Dietary Modification in the Management of Autistic Spectrum Disorders (ASD).

A non-randomized Intervention Study.

'A major project submitted in partial fulfilment for the award of the degree Masters of Science (Nutrition & Dietetics), University of Wollongong.'

Elizabeth Parker
Student #2873746

Department of Biomedical Sciences
University of Wollongong

Table of Contents

Acknowledgements……..p3
List of Figures……...p4
List of Tables……p4
List of Appendices……..p5
ABSTRACT…….....p6
1. INTRODUCTION…….p7
2. METHODS
2.1 Previous Research……p9
2.2 Study Design……...p9
2.3 Recruitment & Selection …….....p10
2.4 Ethics Approval…….....p10
2.5 Consent & Confidentiality……..p10
2.6 Questionnaires…….....p11
2.7 Straw Poll……p12
2.8 Data Analysis……..p12
3. RESULTS
3.1 General Characteristics……p13
3.2 Follow-Up……p14
3.3 DASS-42…….....p15
3.4 CPRS-48……... ……...p15
3.5 CEBASS-50……...……....p15
3.6 PDDBI-C……p16
3.7 Straw Poll…….....p18
4. DISCUSSION…….....p19
5. CONCLUSIONS/RECOMMENDATIONS……..…….....p23
6. REFERENCE LIST……..…...p24
7. APPENDICES…….....p27

Acknowledgements

This research was conducted under the supervision of Dr Anne Swain, Dr Velencia Soutter, and Dr Robert Loblay at the RPAH Allergy Unit. I wish to express my deepest appreciation to my supervisors for their constant guidance and patience, and generous provision of facilities and funding for this project. I feel incredibly privileged to have been given the opportunity to learn from the expertise and knowledge you all have in this field. A sincere thank you to Tim Watkins for his work in developing the study database and to Marijka Batterham for her assistance in statistical analyses. I would like to extend my appreciation to my student colleagues for their support. Finally I would like to thank the parents who gave their time to participate in this study and for their open honesty.

List of Figures

Figure 1 – Participants Undertaking Dietary Modification

Figure 2 – Improvement in Behavioural and GI Symptoms

Figure 3 – Improvement in Maladaptive Behaviours

Figure 4 – Improvement in Adaptive Behaviours

List of Tables

Table 1 – Overall Parent Rated Improvement

Table 2 – Straw Poll Results

List of Appendices

Appendix A – Ethics Approval

Appendix B – Information for Parents

Appendix C – Expression of Interest Form (EOI)

Appendix D – Parent Depression Anxiety & Stress Scale (DASS-42)

Appendix E – Connors' Rating Scales (CPRS-48)

Appendix F – The PDD Behaviour Inventory (PDDBI) – Renamed 'Social Interaction & Communication Skills'

Appendix G – Children's Eating Behaviour & Appetite Scale (CEBAS-50)

Appendix H – Follow-up Questionnaire

Appendix I – Change in Behaviour & GI Symptoms within groups

Appendix J – Change in Behaviour & GI Symptoms between groups

Appendix K – Change in DASS-42 subscale scores within groups

Appendix L – Change in DASS-42 subscale scores between groups

Appendix M – Change in CPRS-48 subscale scores within groups

Appendix N – Change in CPRS-48 subscale scores between groups

Appendix O – Change in CEBASS-50 scores within groups

Appendix P – Change in CEBASS-50 scores between groups

Appendix Q – Change in PDDBI-C subscale scores within groups

Appendix R – Change in PDDBI-C subscale scores between groups

Abstract

In the absence of a known cause or cure for autism, attention has been focused on strategies to treat the associated symptoms. Dietary modification has emerged as a possible 'alternative treatment' for autism and related spectrum disorders. It has been suspected that autistic children may experience increased food sensitivity to a wide range of foods, and dietary exclusion of suspect foods may result in behavioural improvements and decreased gastrointestinal symptoms. The gluten- and casein-free diet is one such diet that has gained strong parental interest and support, despite the lack of strong large-scale evidence to support its effectiveness at improving behaviour. The primary aim of this study was to investigate the effectiveness of dietary modification at reducing behavioural and health problems associated with autism. This follow-up study was conducted by questionnaire, with parent rated scores of specified autistic traits and symptoms compared with results obtained prior to dietary modification. Mean scores of parametric data obtained were compared using the Paired *t*-test and Independent *t*-test, and non-parametric data was compared using the Wilcoxon Sign-Ranked Test and Mann-Whitney U test. Significant improvements ($p<0.05$) were observed in maladaptive behaviours, sleep disturbances and gastrointestinal symptoms in the Diet Group, particularly those undertaking the gluten and casein free diet combined with low chemical. No significant changes were found in the Control Group. Overall, increased food sensitivity appears to affect a sub-population of autistic children, and dietary modification may help aid in the management of ASD, resulting in improvements in behaviour and gastrointestinal symptoms.

Funding Source: The RPAH Allergy Unit

1. Introduction.

Autistic spectrum disorders (ASD) describe a large group of complex developmental disorders including Autism, Asperger's syndrome and other pervasive developmental disorders (PPD). Autism is the most common disorder affecting 1 in 1000, and is seen four times more frequently in males[1]. Autism is often characterized by a 'triad of impairments' affecting social interaction, communication, and development of imagination[2]. Further symptoms reported include repetitive behaviour[3], selective eating [4],[5], sleep disturbances[6], and hyperactivity[7] . Diagnosis is usually made by the age of three, and symptoms are heterogeneous and persist throughout life[8].

Uncertainty surrounds whether increased gastrointestinal (GI) symptoms are common with ASD. Despite reports of a higher frequency of GI symptoms in autism[9, 10] [11] epidemiological data does not support this claim[9, 12]. Inflammation in both the upper[13] and lower GI tract[14-16] has been detected in autistic children with GI complaints. In another study, 9 of 21 (43%) autistic children with no GI complaints had increased intestinal permeability[17].

An area of controversy that evolved from the concept of a 'gut-brain axis' was the proposal of a new variant of autism characterized by GI symptoms and developmental regression[14]. The same authors speculated a possible causal association with the measles, mumps & rubella (MMR) vaccine, however subsequent studies have found no association between the MMR vaccine and the onset of autism[18-22].

