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Food Intolerance and Dietary Intervention in Children with ASD

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Abstract

Background: Autistic Spectrum Disorder (ASD) encompasses a range of developmental disorders, characterised by a triad of symptoms including impaired social interaction skills, communication skills and symbolic or imaginative play. The aetiology of ASD is complex and not yet well understood. Amongst others, diet is one factor implicated as potentially causative in ASD. Gluten-free and casein-free diets have been reported to result in improvements in the symptoms of ASD. Absence of these dietary proteins alone cannot be conclusively said to be the cause of these improvements, as removing foods containing casein and gluten from the diet is also likely to result in an altered intake of other food chemicals. It appears that parents turn away from conventional medical practitioners and seek advice on dietary modification from alternate sources, possibly because of a lack of support from the medical profession.

Objective: To document the food intolerances and symptoms observed in children with ASD, as well as the efficacy and sustainability of dietary modification in those children with ASD who have food intolerances.

Design: Children with ASD were compared with milk intolerant children and children with neither ASD nor food intolerance in a questionnaire based study. Issues examined include symptoms potentially related to food intolerance, any history of adverse reactions to foods or dietary modification and the impact of the children on their families.

Results: The children with ASD exhibited a number of behavioural and gastrointestinal symptoms, which may relate to food intolerance. These symptoms generally began early in life, before abnormal/ASD behaviours and persisted into the present in many cases. Dietary modification had taken place in 100% of the children with ASD and was currently in place for 87.50%. A range of foods were reported as responsible for adverse reactions and many different symptoms were improved with dietary modification.

Conclusion: A group of children with ASD exhibiting symptoms of food intolerance to a range of foods has been described. Dietary modification was generally effective and sustainable in these children. Continued research is needed to further elucidate the role and prevalence of food intolerance in children with ASD.

Introduction

Autistic Spectrum Disorder (ASD) comprises a number of Pervasive Developmental Disorders (PDD), the two most common being Autistic Disorder and Asperger's Disorder(1). As the name implies, ASD corresponds to a spectrum of common characteristics, with each individual affected to a varying extent. The defining characteristics of ASD are abnormal or impaired development of (i) social interaction skills, (ii) communication skills and (iii) symbolic or imaginative play, the latter generally presenting as restricted, repetitive and stereotyped patterns of behaviour, interests and activities(1). Special or extraordinary skills in one particular area may also be observed, as well as some degree of mental retardation(2). Autistic Disorder and Asperger's Disorder affect approximately 59 in 10,000 people in Australia(3). Epidemiological studies have generally found that males are affected more often than females, at a ratio of between 2 and 5(4).

The aetiology of ASD is complex and unclear. Genetic factors, metabolic and neurochemical abnormalities, the Measles Mumps Rubella (MMR) vaccine, gastrointestinal abnormalities and diet have all been implicated as having a causative role in ASD. It is now fairly clear that a predisposition for ASD is largely genetic, however the exact genetic basis is still unknown and the search for genes linked to ASD continues(5-7). Whilst the MMR vaccine has been largely dismissed as a probable cause of ASD(8-12), the respective impacts of diet and gastrointestinal abnormalities in ASD still require further investigation.

The role of diet in ASD has been in question since abnormalities in urinary peptide excretion in ASD subjects were first investigated in the early 1980s(13). These peptides have been subsequently shown to derive in part from the dietary proteins casein and gluten. Further investigation has resulted in the hypothesis that opioid derivatives of these dietary proteins are able to enter the blood though a so-called "leaky gut" and then cross the blood-brain barrier to act at opioid receptors in the brain, causing the neurological abnormalities seen in ASD(13-17). Whilst one recent study failed to replicate the previously

reported abnormalities in urinary peptides(18), others have reported that casein-free and gluten-free diets lead to clinical improvements in the symptoms of ASD(14-16) and anecdotal evidence to this effect is widely available on the internet.

Parents of children with ASD commonly report gastrointestinal symptoms in their children(17, 19, 20). Recent research has drawn attention to the prevalence of gastrointestinal symptoms in ASD, the potential impact of these symptoms on behaviour and possible treatment regimes(17, 20-22). This research has shown that a higher percentage of children with ASD suffer diarrhoea, gaseousness, abdominal pain, bloating, reflux and reflux esophagitis than children not affected by ASD (17, 20, 21). It has also been reported that children with ASD have an increased secretory response to secretin compared to controls(20, 21). One major area of gastrointestinal research in ASD has been the potential therapeutic benefit of secretin. Secretin is a 27 amino acid peptide hormone released by the duodenum, in response to the acidic contents of the stomach, its action is to increase pancreatic secretion(22). Although secretin has been widely touted as a treatment for ASD, most research now indicates that it is not beneficial to symptoms(22-28).

Food intolerances often manifest in ways that may result in altered and difficult behaviours, skin rashes and gastrointestinal symptoms due to the natural salicylates, amines and glutamates in food, as well as added MSG, colours and preservatives(29). The role of food intolerance in ASD needs to more broadly examined. Change to the diet resulting from removal of foods containing casein or gluten is not exclusive to these proteins. It will also result in changes to the overall intake of a range of natural and added chemicals found in foods, including those which can cause food intolerance. For this reason, if removal of casein and/or gluten is the only dietary change made, it is impossible to state with certainty that these proteins alone are the root of the problem. The only way to establish which dietary components are a problem for an individual is a full elimination diet, followed by challenges. The diet minimises the intake of

all food intolerance chemicals, as well as casein and gluten. Once all symptoms have subsided, challenges with individual components of foods to determine which chemicals the individual is sensitive to(29). Hence, it appears that food intolerance chemicals are a possible cause of symptoms in children with ASD and that diet may impact on ASD behaviours both directly, through peptides produced during digestion and indirectly, through food intolerance reactions.

Children with ASD are commonly reported by parents as suffering sleep problems and research indicates that they experience more sleep problems than normally developing children(30-32) and children with other, intellectual disabilities(32). Specifically, when compared to children developing normally, children with ASD have been shown to spend longer in bed before falling asleep, to have decreased total sleep time per night(30, 31), and longer time spent awake if waking during the night(30, 31). They have also been shown to be less ready for bed and to have more unusual bed-time routines(30). Children's sleep may be disturbed as a result of a sleep breathing disorder(33), this may relate to nasal or sinus congestion, a potential symptom of food intolerance(29). As such, sleep difficulties appear to be part of the neurological manifestation of ASD but may also relate to the occurrence of food intolerance in children with ASD, and result in added stress and impact on their entire family.

The impact of a child with ASD on their family may be extensive. Stress on parents, strain on marital relationships, financial costs and effects on sibling relationships have all been described(34-36). This may make the implementation of dietary modification in children ASD more difficult.

Modification or restriction of the diet of a child with ASD is more commonly undertaken without the guidance of a qualified health professional, than with such guidance. Instead of sound advice, most parents rely on secondary sources of information, such as books, magazines, the internet, family/friends or alternative practitioners such as naturopaths(37, 38). Furthermore, imprudent dietary changes may

compromise nutritional adequacy in a child who already has difficult eating behaviour(37, 38). This trend to rely on peripheral sources of information rather than evidence-based practice may well result from a lack of reliable information and conclusive research.

It is clear that the roles of both diet and gastrointestinal disturbance in ASD need to be further examined. The aim of this investigation is to document the dietary intolerances observed in children with ASD, as well as the efficacy and sustainability of dietary modification in those children with ASD found to have dietary intolerances. This will help determine whether the "leaky gut" theory is valid or whether food intolerance chemicals are more likely culprits than casein and gluten only. That is, whether the improvements reported in children with ASD, following a casein-free and gluten-free diet, are related specifically to the removal of these proteins from the diet or to the reduced intake of food intolerance chemicals occurring with casein and gluten in foods.

