keeping a diet record and be honest! This record will give us important information about your child's diet, and help us identify any possible deficiencies which we can then help you correct.***



Example day

<b>Time food was eaten</b>	<b>Complete description of food (food and beverage name, brand, variety, preparation method)</b>	<b>Amount consumed (units, measures, weight)</b>
<i>Example</i> 7:55am	Sanitarium Weetbix	2 weetbix
" "	Anchor Blue Top milk	150ml
" "	Chelsea white sugar	2 heaped teaspoons
" "	Orange juice (Citrus Tree with added calcium – nutrition label attached)	1 glass (275 ml)
10.00am	Raw Apple (gala)	Ate all of apple except the core, whole apple was 125g (core was ¼ of whole apple)
12.00pm	Home made pizza (recipe attached)	1 slice (similar size to 1 slice of sandwich bread, 2 Tbsp tomato paste, 4 olives, 2 rashers bacon (fat removed), 1 Tbsp chopped spring onion, 3 Tbsp mozzarella cheese)
1.00pm	Water	500ml plain tap water
3.00pm	Biscuits	6 x chocolate covered Girl Guide biscuits (standard size)
6.00pm	Lasagne	½ cup cooked mince, 1 cup cooked Budget lasagne shaped pasta , ½ cup Wattie's creamy mushroom and herb pasta sauce, ½ cup mixed vegetables (Pam's carrots, peas and corn), 4 Tbsp grated Edam cheese
6.30pm	Banana cake with chocolate icing (homemade, recipe attached)	1/8 of a cake (22cm diameter, 8 cm high), 2 Tbsp chocolate icing
" "	Tip Top Cookies and Cream ice cream	1/2cup (g) (125g)




Date \_\_\_\_\_

DAY 1 continued

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed





## Appendix B: Dietary Questionnaire

The following questions will enable us to find out information about any exclusion diets and supplements you might have your child on.

1. Is your child on an exclusion diet? Yes      No
  
2. If yes, which of the following diets is your child currently on?
  - Gluten free
  - Dairy free
  - Yeast free
  - Sugar free
  - Egg free
  - Gluten-free/dairy free
  - Specific Carbohydrate diet
  - Feingold diet
  - Low GI diet
  - Additive and preservative free
  - Caffeine free
  - Other \_\_\_\_\_  
\_\_\_\_\_
  
3. If you selected any of the above diets, what were your reasons for adopting the use of a exclusion diet?
  - To improve your child's health and behaviour
  - To improve your child's health
  - To improve developmental levels
  - Health professionals recommendation
  - Other \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_
  - N/A
  
4. What changes did you observe, if any, in regards to the change in your child's diet?
  - Improved behaviour
  - Improved communication skills
  - Improved sleep pattern
  - Improved social interaction
  - Other \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

- No notable changes
- N/A

5. If you selected any of the above diets, please advise where you received your information regarding the exclusion diet. (Tick all those that apply)

- TV
- Website
- Magazine
- Book: please specify name -

- 
- Parent support group
  - Relatives and friends
  - Your GP
  - Dietitian
  - Autism NZ
  - Celiac Society
  - Alternative health professional – Naturopath
  - Other \_\_\_\_\_

6. Is your child on any supplements? YES NO

7. If yes, Please give information on any supplements your child is on. (Tick all those that apply)

- Omega 3 capsules
- Probiotics
- Vitamin C
- Vitamin B12
- Vitamin B6
- Vitamin D
- Selenium
- Iron
- Magnesium
- Melatonin
- General multi-vitamin
- Iron
- Calcium
- Echinacea
- Other \_\_\_\_\_
- N/A

8. Where have you received information from regarding the use of supplements? (Tick all those that apply)

- TV
- Internet
- Magazine
- Parent support group
- Relatives and friends
- Your GP
- Dietitian
- Autism NZ
- Celiac Society
- Other \_\_\_\_\_

9. What were your reasons for using supplements?

- To improve your child's health and behaviour
- To improve your child's health
- To improve developmental levels
- Health professionals recommendation
- To prevent nutritional deficiencies
- Other \_\_\_\_\_
- N/A

10. What changes did you observe, if any, in regards to the use of supplements that your child is taking?

- Improved behaviour
- Improved communication skills
- Improved sleep pattern
- Improved social interaction
- Other \_\_\_\_\_
- No notable changes
- N/A



Appendix C: Contact Details Sheet



MASSEY UNIVERSITY  
COLLEGE OF HEALTH  
TE KURA HAUORA TANGATA

## The VIDOMA Study

### CONTACT DETAILS SHEET

Parent/ guardian name: \_\_\_\_\_

First name

Family name

Child's name: \_\_\_\_\_

First name

Family name

Child's gender (Please tick):    Male        Female   

Child's Date of birth: \_\_\_\_\_

Child's age: \_\_\_\_\_

Child's NHI numbers: \_\_\_\_\_

Daytime telephone number: \_\_\_\_\_

Mobile number: \_\_\_\_\_

Email address: \_\_\_\_\_

Address (for the delivery of supplements and information – if you move house during the trial, please let us know)