The cause of Autism remains unknown, although approximately 10% of cases have a strong association with genetic and medical conditions such as Angelman's syndrome, tuberous sclerosis, fragile X syndrome, and congenital rubella[23]. In the absence of curative measures, attention has been focused on the possible strategies to manage the associated symptoms. Conventional treatment involves behavioural therapy and special education, however dietary modification has emerged as an 'alternative' therapy in the management of autism[24]. An increasing number of dietetic referrals regarding ASD have been observed, with particular interest in the gluten and casein free (GF/CF) diet[25]. This controversial proposed 'alternative' treatment for autism has spawned from the 'opioid excess theory'. This theory proposes autistic disorders result from the incomplete breakdown and excessive absorption of peptides from foods containing gluten and casein via a 'leaky gut', which are then able to cross the blood brain barrier (BBB) and exert opioid

like effects on the central nervous system (CNS) [26],[27]. The GF/CF diet aims to address this 'gut-brain axis', and involves the dietary exclusion of foods containing gluten (wheat) casein (dairy products).

Several studies on small populations have demonstrated significant behavioural improvements in children with ASD, following the implementation of the GF/CF diet[2, 28-32], although strong large scale evidence is still lacking. Despite this, widespread attention and growing parental support continues to surround this diet. Increased levels of urinary peptides, believed to be derived from dietary proteins, have been found in autistic subjects, with reports of normalization of urinary profiles in response to the GF/CF diet accompanied by improvements in behaviour[31, 33, 34]. However many of these studies were conducted by the same group of authors, and a separate study was unable to replicate these findings, failing to detect the presence of opioid peptides in the urines of children with autism[35].

The emerging interest in the role of food sensitivity in autism has extended to other types of foods. True immunological food allergy (IgE-mediated) is uncommon, accounting for less than 10% of adverse reactions to food, whereas food intolerance (FI) appears to be responsible for the majority of cases[36-38]. Autistic children or at least a sub-population appear experience food sensitivity to a wide range of foods which may result in an exacerbation of symptoms, including GI symptoms[39]. Food allergy has been detected in autistic children, particularly to cow's milk[40], however FI has been demonstrated in a larger population of ASD children[41-43]. In the absence of a diagnostic laboratory test to determine FI, the best approach is an elimination diet followed by food challenges[37].

Autistic children with increased food sensitivity may benefit from the Kaiser-Permanente (K-P) diet. The K-P diet was developed based on Feingold's hypothesis that foods and drugs containing artificial flavours, colours, and natural salicylates may have an adverse behavioural effect on some children[44]. The K-P diet involved the dietary exclusion of products containing these chemicals, however research investigating the effects of the K-P diet on behaviour have provided mixed results, with the majority concluding the diet is generally ineffective[45-49]. Feingold's K-P diet, published in 1974[44], listed a small number of foods containing salicylates to avoid, however, it was not until a decade later a study investigating the salicylate content of 333 food items found that

foods previously thought of as salicylate- free such as pineapple and grapefruit were in fact very high in salicylates[50]. Therefore Feingold's hypothesis may still be relevant to some children with behavioural problems, including those with ASD, and more research is needed using the updated list of foods to avoid.

The primary aim of this study was to assess the outcomes of dietary modification on behavioural and health problems in children with ASD. Secondary aims of this study were to investigate current beliefs and practices of dietitians around Australia relating to diet and autism, and to assess the effect of dietary modification on parental anxiety.

2. Methods

2.1 Previous & Concurrent Research

This follow-up study is part of a non-randomized intervention study investigating 'Dietary Issues in children with ASD' initiated in 2003 by the RPAH Allergy Unit. 103 primary caregiver's of ASD affected children completed and returned the original questionnaire booklet detailing background information on the child's development, general health and behaviour, and parental anxiety at the beginning of the study. Urine samples of some of these participants have been collected before and after dietary modification and will be analysed for the presence of abnormal peptides.

2.2 Study Design

A postal follow-up questionnaire was sent out at the end of July 2005 to caregivers of children with ASD who had completed and returned the original questionnaire. Completed follow-up questionnaires were accepted until 1st October, 2005.

Participants were categorized according to type of dietary modification undertaken, and results from the follow-up questionnaire were compared with results obtained from the original questionnaire booklet at the beginning of the study.

A subsection of this study investigated the current beliefs and practices of dietitians around Australia regarding diet and autism via an internet straw-pole conducted through nut-net.

2.3 Participant Recruitment & Selection

Recruitment of participants was through the ASD Association NSW and patients previously seen at the RPAH Allergy Clinic.

Inclusion Criteria

Inclusion criteria were primary caregiver's of children diagnosed as ASD as per DSM IV criteria, aged 3-10 years at the time of completing the original questionnaire booklet, and toilet trained. Only participants that completed and returned the original questionnaire were included in the follow-up study.

Exclusion Criteria

Exclusion criteria were primary caregiver's of children without ASD, and with any other metabolic disorder other than celiac disease or asthma. Primary caregiver's that interacted with the child less than 4 days per week were also excluded as the self-administered questionnaire filled out on behalf of the child, required adequate knowledge of the child's diet and behaviour.

2.4 Ethics Approval

Ethics approval was granted from the Ethics Review Committee (RPAH Zone) of the Central Sydney Area Health Service (Appendix A).

2.5 Subject Consent & Confidentiality

The aims and nature of this follow-up study were conveyed to potential participants in an information sheet (Appendix B) posted out to participants with the follow-up questionnaire booklet and a reply-paid envelope. Participants were instructed to keep the information sheet and refer to them. Contact details of the paediatrician were provided on the information sheet and participants were encouraged to ask questions regarding any concerns. Written consent was obtained from primary caregivers at the start of this project, via an expression of interest form (Appendix C).

Information obtained from participants was collected and stored in a confidential manner, and only the research team involved in the study had access to this information. Participants were informed

that they had been assigned a study code number which was recorded on their questionnaires in order to protect their identity.

2.6 Questionnaires

The follow-up questionnaire booklet comprised of 5 self-administered questionnaires designed to gather information about the child's eating behaviour, social interaction & communication skills, parental anxiety, dietary modification undertaken and any observable changes in behaviour and health. Each questionnaire primarily consisted of a rating score of 0-3 indicating severity or frequency of the specified behaviour or symptom.

The Parent Depression Anxiety Stress Scale (DASS-42) is a validated questionnaire comprising of 42 questions designed to measure parental depression, anxiety and stress[51] (Appendix D). The Conner's Rating Scale (CPRS-48) is a validated behaviour assessment tool for children aged 3 to 17 years[52] (Appendix E). It contains 48 questions on various specified behavioural traits and divides results into 6 subscales measuring conduct problem, learning problem, psychosomatic, impulsive-hyperactive, anxiety, and hyperactivity index. Children's adaptive and maladaptive behaviours were assessed using the validated PDD Behaviour Inventory (PDDBI-C; renamed 'Social Interaction and Communication Skills') (Appendix F) where maladaptive behaviours are divided into 7 subscales measuring sensory/perceptual approach behaviours; ritualisms/resistance to change; arousal regulation problems; specific fears; aggressiveness; social pragmatic problems; and semantic/pragmatic problems; and adaptive behaviours are divided into 4 subscales measuring social approach behaviours; learning, memory and receptive language; phonological skills; and semantic/pragmatic ability[53, 54].