Methods

Subject Selection and Recruitment

Subjects were recruited to form three distinct groups, selection as follows:

1. ASD GROUP – Children aged 3-10 years, with a diagnosis of ASD, previously seen by Dr Velencia Soutter at either the RPAH Allergy Unit or the Developmental Assessment Service at Kogarah.
2. MILK INTOLERANT GROUP – Children aged 3-10 years who have been assessed at the RPAH Allergy Unit and found to be milk intolerant but non-atopic and prescribed Neocate as a result of their milk intolerance. This forms an age-matched, non-ASD control group with known intolerance to milk but no allergies.
3. CONTROL GROUP – Children aged 3-10 years without ASD, either known to study investigators or attending Childcare Centres or Kindergartens in the Central Sydney Area Health Service region or siblings of ASD or Milk Intolerant subjects. This formed an age-matched control group considered to be a "well" control group, in that the subjects had not been diagnosed with ASD nor food intolerance or allergy.

Ethical Approval, Subject Consent and Confidentiality

Ethics approval for the study was sought and granted by the CSAHS Ethics Review Committee (RPAH Zone). Subjects' parents were initially contacted by telephone, if patients of the clinic, or through childcare centres and kindergartens. After an initial interest in the study was expressed, parents were provided with an expression of interest form along with the questionnaire booklet. A completed expression of interest form indicated subject consent and also allowed participants to register for participation in further involvement in the study. Questionnaire booklets and expression of interest forms were distributed already coded with a study identification number. After the booklets were returned only the identification number and child's initials were used to identify subjects.

Questionnaire Booklet Design

This investigation is one section of a broader study, "Dietary Issues in Children with and without Autistic Spectrum Disorder." As such, the questionnaire booklet covered a wide range of areas, not all of which are examined by this section of the study. As all participants completed all sections of the booklet, the process of developing the entire questionnaire booklet is described below.

Initially, questionnaires from various sources covering topics such as the child's developmental history; Autistic behaviours; general behaviours; eating behaviours; the impact of the child on the family; parental depression, anxiety and stress and parental eating disorder history. In addition, a Food Frequency Questionnaire (FFQ) was sourced, to be used as a reference for developing our own FFQ. As some existing questionnaires were not considered suitable for the intended purpose, it was decided that new questionnaires would need to be developed, whilst others could be used unchanged, or with changes to formatting but not content. The final questionnaire booklet is reproduced in Appendix 1, with the exception of the FFQ.

The Conners' Rating Scale is a validated childhood behaviour assessment tool and the fact that its format is scoring-inclusive ensured that it was used in its existing form(39). The Parental Depression Anxiety Stress Scale (DASS-42) (40), the Impact on Family Scale (IFS-24) (41) and the Pervasive Developmental Disorder Behavior Inventory for Parents (PDDBI-C, renamed "Social Interaction and Communication Skills") are all validated assessment tools(42, 43). For each of these, the content of existing questionnaires was retained but the questionnaires reformatted for consistency with the other questionnaires. The existing scoring systems, developed in conjunction with the questionnaires, were used to score the Conners' Rating Scale, the DASS-42, the IFS-24 and the PDDBI-C. In each case, answers to specific combinations of questions were tallied to give scores on a number of subscales.

The Children's Sleep Disturbance Scale was adapted from a scale developed and validated as described in 1996(33). Three questions were omitted as not considered essential to the current study, the rating scale for responses was changed from a five point to a three point scale to maintain consistency with the other questionnaires and the formatting was modified, again for consistency. Scoring was based on the existing system, which classifies sleep disturbances into 6 categories.

New questionnaires were developed to gather information on the child's developmental history ("Background Questionnaire"), food related symptoms and any history of dietary modification ("General Health and Behaviour Checklist"), eating behaviours ("Childhood Eating Behaviour and Appetite Scale") and current dietary intake (FFQ – "Your Child's Eating Preferences"). Whilst the question of food intolerance and allergy is central to this study, existing questionnaires did not gather sufficient information in this area. Hence, the new questionnaires developed were specifically designed to identify issues relating to food intolerance. This includes food related symptoms, treatments pursued for food intolerance, fussy eating behaviours and the overall load of food chemicals in the child's diet. Much of the data gathered by the Background Questionnaire and General Health and Behaviour Checklist was qualitative and therefore not scored. However, responses for "Health problems in the First Two Years," food reactions in the first year (both of these were in the Background Questionnaire) and the General Health and Behaviour Checklist were categorised into groups by symptom type or food cause.

Data Analysis

Data were entered using Microsoft Access 2002, computed using Microsoft SQL Query Analyser 2000 and Microsoft Excel 2002. Once entered into the study database, the data were checked by for accuracy. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) Version 11.5.

Data for subject age, gender, age at weaning, age at introduction of solids, the Background Questionnaire and the General Health and Behaviour Checklist were entered into the database and computed manually. Age for each subject was calculated at June 2003 and a oneway ANOVA performed with a Scheffe post-hoc test in order to determine mean age for each group and statistical significance for the differences in mean age between study groups. Gender was assessed as the percentage of males in each group, this was not assessed statistically. Data for Health Problems in the First Two Years (Background Questionnaire) were compiled and total number of percentage of subjects affected calculated for two categories for each group; duration longer or shorter than 1 year. No statistical analysis was performed on this data. The data from the scored questions of the General Health and Behaviour Checklist were manually assessed to determine the number of subjects in each group scoring "Sometimes" or "Often" (2 or 3) for five categories of symptoms. No statistical analysis was performed on this data. Data for foods causing adverse reactions were compiled and graphed using Excel, no statistical analysis was performed.

For questionnaires scored using subscales (Conners' Rating Scale, DASS, IFS, Children's Sleep Disturbance Scale), individual answers to questions were entered into the study database and Query Analyser was used to compute the scores for the various subscales. Oneway ANOVA analysis with a Scheffe post-hoc test was performed to determine any statistically significant differences between each of the study groups for the mean score on each subscale.

Results

Participation

63 completed questionnaire booklets were analysed. These were from 16 ASD subjects, 21 milk intolerant subjects and 26 control subjects. 29 ASD, 56 milk intolerant and 116 control subjects were approached. 25 booklets were distributed to ASD subjects, 46 to milk intolerant subjects and 116 to control subjects. Hence 86.21% of ASD subjects, 82.14% of milk intolerant subjects and 100.00% of control subjects approached were sent booklets. 64.00% of booklets distributed to ASD subjects were completed, 45.65% of those distributed to milk intolerant subjects and 22.42% of those distributed to control subjects. 5 booklets were returned completed after the data analysis for this stage of the study was completed. These were in addition to the above and will be included in future investigations in this study.

Of the 4 ASD subjects approached who did not receive booklets, 3 declined because the child's diagnosis was not ASD and was incorrect on clinic records. The fourth did not wish to participate.

Characteristics of the sample groups

Table 1 shows the mean age, gender and Conners' Childhood Behaviour Rating Scale T scores for the three study groups. Differences between the ASD group and either of the other groups that were shown to be statistically significant are indicated with symbols and p values shown with the table. The mean age of the ASD group (7.33 years or 7 years, 3 months) was significantly greater than the mean age of the milk intolerant group (5.5 years, $p = 0.029$) and the control group (5.4 years, $p= 0.013$). The ASD group was entirely male, considerably more so than both the milk intolerant and control groups, 52.38% and 73.08% male respectively. The Conners' Childhood Behaviour Rating Scale is scored with T scores on 6 subscales. Mean T scores on all subscales were within one standard deviation of the population mean (T score between 40 and 60) for all 3 groups excepting those listed below.