House Number: \_\_\_\_\_

Street Name: \_\_\_\_\_

Suburb: \_\_\_\_\_

City: \_\_\_\_\_

Postcode: \_\_\_\_\_

GP Name: \_\_\_\_\_

GP Address: \_\_\_\_\_

GP Contact Number: \_\_\_\_\_

OK for us to send results:

*Appendix D: Medical History Questionnaire*



**MASSEY UNIVERSITY**  
COLLEGE OF SCIENCES  
TE WĀHANGA PŪTAIAO

**The VIDOMA Study**

**Medical History**

**Confidentiality**

Your personal information will be kept in a secure location separate from the main questionnaire data.

Your answers are completely confidential. No personal information such as your name or address will be shared with any other individual or agency.

**1. Does your child have or has your child had any chronic (that is ongoing) medical conditions?**

**E.g asthma, eczema, allergies, ear or throat infections**

If yes, please describe: \_\_\_\_\_

**2. Is your child currently taking any medication?**

If yes, please list (medication name & dosage, reason for taking) \_\_\_\_\_

\_\_\_\_\_

**3. Is your child currently having any therapy related to autism, such as behavioural or speech therapy?**

If yes, please describe it (what type of therapy, who with, how often) \_\_\_\_\_

\_\_\_\_\_

**4. Has your child ever had or been treated for low iron stores, iron deficiency or iron deficiency anaemia?**

Diagnosis, date, diagnosed by, any further details: \_\_\_\_\_

\_\_\_\_\_

**5. Has your child had any blood loss in the past 6 months**

**e.g. from medical conditions, injuries, nose bleeds, etc**

If yes, please describe

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**6. During pregnancy, did the mother of the child experience any infections or illnesses?**

e.g gestational diabetes, iron-deficiency anaemia, severe morning sickness

Yes

No

If yes, please specify condition

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**7. After birth, did the child experience any infections, illnesses or anything else of note?**

e.g jaundice, respiratory tract infections, ear infections, rickets

Yes

No

If yes, please specify condition

---

**8. Which ethnic group or groups does your child belong to?:**

New Zealand European

Maori

Pacific  Please specify \_\_\_\_\_

South Asian

Chinese

Korean

Southeast Asian  Please specify \_\_\_\_\_

Other ethnicity  Please specify \_\_\_\_\_

**9. In the last 12 months what was your annual household income (after tax)?**

Below \$60,000

\$60,000 – 140,000

Over \$140,000

10. How many other children live in your household? \_\_\_\_\_

11. What are their ages? \_\_\_\_\_

12. Have any of your other children been diagnosed with autism? Yes  No

---

13. If your child was breastfed, how long were they exclusively breastfed for?

Not breastfed

Exclusively breastfed for \_\_\_\_\_ months

14. During the first 18 months of life, which, if any, of the following did your child regularly consume:

Infant formula

Follow-on formula or toddler milk

Blue top cow's milk

Orange top cow's milk

Water

Juice

*Appendix E: IOTF BMI Cut-off Points*

International Obesity Task Force BMI cut-offs for children and young people (aged 2-18 years)

Age	BMI 18.50		BMI 25.00		BMI 30.00		BMI 35.00		BMI 40.00	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
2	15.24	14.96	18.36	18.09	19.99	19.81	21.20	21.13	22.12	22.16
2.5	15.02	14.77	18.09	17.84	19.73	19.57	20.95	20.90	21.89	21.96
3	14.83	14.60	17.85	17.64	19.50	19.38	20.75	20.74	21.72	21.83
3.5	14.66	14.44	17.66	17.48	19.33	19.25	20.61	20.65	21.62	21.78
4	14.51	14.30	17.52	17.35	19.23	19.16	20.56	20.61	21.61	21.81
4.5	14.38	14.16	17.43	17.27	19.20	19.14	20.60	20.67	21.73	21.94
5	14.26	14.04	17.39	17.23	19.27	19.20	20.79	20.84	22.03	22.23
5.5	14.15	13.93	17.42	17.25	19.46	19.36	21.15	21.16	22.57	22.70
6	14.06	13.85	17.52	17.33	19.76	19.61	21.69	21.61	23.35	23.37
6.5	14.00	13.81	17.67	17.48	20.15	19.96	22.35	22.19	24.32	24.21
7	14.00	13.83	17.88	17.69	20.59	20.39	23.08	22.88	25.38	25.19
7.5	14.05	13.90	18.12	17.96	21.06	20.89	23.83	23.65	26.44	26.28
8	14.13	14.00	18.41	18.28	21.56	21.44	24.61	24.50	27.55	27.49

Source: (Cole, 2007; Cole et al., 2000; Cole & Lobstein, 2012)