The Children's Eating Behaviour & Appetite Scale (CEBAS-50) was developed by the RPAH Allergy Unit to assess the eating habits and behaviours related to food (Appendix G). It consisted of 50 questions, however only questions 1, 3, 5, 8-17, 40, 42-45 were analysed. These questions were chosen based on the literature discussing typical ASD related eating difficulties [55]. A new follow-up questionnaire was developed to summarize type of dietary modification and challenges undertaken and changes in behaviour and health (Appendix H). It also provided parents the opportunity to indicate the time taken to notice any effects of dietary modification.

2.7 Straw Poll

A straw poll was developed and conducted in August-September 2005, to investigate the current beliefs and practices of dietitian's around Australia in the area of autism and diet. The straw poll comprised of 4 closed questions, with question 4 subdivided into 4 separate questions, and was distributed via the Nutritionist's Network of Health Professionals ('Nutnet'), an internet discussion group that professionals in nutrition and dietetics around Australia subscribe to.

2.8 Data Analysis

Data was entered into Microsoft SQL Query Analyser 2000 (Microsoft Corp, USA) and Microsoft Excel 2002 (Microsoft Corp, USA). Participants were categorized according to dietary modification undertaken. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) Version 13.0, with a significant difference indicated by $p<0.05$ for all analyses.

Before and after scores were compared within each group to determine if a significant change had occurred following dietary modification. Mean scores of parametric data obtained from the validated questionnaires DASS-42, CPRS-48, and PDDBI-C were compared using the Paired *t*-test, and non-parametric data from the CEBASS-50 and Follow-up Questionnaire was compared using the Wilcoxon Sign rank test.

To determine changes between diet groups, each 'after' score was subtracted from the corresponding 'before' score to obtain a 'difference' score. The mean difference score of diet groups were compared with the mean difference score of the 'no diet group', using the Independent *t*-test for parametric data and the Mann-Whitney U test for non-parametric data. All questionnaires with the exception of section 2 of the PDDBI-C, contained negatively geared questions, whereby a positive difference indicated an improvement, whilst a negative difference score indicated a worsening of symptoms, and the reverse applying for the positively geared questions.

3. Results

General Characteristics

Forty-five completed questionnaires were returned of the total 103 posted out, a response rate of 43.7%. Of the 32 children already on some dietary modification at the beginning of the study, 14 (43.75%) were on the GF/CF diet. The sex ratio in the total sample was five boys to one female, similar to the average 4:1 given in the literature[23].

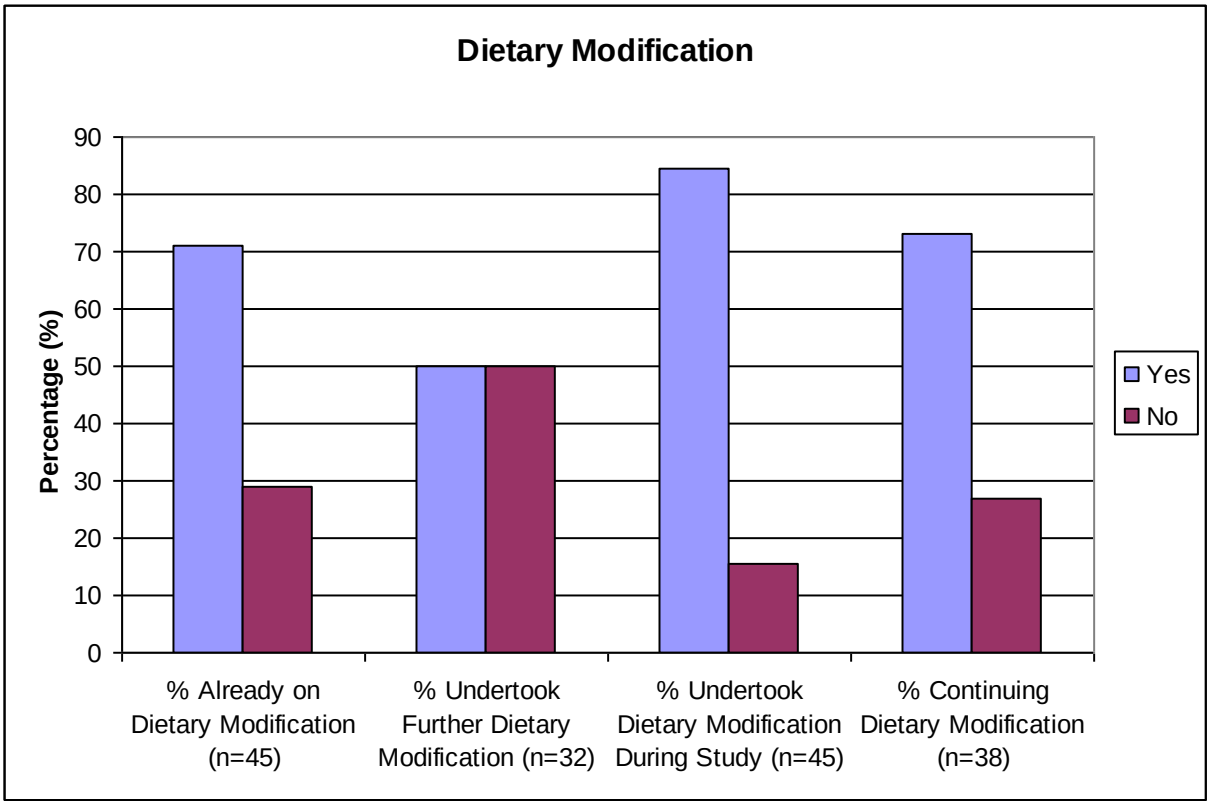

Figure 1. Participants Undertaking Dietary Modification

During the study period, 38 children (84.5%) undertook dietary modification (mean age 7). The mean age of the 'no diet' group was 10 years (n=7). Some children in the diet group were further categorised into the following subgroups: dairy-free (n=4, mean age 7); strict low chemical (n=5, mean age 7); and highly modified diet (n=24, mean age 6). The highly modified diet subgroup included children undertaking a combination of the GF/CF with low chemical diet.