TABLE 1. Sample size; age, gender and behavioural characteristics of the study groups

	ASD	MI	Control
Number of subjects (n)	16	21	26
Age (years) – mean ± SD	7.33 ±2.17	5.54 ±2.06#	5.41 ±1.76∞
Gender – % male	100.00	52.38	73.08
Conners' T Scores:			
Oppositional – mean ± SD	47.13 ±19.16	54.80 ±17.72	47.27 ±6.86
Learning Difficulties – mean ± SD	77.07 ±22.82	52.45 ±14.18*	47.12 ±11.72*
Psychosomatic – mean ± SD	50.06 ±24.41	75.90 ±19.96**	60.96 ±20.44
Impulsive–Hyperactive – mean ± SD	61.06 ±21.45	54.35 ±15.01	47.88 ±8.89***
Anxiety – mean ± SD	46.56 ±18.88	55.35 ±13.58	46.54 ±7.91
Hyperactivity – mean ± SD	69.13 ±19.05	51.65 ±17.08****	45.92 ±9.43*

p = 0.029; ∞ p = 0.013; * p = 0.000; ** p = 0.003; *** p = 0.027; **** p = 0.005.

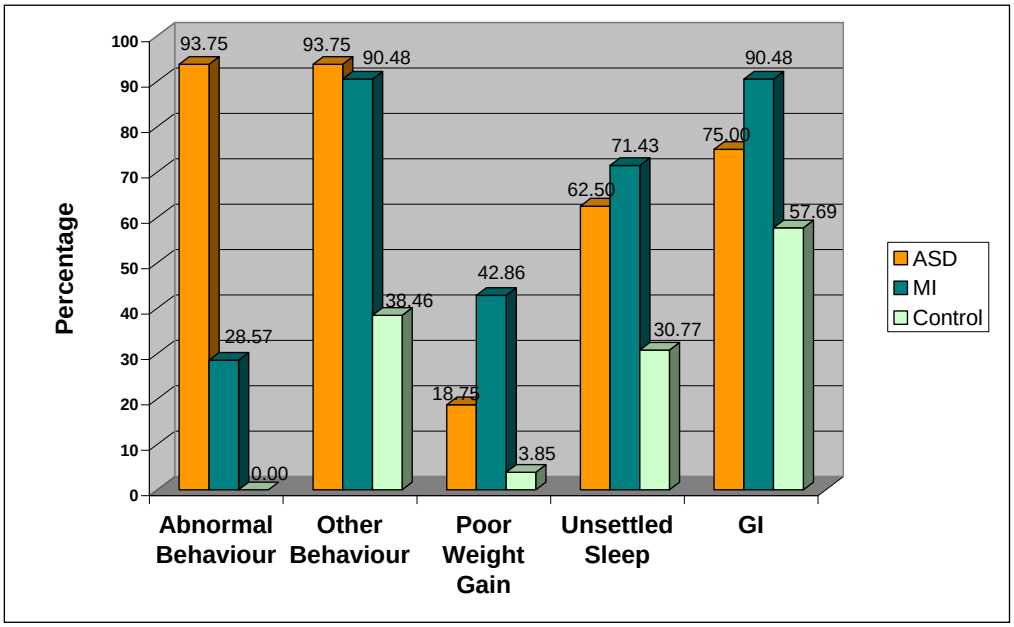
The mean T Score of the ASD group for Learning Problems was 77.07, which is more than two standard deviations above the mean and puts this group above the 98th percentile. The ASD group showed significantly more difficulty with learning than the milk intolerant (p = 0.000) and control groups (p = 0.000). The milk intolerant group scores greater than two standard deviations above the mean (above the 98th percentile) on the Psychosomatic subscale, significantly higher than the ASD group (p = 0.003) but not the control group. The ASD group scored greater than one standard deviation above the mean for both the Impulsive-Hyperactive and Hyperactivity subscales, placing them between the 86th and 94th percentiles for Impulsive-Hyperactive and between the 95th and 98th percentiles for Hyperactivity. The ASD group was significantly more impulsive than the control group (p = 0.027) and more hyperactive than both the milk intolerant (p = 0.005) and control (p = 0.000) groups (39).

Health Problems Observed in the First Two Years of Life

Using check boxes, respondents indicated periods in the first two years of life that their child experienced any of a range of symptoms. These are all common symptoms of food intolerance and may indicate that the child suffered from food intolerance. Data for the total number of subjects affected by each type of symptom is shown in Figure 1. Data for ear infections and frequent antibiotic use is shown in Figure 2.

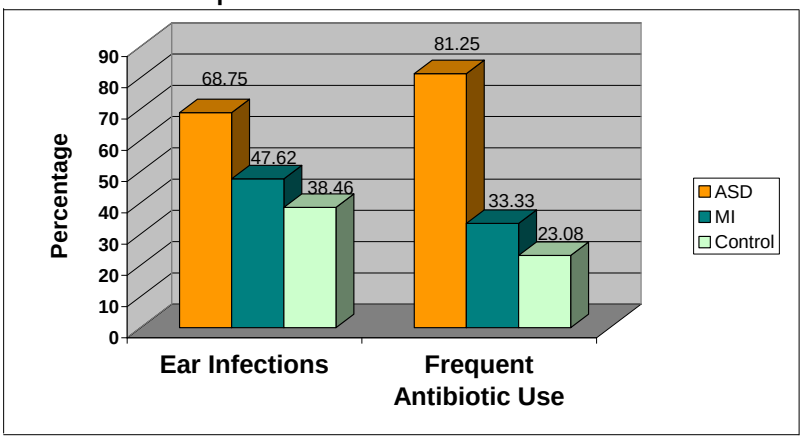
Complete data, which details the number and percentage of subjects experiencing symptoms for longer than one year and less than one year, is shown in Appendix 2.

FIGURE 1. Health Problems in the First Two Years



After compiling this data, most noteworthy are behavioural issues, unsettled sleep, Gastrointestinal (GI) symptoms, ear infections and frequent antibiotic use. The ASD group exhibited a considerably higher prevalence of abnormal behaviour than both milk intolerant and control groups. All but 1 (93.75%) of the ASD group showed signs abnormal behaviour within their first two years and 13 of 16 (81.25%) for longer than one year of their first two. Substantially more ASD and milk intolerant subjects showed other behavioural symptoms (demanding behaviour, screaming/colic and feeding difficulty) than control subjects. 15 of 16 ASD subjects (93.75%) and 19 of 21 milk intolerant subjects (90.48%) showed at least one of these symptoms within the first two years, compared with 10 of 26 control subjects (38.46%). The majority of those had symptoms for longer than one year (12 ASD subjects, 17 milk intolerant subjects). Milk intolerant subjects had appreciably higher prevalence of GI symptoms (Vomiting/reflux, blood/mucous in vomit, frequent loose stools, blood/mucous in stools and nappy rashes/burnt bottom) than ASD subjects who in turn had a higher rate of GI symptoms than control subjects.

FIGURE 2. Percentage of Each Group Affected by Ear Infections and Frequent Antibiotic Use

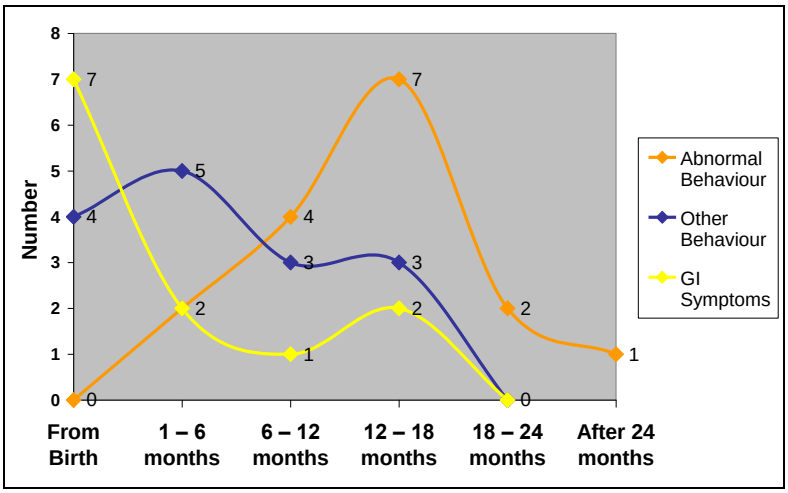


More ASD subjects suffered ear infections (68.75%), during their first two years, than both milk intolerant (47.62%) and control subjects (38.46%). Even more striking was their frequent antibiotic use. 13 of 16 ASD subjects (81.25%) had antibiotics frequently at some stage in their first two years, for 7 of these (43.75%) antibiotics were used frequently for longer than one year. Comparatively, 7 (33.33%) milk intolerant and 6 (23.08%) control subjects used antibiotics frequently during their first two years.

Onset of Abnormal Behaviour (ASD group)

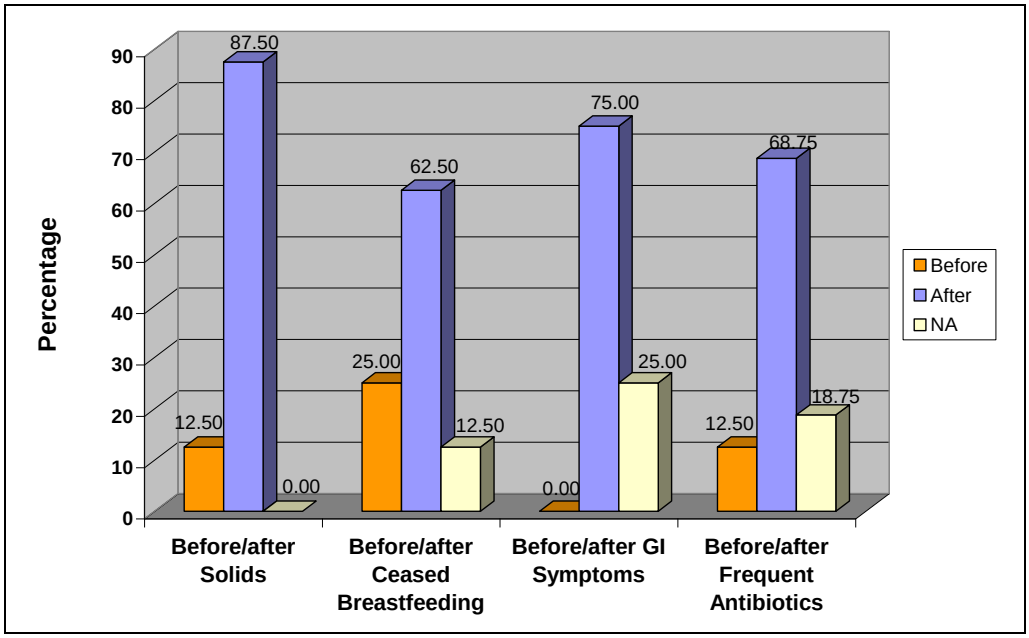
The age at onset of abnormal behaviour and other behaviours (demanding behaviour; screaming/"colic") are compared in Figure 3. Most ASD subjects first exhibited abnormal behaviour between 9 and 24 months (75% of all subjects) whilst the other behaviours more often began either from birth or within the first 9 months of life. 9 of 15 parents reporting other behavioural symptoms (60.00%) reported that these began before 6 months of age, 12 of 15 (80.00%) began before 12 months and none after 18 months. This data is shown in full in Appendix 3.

FIGURE 3. Age at onset of Abnormal Behaviour, Other Behaviour and GI Symptoms (ASD subjects)



The timing of the onset of abnormal behaviour was also compared with other factors, including the introduction of solid foods, cessation of breastfeeding and onset of other symptoms. These results are shown in Figure 4. The onset of abnormal behaviour was more often after than before the introduction of solids, cessation of breastfeeding, onset of GI symptoms and frequent antibiotic use.

FIGURE 4. Onset of Abnormal Behaviour – Before or After Other Factors

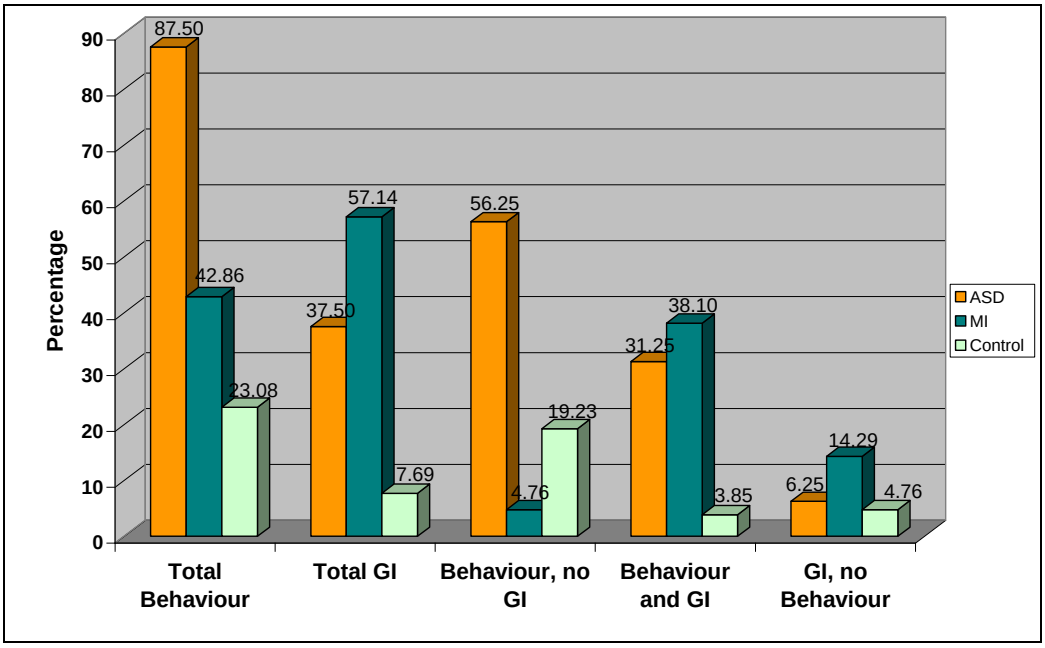


Current Symptoms

Current health issues which may be indicative of food intolerance were investigated. These included behavioural symptoms, GI symptoms grouped by type, sleep problems, having a blocked nose and ear conditions. Data were calculated by the number and percentage of subjects in each group affected, that is scoring 2 or 3 on the General Health and Behaviour Checklist for each symptom type.

Data for behavioural symptoms (hyperactivity; staring blankly/glazing over; irritability) and GI symptoms (abdominal pain or distension; reflux or swallowing difficulty, nausea or vomiting; diarrhoea or loose bowel motions; constipation) are shown in Figure 5. Data for sleep problems, blocked nose and ear problems (ear infections and glue ear) are shown in Table 2.

FIGURE 5. Current Symptoms – Percentage of Each Group Affected by Behavioural and Gastrointestinal Symptoms



As shown in Figure 5, a considerably greater percentage of the ASD group (87.50%) were affected by the behavioural issues examined than both the milk intolerant (42.86%) and control groups (23.08%).

Furthermore, the percentage of milk intolerant subjects who have behavioural problems was greater than the percentage of control subjects. Data shown in this figure are composite and data for individual behavioural and GI symptoms are shown in Appendix 4. Constipation was the only GI symptom exhibited by a higher percentage of ASD subjects (12.50%) than milk intolerant subjects (9.52%). The trend for all other GI symptoms (abdominal pain/distension, reflux/swallowing difficulty, nausea/vomiting and diarrhoea) was that the percentage of milk intolerant subjects affected was greater than for the ASD group, which was in turn greater than for the control group. The only exception to this was nausea and vomiting which affected no subjects in either the ASD or control group.