Follow-Up Questionnaire

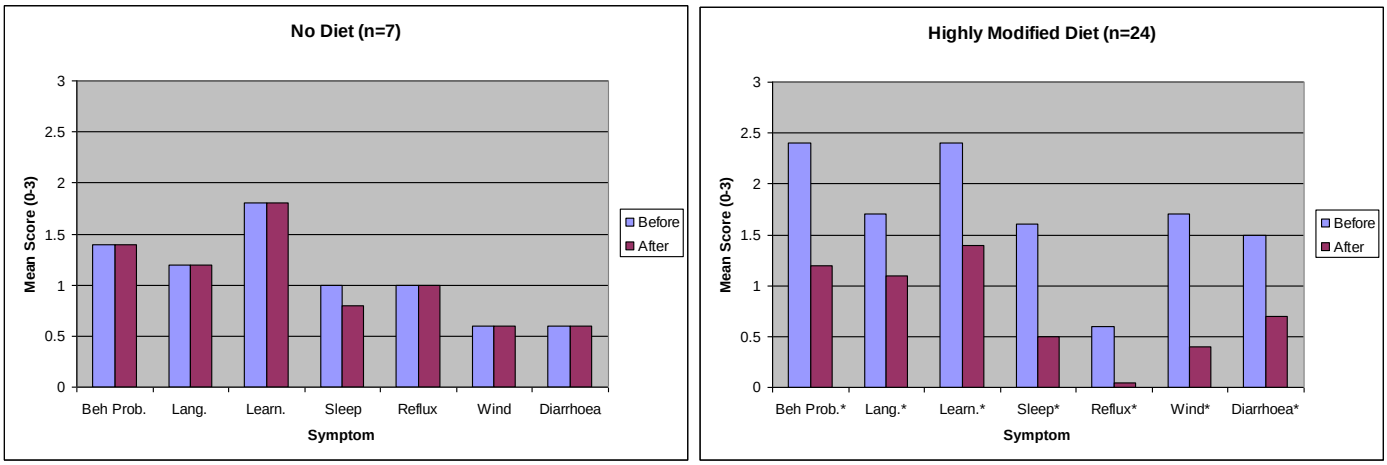

Figure 2. Behavioural Problems and GI Symptoms. Before and After mean scores for the No Diet and Highly Modified Diet Subgroup were compared for each symptom using the non-parametric Wilcoxon Sign-Rank Test. A decreased score after the experimental period indicates improvement in symptom. Significant change ($p<0.05$) for each symptoms is marked as *.

Both no diet and diet groups suffered from behavioural and GI symptoms. Learning difficulties affected 100% of participants in both the diet and no diet groups. Significant improvements ($p<0.05$) were observed in the diet group for all symptoms, in particular the highly modified diet group; behaviour problems and learning difficulties p=0.000; language p=0.014; sleep disturbances and wind/abdominal pain p=0.001; reflux/vomiting p=0.026; diarrhoea p=0.007. No significant changes were observed in the no diet group or dairy-free and strict low chemical diet subgroups (Appendix I). Significant differences in behavioural problems (p=0.009) and learning difficulties (p=0.009) were found between the highly modified diet and no diet groups (Appendix J).

Table 1. Parent Rated Child's Condition Now

	No Diet	Diet	Dairy	Low Chemical	Highly Modified
Child's Condition Now Compared With Before					
Better†	5	30	2	4	21
Same	1	8	2	1	3
Worse	1	0	0	0	0
† **Time to notice improvement**	6 months	2 days – 6 months	2 - 6 weeks	2 days - 2 months	1 day – 6 months

DASS-42

No significant changes were found in no diet group or in any of the diet groups for parental anxiety, stress, or depression (Appendix K), and there were no significant differences found between groups (Appendix L).

CPRS-48

No significant changes were found in any of the diet groups or control regarding the subscales for conduct problem, learning problem, psychosomatic, impulsive-hyperactive, anxiety, and hyperactivity index (Appendix M). No significant changes were found between groups (Appendix N).

CEBASS-50

Only questions 8 and 17 showed a significant improvement in the highly modified diet subgroup, indicating increased variety of food choices ($p=0.031$) and improvement in fear of trying new foods ($p=0.033$) (Appendix O).

Section 1: Maladaptive Behaviours

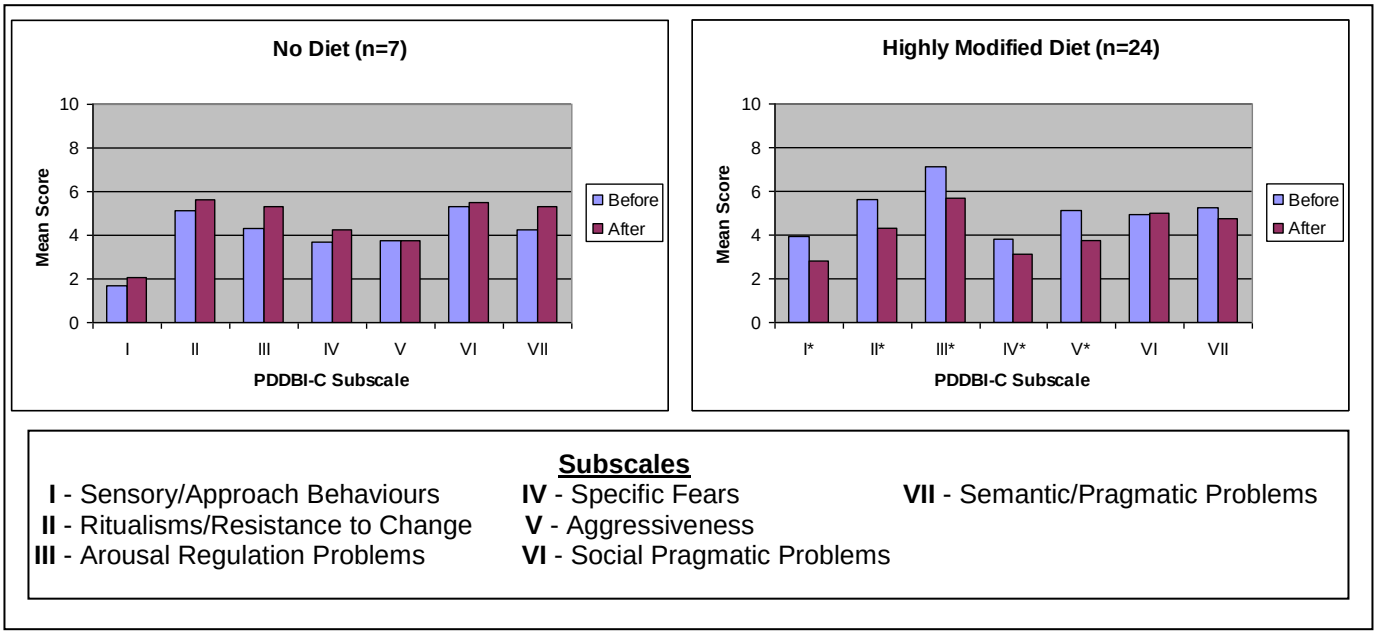

Figure 3. PDDBI-C Section 1. Before and After mean scores for the No Diet and Highly Modified Diet Subgroup were compared for each subscale using the Paired *t*-test. A decreased score after the experimental period indicates improvement. Significant change ($p<0.05$) for each symptoms is marked as *.