As shown in Table 2, considerably more ASD subjects suffer from a blocked nose (31.25%) than milk intolerant (9.52%) and control subjects (0.00%), whilst a substantially greater percentage of milk intolerant subjects (38.10%) than ASD (6.25%) or control (3.85%) subjects have sleep problems. More milk intolerant subjects (9.52%) experience ear infections or glue ear than ASD (6.25%) subjects and controls (7.69).

TABLE 2. Current Symptoms – Percentage of Each Group Affected by Sleep Problems, Blocked Nose, Ear Problems

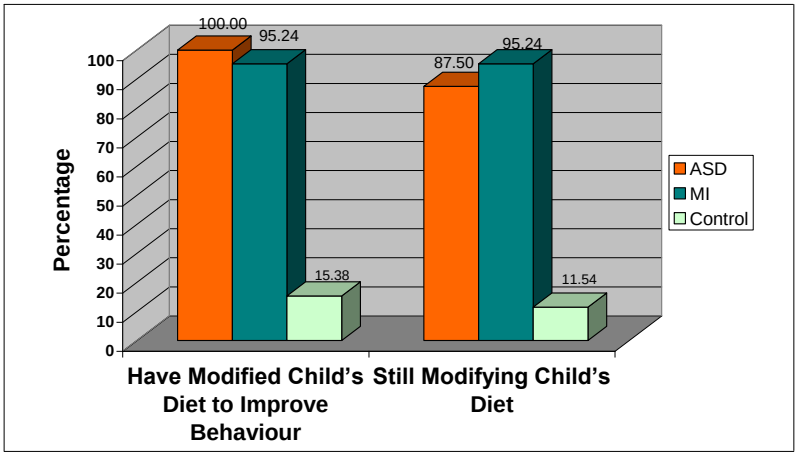
	ASD (n = 16)	**MI** (n = 21)	**Control** (n = 26)
Sleep Problems	6.25%	38.10%	3.85%
Blocked Nose	31.25%	9.52%	0.00%
Ear Infections or Glue Ear	6.25%	9.52%	7.69%

Sleep problems and breathing issues are also examined by the Children's Sleep Disturbance Scale. On this scale, the total score for sleep disturbance was significantly higher for milk intolerance subjects (15.75 out of a possible 69) than both ASD subjects (8.00) and control subjects (9.00). Differences were significant between milk intolerant and ASD groups ($p = 0.032$) and between milk intolerant and control subjects ($p = 0.023$). This confirms the results of the General Health and Behaviour Checklist, in that milk

intolerant subjects do appear to have more sleep disturbance issues than the other group. With respect to sleep breathing disorders, which may be linked to nasal passages being blocked, the Children's Sleep Disturbance Scale contradicts the results presented in Figure 5. Milk intolerant subjects had the highest mean score for sleep breathing disorders, 1.15 out of a possible 9, compared with 0.87 for ASD subjects and 0.67 for control subjects, although these differences were not statistically significant. Full data for the Children's Sleep Disturbance Scale is shown in Appendix 5.

History of Dietary Modification

FIGURE 6. History of Dietary Modification



Respondents were asked if they had modified their child's diet in order to change their child's behaviour and if they were still modifying their child's diet. The results are shown in Figure 6. It was found that 100.0% of the parents of ASD subjects had modified their child's diet to improve behaviour, as had 95.24% of parents of milk intolerant subjects and 15.38% of parents of control subjects. 87.50% of ASD subjects currently had diets modified in some way, as well as 95.24% of milk intolerant subjects and 11.54% of control subjects.

9 of the ASD group (56.25%) had completed the elimination diet and food/chemical challenges through the RPAH Allergy Unit. 7 of the group (43.75%) had been prescribed Neocate, an amino acid based formula.

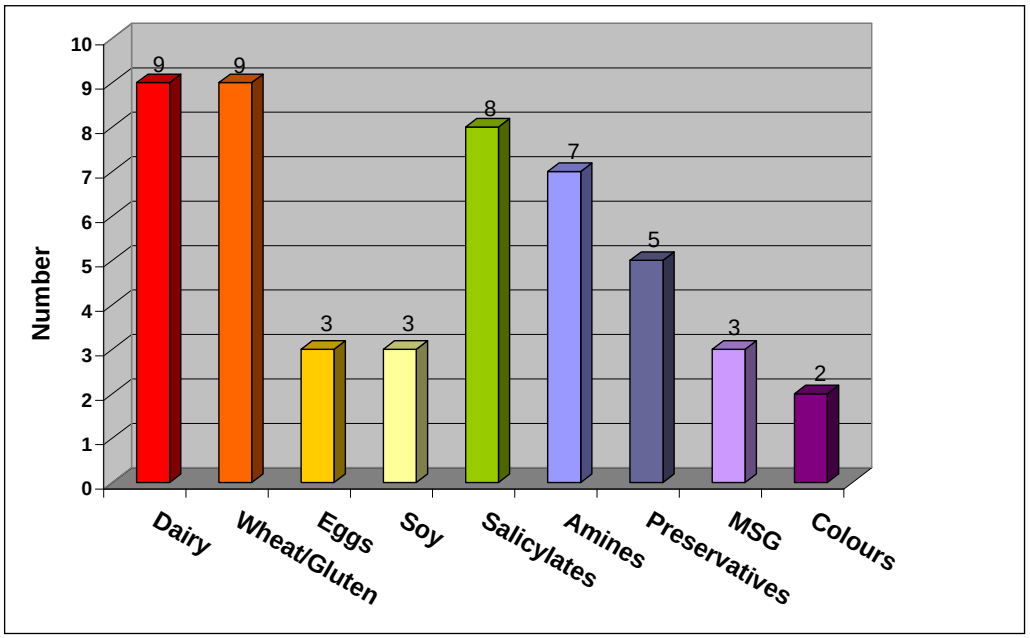
Intolerances Observed in Children with ASD who have Attended the RPAH Allergy Unit

Parents were asked to list any foods or food chemicals which they consider affect their child's behaviour or well-being. These are shown in Figure 7 and a full list is shown in Appendix 6. One subject had allergies to milk and eggs and these two foods were excluded from the data, all other reactions for all subjects were

non-allergic. The foods parents indicated that their child tolerated in their first year and those which caused adverse reactions were also analysed. These data are also presented in Appendix 6.

The dominant foods cited by parents were dairy foods (9 of 16 subjects; 56.25%) and wheat or gluten (9 of 16; 56.25%). These are likely to be food protein mediated reactions. Also commonly listed were the natural food chemicals of the salicylate (8 of 16; 50.00%) and amine (7 of 16; 43.25%) groups and preservatives added to foods; proprionate, sulphites, antioxidants (5 of 16; 31.25%). Many of the group were intolerant to multiple foods. The foods reacted to by each subject and a tally of the number of foods affecting individual subjects is shown in Appendix 6. 8 of 16 subjects (50.00%) were reported to have reactions to more than 3 foods by parents and 4 (25.00%) were reported to be to have non-allergic reactions to 3 or more food proteins (dairy, soy, wheat/gluten, eggs, nuts). These 4 were all prescribed Neocate.

FIGURE 7. Foods Affecting Behaviour or Well-Being of ASD Subjects (parent-reported)



The major difference between the foods reported to currently affect behaviour or well being and those reacted to in the first year of life was the wheat/gluten group. Only 2 children were reported to have

reacted to wheat or gluten containing foods in their first year of life, whilst 9 were reported to be affected by wheat or gluten at the time the questionnaire was completed. The other point of note was that 2 children reportedly reacted to protein rich foods, meat or fish, in their first year. No current reactions to meat or fish were reported. For all other foods and food chemicals the number of children who reacted within their first year of life was comparable to, and possibly slightly fewer than, the number that have current reactions.

Outcomes of Dietary Modification in Children with ASD who have Attended the RPAH Allergy Unit

14 of 16 parents (87.5%) reported improvements in symptoms following dietary modification. Many different symptoms were listed, these are summarised in Table 3, with the number of parents listing improvement for each.

Table 3. Symptoms Reported by Parents to Improve with Dietary Modification (ASD Group)

Improvement in:	Number of Subjects
Behaviour in general	3
Irritibility/aggression	3
Hyperactivity	3
Concention/learning	2
Social Interaction	2
Communication	2
Overly emotional behaviour	1
Bowel habit	5
Bloating/pain/gas	2
Bladder control	2
Sleep	2
Skin symptoms	3
Tremor	1
General health	1

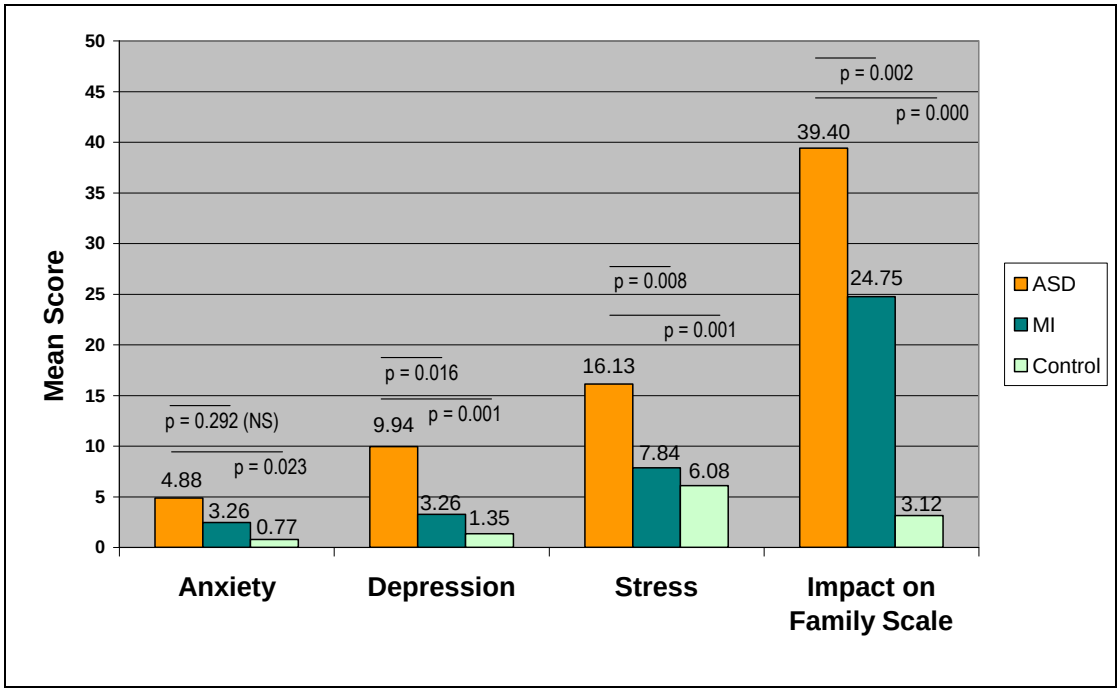
Overall the behavioural improvement, including reduced hyperactivity, irritability/aggression, overly emotional behaviour, as well as social interaction, communication and concentration/learning were the most improved, flowed by bowel habit, which was generally improvement in stool firmness. Sleep, skin and bladder control problems also improved. The two parents who had ceased dietary modification did so

as they could not see any significant improvement in symptoms and could not pinpoint any foods as responsible for symptoms.

Impact of a Child with ASD on Parents' State of Mind and on the Family as a Whole

The effect of the three groups of children on their parent's levels of depression, anxiety and stress was assessed using the DASS-42, as well as their impact on their family as a whole. Impact on the family was measured using the IFS-24 a scale which measures levels of financial strain, familial or social strain, personal strain on parents and the level of mastery demanded of parents. Full data for the IFS-24 are shown in Appendix 7. Mean scores for depression, anxiety, stress and total score of the IFS-24 are shown in Figure 8. Each DASS-42 subscale is scored out of 42 and the maximum total score for the IFS-24 is 72. Significance between the ASD and milk intolerance groups and between the ASD and control groups is indicated on the Figure by p values.

FIGURE 8. Parental Depression, Anxiety, Stress and the Impact of the Child on the Family



`The mean score for anxiety was highest for parents in the ASD group, although the difference was significant only between the ASD and control groups ($p = 0.023$). Mean score for depression in the ASD group were significantly higher than the milk intolerant ($p = 0.016$) and control ($p = 0.001$) groups. Stress was significantly higher in parents of ASD subjects than milk intolerant subjects ($p = 0.008$) and control subjects ($p = 0.001$). Using the DASS Profile Sheet(40), scores for depression, anxiety and stress can be rated in terms of severity. The ASD group mean scores indicate mild depression, normal anxiety and mild stress levels. The IFS-24 total score was significantly higher for ASD subjects than both milk intolerant subjects ($p = 0.002$) and control subjects ($p = 0.000$).

Discussion

GI and behavioural symptoms were very common in the ASD group studied in this investigation. 37.50% currently had GI symptoms, whilst 75.00% had experienced GI symptoms in their first two years of life. This decrease may reflect the impact of dietary modification on symptoms, as the vast majority of the group currently have some modification made to their diet. Behavioural symptoms such as hyperactivity and irritability currently affect 87.50% of subjects and 93.75% were demanding or "colicky" (irritable) at some point prior to age two. Both GI and behavioural symptoms have been linked with ASD in the literature. GI issues including abnormal intestinal permeability and histology, enzyme deficiencies, as well as symptoms including diarrhoea, abdominal pain, nausea and vomiting have been described in children with ASD(17, 20, 21). One study reported that 76% of a group of children with ASD experienced at least one GI symptom(21). Features of attention-deficit hyperactivity disorder (AHDH), including overactivity, impulsivity and inattention, are commonly displayed by children with ASD(44-46). Correlations between GI symptoms and behaviour in ASD have been described. Wakefield et al express the view that behavioural symptoms are likely to be intestinal in origin(17) and Horvath et al showed that in ASD children suffering reflux oesophagitis had increased levels of unexplained daytime irritability, compared with those with no oesophagitis(21).

Dietary modification has been shown to benefit children with ASD. For 87.50% of ASD subjects in this study, the outcomes of dietary modification warranted its continuation. Elsewhere, clinical improvements have been reported in Children with ASD, following implementation of Gluten-free and Casein-free diets(14, 15, 47) and mechanisms for these improvements proposed(47). The areas demonstrating improvement included aloofness, concentration and response to teaching(15), all of which were reported by parents of ASD subjects in this study as improving following dietary modification.

Hence, there is paradox in the ASD literature: links have been made between both behavioural and GI symptoms and ASD, and between clinical improvements and dietary modification, yet no link with food intolerance has been postulated. Behavioural(46) and GI(17) symptoms have been almost considered to be a manifestation of the disorder itself, rather than having a role in their own right.

The evidence presented in this investigation strongly suggests diet may affect some of the symptoms of children with ASD. Firstly, symptoms such as diarrhoea, vomiting, irritability and hyperactivity, which have been shown to affect children with ASD, both in this investigation and elsewhere(20, 21), may be exacerbated by food intolerance(29, 48). Secondly, behavioural and GI symptoms tend to begin within the first six months, and generally precede any abnormal/ASD behaviour in most cases. This implies that many of these symptoms are related to food intolerance rather than to ASD.