The diet group showed a significant improvement ($p<0.05$) in maladaptive behaviours in subscales (I-V), in particular the highly modified diet subgroup: subscale I ($p=0.001$); II ($p=0.003$); III ($p=0.000$); IV ($p=0.021$); and V ($p=0.002$). No significant differences were found in the no diet group or other subgroups (Appendix Q). A significant difference was found between the highly modified diet subgroup and no diet group in subscales: I ($p=0.013$); II ($p=0.039$); III ($p=0.004$); and IV ($p=0.049$).

Section 2: Adaptive Behaviours

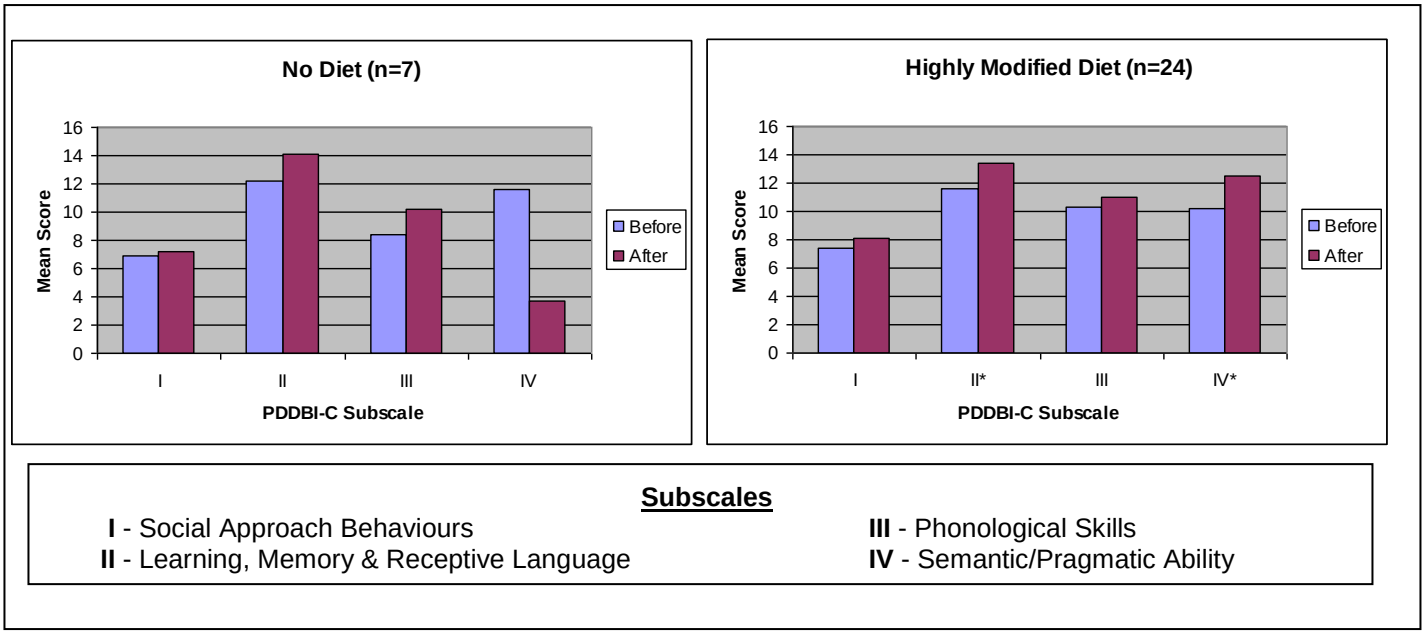

Figure 4. PDDBI-C Section 2. Before and After mean scores for the No Diet and Highly Modified Diet Subgroup were compared for each subscale using the Paired *t*-test. An increased score after the experimental period indicates improvement. Significant change ($p<0.05$) for each symptoms is marked as *.

Significant improvements were found in the highly modified diet subgroup for subscales II ($p=0.002$) and IV ($p=0.011$), however the overall change in scores was not significantly different compared with the no diet group ($p=0.950$ and $p=0.971$ respectively) (Appendix R).

Table 2. Results of a straw poll of dietitian's practices in the area of autism and diet conducted via 'Nutnet'

No. of Respondents: 40

	Question	Yes (%)	No (%)	No response (%)
1	Do you think that a gluten/wheat-free and casein/milk-free diet has a role in the management of children with Autistic Spectrum Disorder (ASD)?	62.5	20	17.5
2	Do you see families who have a child with ASD seeking dietary advice?	65	35	0
3	If yes, do you recommend a trial of a gluten/wheat-free and casein/milk-free diet?	58	23	19
4	If a child with ASD is already on a diet modified in this way when you see them, do you:			
a	Advise them to return to an unrestricted diet?	11.5	54	34.5
b	Advise them to continue, giving general advise on adequate nutrition?	73	8	19
c	Formally analyse their diet for nutritional sufficiency (e.g. using a computer program such as Serve or Food Works)?	42	38.5	19.5
d	Advise formal challenge testing with wheat and/or dairy?	65	12	23

4. Discussion

The results from this study strongly suggest diet may affect some of the symptoms of children with ASD. Significant improvements in behaviour (p=0.000) and GI symptoms (p=0.001) were reported in children with ASD, following the implementation of a highly modified diet involving the dietary exclusion of foods containing gluten, casein, and high levels of artificial and natural chemicals.

GI symptoms were reported by many parents in this group of ASD children (Figure 2), and may be the result of food intolerance (FI) to a variety of foods. The follow-up questionnaire did address milder forms of GI symptoms such as wind and reflux, however it did not specify constipation. This may be a limitation as the literature reports constipation is a frequent and under diagnosed problem in ASD children [11]. The presence of GI symptoms and pathology in ASD children have been demonstrated elsewhere[10], yet it still remains unclear if GI symptoms occur more frequently, although they do appear to effect at least a sub population of autistic children. An epidemiological survey found no evidence of increased GI disorders in children with autism[9], however the survey only investigated serious GI disorders such as celiac disease and did not take into account milder sub-clinical GI symptoms such as abdominal pain, bloating, and wind.