Food intolerance is dose related and tolerance levels are variable and idiosyncratic(29). As such, the most sensitive individuals have the lowest tolerance threshold and react earlier in life, often from birth. Others who are less sensitive have higher tolerance levels and react later in life. This can occur as a result of widening of the diet with the introduction of solids. The increase in variety of the diet from exclusive breast or formula feeding leads to an increase in the overall load of foods which may cause intolerance.

The foods and food chemicals reported by parents as causing reactions in ASD subjects are numerous and varied. This indicates that the role of diet in the aetiology of symptoms in these children may involve more than just gluten and/or casein.

First of all, there was a high prevalence of parent-reported intolerance to dairy and wheat/gluten as well as eggs and soy products. Reactions may be to the protein component of each of these foods and as such,

this may relate to the concept of Multiple Food Protein Intolerance of Infancy (MFPI) which has been described by others(48). Hill et al define MPFI as intolerance to soy formula, extensively hydrolysed formula and several other common food proteins including cow's milk protein, egg and nut proteins. As infants affected by MPFI tend to exhibit intolerance to numerous different foods they present a complex nutritional problem and require an elemental amino acid formula, such as Neocate(48). In this investigation, 25% of children with ASD were intolerant to three or more of these protein foods and all were prescribed Neocate. It is also possible that the reason for reactions to dairy may be in part be related to lactose intolerance, Horvath et al found lactase levels to be low in 14 of 36 children with ASD(20).

Furthermore, reactions to salicylates, amines, MSG and various preservatives were reported in many of the ASD subjects. This shows that as well as observed food protein reactions, reactions to these natural and added food chemicals are also common in this group of children with ASD.

As indicated by the results of the DASS-42 and IFS-24, parents of children with ASD are significantly more depressed, stressed and anxious than the other study parents and children with ASD have a significantly greater impact on their family than the other study children. Dietary modification may be more difficult for parents of children with ASD to implement for these reasons. Furthermore, behavioural, GI and other symptoms resulting from food intolerance may increase the already heavy burden of having a child with ASD. These issues are largely beyond the scope of this investigation but certainly warrant further research. To this end, the major focus of another investigation in this study has been the effect of a child with ASD on their parents' mental health and their family, with focus groups held to gather information on the major food or dietary issues faced by parents of children with ASD.

In this study, we did not find significant sleep problems in the ASD group as documented by others(30-32). Their mean score from the Children's Sleep Disturbance Scale indicates that they had sleep problems less

commonly than the milk intolerant group. Furthermore, although many of the ASD group were reported to have a blocked nose, they had a lower mean score for sleep breathing disorders.

Limitations/Future Research

This study has four main areas for further research. Firstly, the small sample size. With only 16 subjects in the ASD group it is difficult to make firm conclusions, further subjects need to be recruited to better document the findings. Additionally, with increased numbers, sub-group analysis could be undertaken to exclude the possible effect of age and gender differences between groups on results. Secondly, the sample group was a selected group of children with ASD. As all ASD subjects had attended the RPAH Allergy Unit for assessment of possible food intolerance or allergy, this group is likely to be more affected by food intolerance, behavioural or GI symptoms than other children with ASD. Further research needs to be undertaken looking at children with ASD as a whole, in order to determine the range of food intolerances of children with ASD and whether there is a high prevalence of food intolerance in children with ASD. Thirdly, some data were rendered unusable as the questions were not always answered in the manner intended; there was variation in how the questions' were interpreted by respondents. Fourth, the data for food intolerances in subjects with ASD were parent reported. Ideally, reactions to challenges would be determined by elimination diet and challenge for each subject. This would determine the range of food intolerances affecting children with ASD more accurately.

These difficulties can be addressed as the study continues, with further subjects recruited from children with ASD in the general population. This will allow both the first and second limitations to be addressed; achieving an increase in sample size and allowing a comparison of a group of ASD subjects from the general population with the group described in this investigation.

Conclusion

A group of children with ASD exhibiting food intolerance to a wide range of foods/food chemicals has been described. Many of these children exhibit behavioural and/or gastrointestinal symptoms common in patients with food intolerance and dietary modification has been shown to improve these children's symptoms in many cases. This implies a potential role for food intolerance in the aetiology and management of symptoms in children with ASD. Future research is needed in order to determine the nature and prevalence of food intolerance in children with

References

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th Ed, Text Revision. Washington, DC: American Psychiatric Association, 2000.
2. Volkmar FR, Pauls D. Autism. The Lancet. 2003;362:1133-1141. Last accessed, 29/10/2003.
3. Autism Council of Australia Ltd. http://www.autismaus.com.au/what/index.html.
4. Fombonne E. Epidemiological Surveys of Autism and Other Pervasive Developmental Disorders: An Update. Journal of Autism and Developmental Disorders 2003;33:365-382.
5. Folstein SE, Rosen-Sheidley B. Genetics of autism: complex aetiology for a heterogeneous disorder. Nature Reviews Genetics. 2001;2:943-55.
6. Lauritsen M, Ewald H. The genetics of autism. Acta Psychiatrica Scandinavica. 2001;103:411-27.
7. Monaco AP, Bailey AJ. Autism. The search for susceptibility genes. Lancet. 2001;358:S3.
8. Madsen KM, Hviid A, Vestergaard M, et al. A Population-Based Study of Measles, Mumps, and Rubella Vaccination and Autism. New England Journal of Medicine. 2002;347:1477-1482.
9. Fombonne E, Chakrabarti S. No evidence for a new variant of measles-mumps-rubella-induced autism. Pediatrics. 2001;108:E58.
10. Fombonne E, Cook EH. MMR and autistic enterocolitis: consistent epidemiological failure to find an association. Molecular Psychiatry. 2003;8:133-134.
11. Taylor B, Miller E, Farrington CP, et al. Autism and measles, mumps, and rubella vaccine: no epidemiological evidence for a causal association. Lancet. 1999;353:2026-9.
12. Taylor B, Miller E, Lingam R, Andrews N, Simmons A, Stowe J. Measles, mumps, and rubella vaccination and bowel problems or developmental regression in children with autism: population study. BMJ 2002;324:393-396.
13. Reichelt KL, Hole K, Hamberger A, et al. Biologically active peptide-containing fractions in schizophrenia and childhood autism. Advances in Biochemical Psychopharmacology. 1981;28:627-43.
14. Knivsberg AM, Reichelt KL, Hoien T, Nodland M. A randomised, controlled study of dietary intervention in autistic syndromes. Nutritional Neuroscience. 2002;5:251-61.
15. Knivsberg AM, Reichelt KL, Nodland M. Reports on dietary intervention in autistic disorders. Nutritional Neuroscience. 2001;4:25-37.
16. Seim AR, Reichelt KL. An enzyme/brain-barrier theory of psychiatric pathogenesis: unifying observations on phenylketonuria, autism, schizophrenia and postpartum psychosis. Medical Hypotheses. 1995;45:498-502.
17. Wakefield AJ, Puleston JM, Montgomery SM, Anthony A, O'Leary JJ, Murch SH. Review article: the concept of entero-colonic encephalopathy, autism and opioid receptor ligands. Alimentary Pharmacology & Therapeutics. 2002;16:663-74.
18. Hunter LC, O'Hare A, Herron WJ, Fisher LA, Jones GE. Opioid peptides and dipeptidyl peptidase in autism.[comment]. Developmental Medicine & Child Neurology. 2003;45:121-8.
19. Wakefield AJ, Murch SH, Anthony A, et al. Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. Lancet. 1998;351:637-41.