Two separate studies have detected inflammation in the upper[13] and lower GI tract[14-16] of children with ASD and GI symptoms. Ileo-lymphoid nodular hyperplasia was found in 129 of 144 ASD (90%) compared with 8 of 21(30%) controls (p<0.0001) [16]. The same authors subsequently proposed a new variant form of autism associated with the MMR vaccine[15]. This speculated association was fuelled by an apparent 'epidemic' of autism coinciding with the introduction of the MMR vaccine in the UK in 1988. Better diagnosis and greater awareness within the medical community are likely to have contributed to this perceived increase, as well as the broadening of the term ASD for the inclusion of less severe forms. The loss of confidence in the vaccine could have serious implications on public health and so numerous studies were subsequently undertaken and all concluded no causal link to the vaccine[18-22], and 9 of the 11 original authors released a formal 'retraction of an interpretation' regarding the vaccine[56]. The MMR vaccine controversy appears to have overshadowed the findings of gut pathology by these authors, and unfortunately very few studies if any, have gone on to investigate the increased presence of GI inflammation initially reported.

Increased intestinal permeability has been detected in asymptomatic children with ASD[17]. Altered intestinal permeability can be a sign of gut mucosal damage, and factors contributing to this breakdown can include food intolerance and food allergic responses, as well as nutrient deficiencies and viral or bacterial infections[1]. Although the study described found gut pathology in autistic children with no GI complaints, it is possible that due to impaired communication and language skills, autistic children may be unable to communicate pain and discomfort which may contribute to an underestimation of reported GI problems.

Taken together these findings suggest that significant and widespread gut pathology may accompany autism, at least within a subpopulation of patients. It remains unclear if the pathology is central to the cause of autism, as suggested in the 'opioid excess theory', or is simply a consequence of the disorder in those with GI pathology and/or symptoms. Furthermore, sudden outbursts, irritability, and other behavioural problems related to pain, may be mistaken as bad behaviour, and sleep disturbances may also be a sign of GI pain and discomfort. The results from this current study support this possibility as improvement in GI symptoms were accompanied by a significant improvement in sleep disturbances ($p=0.001$) and behavioural problems ($p=0.000$) in the diet group, particularly in those implementing the highly modified diet ($p=0.001$ and $p=0.000$, respectively).

Dietary approaches, in particular the GF/CF diet, have increased in popularity partly due to the wide dissemination of information available on the internet. This was reflected in the study population as many participants were already implementing some form of dietary restriction (Figure 1), mainly the GF/CF diet, when they joined the study. Although there is an abundance of information on the GF/CF diet available, a Cochrane review found only one article that met the criteria of randomised controlled trial (RCT; high level of evidence), hence was unable to perform a meta-analysis, indicating the lack of strong large scale evidence[29]. The majority of evidence supporting the GF/CF diet has come from a group of authors in Norway[30-34]. These studies have been single-blinded and have involved small samples of autistic children with abnormal urinary profiles. These studies reported a reduction in autistic traits and increased communicative skills in children with ASD, following the implementation of the GF/CF diet, and improvements observed were accompanied by the normalisation of urinary peptide levels[32]

The results of this current study support these findings as significant improvements were found in the highly modified diet subgroup in maladaptive behaviours such as resistance to change (p=0.003) (Figure 3), and communication skills including learning, memory and receptive language (p=0.002) (Figure 4). It remains unclear if the improvements observed in communication skills are independent of improvements in GI symptoms, or if they are due to natural developmental change. A limitation in the Norwegian studies and in this study, is that results were based on parental observations and parents were not blind to the intervention, and this may have influenced their perception of improvement in behaviour. Furthermore, dietary compliance was not monitored throughout the study period and cumulative effects of other therapies were not considered as improvements observed may not have been solely due to diet. Clearly further research is needed involving RCT on larger populations.

It is unclear if the GF/CF diet only works in children with ASD with abnormal urinary profiles and/or GI symptoms. As previously discussed, a separate study on the same study population is currently investigating the change in urinary profiles following dietary modification. It will be interesting to see if the results support the beneficial outcomes of dietary modification found in this study.

Many dietitians still remain uncertain on the effectiveness of the GF/CF diet in children with ASD. With a growing number of referrals to the dietetic service regarding this area[25], more research is needed to provide practicing dietitians with up to date information. Forty dietitians responded to the straw poll with the majority of practicing dietitians (62.5%) believing the GF/CF diet had a role in the management of ASD, however 17.5% did not answer this question indicating the uncertainty in this area (Table 2).

The Feingold K-P diet has also been recommended for children with ASD. As previously discussed, the majority of studies investigating the diet have concluded the diet is generally ineffective[45-49]. A plausible explanation for the mixed results may be that salicylates were not being eliminated from the diet, rather one source of salicylate was being replaced by a different source. Published in 1974, the diet recommended the intake of grapefruit, pears, pineapple, and melon, claiming they were 'salicylate-free'[44, p192], however it was not until a decade later it was found that foods previously thought of as salicylate-free such as pineapple and grapefruit were in fact very high in salicylates[50]. Other foods recommended such as honey, broccoli and avocado[44]

were also proven to be very high in salicylates. Therefore Feingold's hypothesis should not be discredited just yet, and further research is needed using the updated list of foods to avoid. Furthermore the K-P diet did not exclude all natural chemicals such as natural amines and glutamate (MSG). This current study included a strict low chemical diet subgroup which excluded artificial colours, and preservatives, and reduced salicylates and amines. No significant changes were found in this subgroup, however the sample size (n=5) may have been too small to reach statistical significance.

Interestingly, studies investigating the effectiveness of dietary modification in children with ASD appear to have only focussed on the GF/CF diet and Feingold K-P diet separately. At the beginning of this study, 14 participants were already on the GF/CF diet and had noticed improvements in their child's behaviour. All 14 subjects were subsequently placed on further dietary restriction including a low chemical diet, similar to the Feingold K-P diet, and were included in the highly modified diet subgroup that showed significant improvements in behavioural problems and GI symptoms.

Many foods have been hypothesized to adversely affect the behaviour of children, including the foods described above. Dietary intervention may seem as a safe alternative treatment for ASD, however a major concern when undertaking any dietary modification is the fear of under nutrition resulting from the further exclusion of foods to a population with an already selective feeding behaviour. Interestingly the most restricted subgroup, the highly modified diet, reported a significant improvement regarding increased variety in food choices ($p=0.031$) and improvement in fear of trying new foods ($p=0.033$).

ASD is characterised by the heterogeneity of symptoms, meaning affected children have a unique combination of abnormalities. Therefore it can be expected that results of dietary modification vary between subjects. This was observed in the results, as some parents in the diet groups reported no change, whilst others reported small and large improvements (Table1). The timing for improvements also varied, with some respondents noticing immediate improvements, whilst others required several months. Dietary modification should therefore be seen as a complementary strategy to support conventional therapies, rather than as an alternative substitute, as it may only help a subpopulation and perhaps only particular symptoms.

Interestingly, the majority of parents in the 'no diet' group rated their child as better, despite none of the questionnaires detecting a significant improvement. This may have been due to the small sample size, or perhaps the questionnaires did not cover all symptoms, although this follow-up study sought to cover all symptoms by using a range of questionnaires. A limitation of using so many questionnaires is the increased burden on participants which may have contributed to the low response rate, and with a response rate of 43.7%, one must question if only parents in the diet groups that observed a positive result bothered to complete the follow-up questionnaire.

Due to the non-randomised design, larger group numbers could not be guaranteed. An advantage of this follow-up study design was that every person served as their own control, however the study was initiated in 2003, hence some improvements may have been due to natural maturation of the child. The CPRS-48 and PDDBI-C are the only questionnaires that took difference in age into account.

Unlike food allergy, there is no laboratory test to diagnose FI, with the best approach being an elimination diet followed by food challenges to confirm suspect foods. A large proportion of parents did not challenge their child's diet. This may be due to fear of deterioration, however it remains important to formally challenge foods as symptoms of food intolerance are dose related and certain foods may be able to be tolerated at lower levels. The highly modified diet is highly restrictive and very bland, and in some cases such strict compliance may not be necessary. It is therefore important to seek dietetic advice to help confirm suspect foods and ensure nutritional adequacy is met when undertaking such trials.

5. Conclusions/Recommendations

Overall, there is a growing interest in the use of dietary modification in the management of ASD. Results indicate that food sensitivity to wide range of foods is a common finding in a subpopulation of children with ASD. Children with ASD that respond favourably to the GF/CF diet may further benefit from the removal of foods containing high levels of artificial and natural chemicals. In children who responded well to dietary exclusion, it remains unclear if food intolerance has a role in the cause of autism, or is just a symptom of the disorder. Further research is needed using larger numbers and investigating the effects of combining a GF/CF with low chemical diet.

6. Reference List

1. White, J.F., *Intestinal pathophysiology in autism.* Exp Biol Med (Maywood), 2003. **228**(6): p. 639-49.
2. Knivsber, A.M., K.L. Reichelt, and M. Nodland, *Reports on dietary intervention in autistic disorders.* Nutr Neurosci, 2001. **4**(1): p. 25-37.
3. Cornish, E., *Gluten and casein free diets in autism: a study of the effects on food choice and nutrition.* J Hum Nutr Diet, 2002. **15**(4): p. 261-9.
4. Schreck, K.A., K. Williams, and A.F. Smith, *A comparison of eating behaviors between children with and without autism.* J Autism Dev Disord, 2004. **34**(4): p. 433-8.
5. Williams, P.G., N. Dalrymple, and J. Neal, *Eating habits of children with autism.* Pediatr Nurs, 2000. **26**(3): p. 259-64.
6. Wiggs, L. and G. Stores, *Sleep patterns and sleep disorders in children with autistic spectrum disorders: insights using parent report and actigraphy.* Dev Med Child Neurol, 2004. **46**(6): p. 372-80.
7. Aman, M.G., *Management of hyperactivity and other acting-out problems in patients with autism spectrum disorder.* Semin Pediatr Neurol, 2004. **11**(3): p. 225-8.
8. Kidd, P.M., *Autism, an extreme challenge to integrative medicine. Part 2: medical management.* Altern Med Rev, 2002. **7**(6): p. 472-99.
9. Black, C., J.A. Kaye, and H. Jick, *Relation of childhood gastrointestinal disorders to autism: nested case-control study using data from the UK General Practice Research Database.* Bmj, 2002. **325**(7361): p. 419-21.
10. Horvath, K. and J.A. Perman, *Autism and gastrointestinal symptoms.* Curr Gastroenterol Rep, 2002. **4**(3): p. 251-8.
11. Afzal, N., et al., *Constipation with acquired megarectum in children with autism.* Pediatrics, 2003. **112**(4): p. 939-42.
12. Kuddo, T. and K.B. Nelson, *How common are gastrointestinal disorders in children with autism?* Curr Opin Pediatr, 2003. **15**(3): p. 339-43.
13. Horvath, K., et al., *Gastrointestinal abnormalities in children with autistic disorder.* J Pediatr, 1999. **135**(5): p. 559-63.
14. Wakefield, A.J., et al., *Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children.* Lancet, 1998. **351**(9103): p. 637-41.
15. Wakefield, A.J., et al., *Enterocolitis in children with developmental disorders.* Am J Gastroenterol, 2000. **95**(9): p. 2285-95.
16. Wakefield, A.J., et al., *The significance of ileo-colonic lymphoid nodular hyperplasia in children with autistic spectrum disorder.* Eur J Gastroenterol Hepatol, 2005. **17**(8): p. 827-836.
17. D'Eufemia, P., et al., *Abnormal intestinal permeability in children with autism.* Acta Paediatr, 1996. **85**(9): p. 1076-9.
18. Madsen, K.M., et al., *A population-based study of measles, mumps, and rubella vaccination and autism.* N Engl J Med, 2002. **347**(19): p. 1477-82.
19. Taylor, B., et al., *Measles, mumps, and rubella vaccination and bowel problems or developmental regression in children with autism: population study.* Bmj, 2002. **324**(7334): p. 393-6.
20. Fombonne, E. and S. Chakrabarti, *No evidence for a new variant of measles-mumps-rubella-induced autism.* Pediatrics, 2001. **108**(4): p. E58.
21. Smeeth, L., et al., *MMR vaccination and pervasive developmental disorders: a case-control study.* Lancet, 2004. **364**(9438): p. 963-9.

22. Taylor, B., et al., *Autism and measles, mumps, and rubella vaccine: no epidemiological evidence for a causal association.* Lancet, 1999. **353**(9169): p. 2026-9.
23. Kidd, P.M., *Autism, an extreme challenge to integrative medicine. Part: 1: The knowledge base.* Altern Med Rev, 2002. **7**(4): p. 292-316.
24. Page, T., *Metabolic approaches to the treatment of autism spectrum disorders.* J Autism Dev Disord, 2000. **30**(5): p. 463-9.
25. Bowers, L., *An audit of referrals of children with autistic spectrum disorder to the dietetic service.* J Hum Nutr Diet, 2002. **15**(2): p. 141-4.
26. Panksepp, J., *A neurochemical theory of autism.* Trends in Neurochemical Science, 1979. **2**: p. 174-177.
27. Reichelt, K.L. and A.M. Knivsberg, *Can the pathophysiology of autism be explained by the nature of the discovered urine peptides?* Nutr Neurosci, 2003. **6**(1): p. 19-28.
28. Whitely, P., Rodgers, J., Savery, D., Shattock, P. , *A gluten-free diet as an intervention for autism and associated spectrum disorders: preliminary findings.* SAGE, 1999. **3**(1): p. 45-65.
29. Millward, C., et al., *Gluten- and casein-free diets for autistic spectrum disorder.* Cochrane Database Syst Rev, 2004(2): p. CD003498.
30. Knivsberg, A.M., et al., *A randomised, controlled study of dietary intervention in autistic syndromes.* Nutr Neurosci, 2002. **5**(4): p. 251-61.
31. Knivsberg, A.M., Reichelt, K. L., Noland, M. & Hoien, T., *Autistic Syndromes and Diet: a follow-up study.* Scandinavian Journal of Educational Research, 1995. **39**(3): p. 223-236.
32. Knivsberg, A.M., Reichelt, K. L., Hoien, T. & Noland, M., *Effect of a Dietary Intervention on Autistic Behavior.* Focus on Autism & Other Developmental Disabilities, 2003. **18**(4): p. 247-256.
33. Reichelt, W.H., Knivsberg, A.M., Noland, M., Stensrud & Reichelt, K.L. , *Urinary Peptide Levels and Patterns in Autistic Children from Seven Countries, and the Effects of Dietary Intervention after 4 Years.* Developmental Brain Dysfunction, 1997. **10**: p. 44-55.
34. Reichelt, K.L., Ekrem, J. & Scott, H. , *Gluten, Milk Protein and Autism: Dietary Intervention Effects on Behaviour and Peptide Secretion.* Journal of Applied Nutrition, 1990. **42**(1): p. 1-9.
35. Hunter, L.C., et al., *Opioid peptides and dipeptidyl peptidase in autism.* Dev Med Child Neurol, 2003. **45**(2): p. 121-8.
36. Hedges, H.H., *The elimination diet as a diagnostic tool.* Am Fam Physician, 1992. **46**(5 Suppl): p. 77S-84S.
37. Allen, D.H., et al., *Adverse reactions to foods.* Med J Aust, 1984. **141**(5 Suppl): p. S37-42.
38. Loblay, R.H.S., A.R., *Food Intolerance.* Recent Advances in Clinical Nutrition, 1986. **2**: p. 169-177.
39. Renzoni, E., et al., *Brief report: allergological evaluation of children with autism.* J Autism Dev Disord, 1995. **25**(3): p. 327-33.
40. Lucarelli, S., et al., *Food allergy and infantile autism.* Panminerva Med, 1995. **37**(3): p. 137-41.
41. Jyonouchi, H., S. Sun, and N. Itokazu, *Innate immunity associated with inflammatory responses and cytokine production against common dietary proteins in patients with autism spectrum disorder.* Neuropsychobiology, 2002. **46**(2): p. 76-84.
42. Jyonouchi, H., et al., *Dysregulated innate immune responses in young children with autism spectrum disorders: their relationship to gastrointestinal symptoms and dietary intervention.* Neuropsychobiology, 2005. **51**(2): p. 77-85.

43. Jyonouchi, H., et al., *Evaluation of an association between gastrointestinal symptoms and cytokine production against common dietary proteins in children with autism spectrum disorders.* J Pediatr, 2005. **146**(5): p. 605-10.
44. Feingold, B.F., *Why Your Child is Hyperactive*. 1974: Random House Inc., New York.
45. Harley, J.P., et al., *Hyperkinesis and food additives: testing the Feingold hypothesis.* Pediatrics, 1978. **61**(6): p. 818-28.
46. Harner, I.C. and R.A. Foiles, *Effect of Feingold's K-P diet on a residential mentally handicapped population.* J Am Diet Assoc, 1980. **76**(6): p. 575-8.
47. Stare, F.J., E.M. Whelan, and M. Sheridan, *Diet and hyperactivity: is there a relationship.* Pediatrics, 1980. **66**(4): p. 521-5.
48. Wender, E.H., *The food additive-free diet in the treatment of behavior disorders: a review.* J Dev Behav Pediatr, 1986. **7**(1): p. 35-42.
49. Thorley, G., *Pilot study to assess behavioural and cognitive effects of artificial food colours in a group of retarded children.* Dev Med Child Neurol, 1984. **26**(1): p. 56-61.
50. Swain, A.R., S.P. Dutton, and A.S. Truswell, *Salicylates in foods.* J Am Diet Assoc, 1985. **85**(8): p. 950-60.
51. Lovibond, S.H., & Lovibond, P.H., *Parent Depression Anxiety & Stress Scale (DASS-42)*. 1995, UNSW Australia: School of Psychology.
52. Conners, C.K., *Conners' Rating Scales - Revised*. 2001, Multi-Health Systems Inc.: Canada.
53. Cohen, I.L., et al., *The PDD Behavior Inventory: a rating scale for assessing response to intervention in children with pervasive developmental disorder.* J Autism Dev Disord, 2003. **33**(1): p. 31-45.
54. Cohen, I.L., *Criterion-related validity of the PDD Behavior Inventory.* J Autism Dev Disord, 2003. **33**(1): p. 47-53.
55. Ahearn, W.H., et al., *An assessment of food acceptance in children with autism or pervasive developmental disorder-not otherwise specified.* J Autism Dev Disord, 2001. **31**(5): p. 505-11.
56. Murch, S.H., et al., *Retraction of an interpretation.* Lancet, 2004. 363(9411): p. 750.

APPENDIX A. Ethics Approval

APPENDIX B. Information for Parents Sheet

APPENDIX C. Expression of Interest Form

APPENDIX D. Parent Depression Anxiety & Stress Scale (DASS-42)

APPENDIX E. Conner's Rating Scales (CPRS-48)

APPENDIX F. PDD Behaviour Inventory (PDDBI-C) – Renamed ‘Social Interaction & Communication Skills’

APPENDIX G. Children's Eating Behaviour & Appetite Scale (CEBASS-50)

APPENDIX H. Follow-Up Questionnaire

APPENDIX I. Change in Behaviour & GI Symptoms during the study period within groups

APPENDIX J. Change in Behaviour & GI Symptoms during the study period between DIET groups and NO DIET group

APPENDIX K. Change in DASS-42 Subscale Scores during the study period within groups

APPENDIX L. Change in DASS-42 Subscale Scores during the study period between DIET groups and NO DIET group

APPENDIX M. Change in CPRS-48 Subscale Scores during the study period within groups

APPENDIX N. Change in CPRS-48 Subscale Scores during the study period between DIET groups and NO DIET group

APPENDIX O. Change in CEBASS-50 Scores during the study period within groups

APPENDIX P. Change in CEBASS-50 Scores during the study period between DIET groups and NO DIET group

APPENDIX Q. Change in PDDBI-C Subscale Scores during the study period within groups

APPENDIX R. Change in PDDBI-C Subscale Scores during the study period between DIET groups and NO DIET group