20. Horvath K, Papadimitriou JC, Rabsztyn A, Drachenberg C, Tildon JT. Gastrointestinal abnormalities in children with autistic disorder. Journal of Pediatrics. 1999;135:559-63.

21. Horvath K, Perman JA. Autistic disorder and gastrointestinal disease. Current Opinion in Pediatrics. 2002;14:583-7.

22. Sandler AD, Sutton KA, DeWeese J, Girardi MA, Sheppard V, Bodfish JW. Lack of benefit of a single dose of synthetic human secretin in the treatment of autism and pervasive developmental disorder. New England Journal of Medicine. 1999;341:1801-6.

23. Sandler AD, Bodfish JW. Placebo effects in autism: lessons from secretin. Journal of Developmental & Behavioral Pediatrics. 2000;21:347-50.

24. Roberts W, Weaver L, Brian J, et al. Repeated doses of porcine secretin in the treatment of autism: a randomized, placebo-controlled trial. Pediatrics. 2001;107:E71.

25. Molloy CA, Manning-Courtney P, Swayne S, et al. Lack of benefit of intravenous synthetic human secretin in the treatment of autism. Journal of Autism & Developmental Disorders. 2002;32:545-51.

26. Owley T, McMahon W, Cook EH, et al. Multisite, double-blind, placebo-controlled trial of porcine secretin in autism. Journal of the American Academy of Child & Adolescent Psychiatry. 2001;40:1293-9.

27. Unis AS, Munson JA, Rogers SJ, et al. A randomized, double-blind, placebo-controlled trial of porcine versus synthetic secretin for reducing symptoms of autism. Journal of the American Academy of Child & Adolescent Psychiatry. 2002;41:1315-21.

28. Corbett B, Khan K, Czapansky-Beilman D, et al. A double-blind, placebo-controlled crossover study investigating the effect of porcine secretin in children with autism. Clinical Pediatrics. 2001;40:327-31.

29. Loblay RH, Swain AR. Food Intolerance. In: Wahlqvist M, Truswell AS, eds. Recent Advances in Clinical Nutrition, 2nd ed. London: Libbey, 1986.

30. Patzold LM, Richdale AL, Tonge BJ. An investigation into sleep characteristics of children with autism and Asperger's Disorder. Journal of Paediatrics & Child Health. 1998;34:528-33.

31. Diomedi M, Curatolo P, Scalise A, Placidi F, Caretto F, Gigli GL. Sleep abnormalities in mentally retarded autistic subjects: Down's syndrome with mental retardation and normal subjects. Brain & Development. 1999;21:548-53.

32. Richdale AL. Sleep problems in autism: prevalence, cause, and intervention. Developmental Medicine & Child Neurology. 1999;41:60-6.

33. Bruni O, Ottaviano S, Guidetti V, et al. The Sleep Disturbance Scale for Children (SDSC). Construction and validation of an instrument to evaluate sleep disturbances in childhood and adolescence. Journal of Sleep Research. 1996;5:251-61.

34. Tunali B, Power TG. Coping by Redefinition: Cognitive Appraisals in Mothers of Children with Autism and Children Without Autism. Journal of Autism & Developmental Disorders.1` 2002;32:25-34.

35. Wood Rivers J, Stoneman Z. Sibling Relationships When a Child Has Autism: Marital Stress and Support Coping. Journal of Autism & Developmental Disorders 2003;33:383-394.

36. Jarbrink K, Fombonne E, Knapp M. Measuring the Parental, Service and Cost Impacts of Children with Autistic Spectrum Disorder: A Pilot Study. Journal of Autism & Developmental Disorders 2003;33:395-401.

37. Cornish E. Gluten and casein free diets in autism: a study of the effects on food choice and nutrition. Journal of Human Nutrition & Dietetics. 2002;15:261-9.

38. Arnold GA, Hyman SL, Mooney RA, Kirby RS. Plasma Amino Acids Profiles in Children with Autism: Potential Risk of Nutritional Deficiencies. Journal of Autism & Developmental Disorders. 2003;33:449-454.

39. Conners CK. Conners' Rating Scales - Revised. Canada: Multi-Health Systems Inc., 1997.

40. Lovibond SH, Lovibond PF. Manual for the Depression Anxiety Stress Scales. (2nd. Ed.). Sydney: Psychology Foundation, 1995.

41. Stein RE, Riessman CK. The development of an impact-on-family scale: preliminary findings. Medical Care. 1980;18:465-72.

42. Cohen IL, Schmidt-Lackner S, Romanczyk R, Sudhalter V. The PDD Behavior Inventory: a rating scale for assessing response to intervention in children with pervasive developmental disorder. Journal of Autism & Developmental Disorders. 2003;33:31-45.

43. Cohen IL. Criterion-related validity of the PDD Behavior Inventory. Journal of Autism & Developmental Disorders. 2003;33:47-53.

44. Aman MG, Langworthy KS. Pharmacotherapy for hyperactivity in children with autism and other pervasive developmental disorders. Journal of Autism & Developmental Disorders. 2000;30:451-9.

45. Handen BL, Johnson CR, Lubetsky M. Efficacy of methylphenidate among children with autism and symptoms of attention-deficit hyperactivity disorder. Journal of Autism & Developmental Disorders. 2000;30:245-55.

46. Nicolson R, Castellanos FX. Commentary: considerations on the pharmacotherapy of attention deficits and hyperactivity in children with autism and other pervasive developmental disorders. Journal of Autism & Developmental Disorders. 2000;30:461-2.

47. Reichelt KL, Knivsberg AM. Can the pathophysiology of autism be explained by the nature of the discovered urine peptides? Nutritional Neuroscience. 2003;6:19-28.

48. Hill DJ, Heine RG, Cameron DJS, Francis DEM, Bines JE. The Natural History of Intolerance to Soy and Extensively Hydrolysed Formula in Infants with Multiple Food Protein Intolerance. Journal of Pediatrics. 1999;135:118-121.

APPENDIX 1. Questionnaire Booklet

APPENDIX 2. Health Problems in the First Two Years

Percentage of Subjects in Each Group Affected by a Number of Symptoms that May Indicate Food Intolerance

	ASD		MI		Control	
	Lasted Longer than 1 year	Lasted Less than 1 year	Lasted Longer than 1 year	Lasted Less than 1 year	Lasted Longer than 1 year	Lasted Less than 1 year
Abnormal Behaviour	13 (81.25%)	2 (12.5%)	6 (28.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Other Behavioural Symptoms	12 (75.00%)	3 (18.75%)	17 (80.95%)	2 (9.52%)	7 (26.92%)	3 (11.54%)
Poor Weight Gain	2 (12.50%)	1 (6.25%)	6 (28.57%)	3 (14.29%)	0 (0.00%)	1 (3.85%)
Unsettled Sleep	6 (37.50%)	4 (25.00%)	11 (52.38%)	4 (19.05%)	4 (15.38%)	4 (15.38%)
GI Symptoms	8 (50.00%)	4 (25.00%)	17 (80.95%)	2 (9.52%)	6 (23.08%)	9 (34.62%)
Skin Symptoms	6 (37.50%)	7 (43.75%)	10 (47.62%)	5 (23.81%)	6 (23.08%)	4 (15.38%)
Respiratory Symptoms	2 (12.5%)	1 (6.25%)	1 (4.8%)	9 (42.9%)	4 (15.4%)	2 (7.7%)
Ear Infections	8 (50.00%)	3 (18.75%)	7 (33.33%)	3 (14.29%)	8 (30.77%)	2 (7.69%)
Frequent Antibiotics	7 (43.75%)	6 (37.50%)	4 (19.05%)	3 (14.29%)	6 (23.08%)	0 (0.00%)
Fits/Seizures	1 (6.25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
No symptoms in 1st 2 years	0 (0.00%)		0 (0.00%)		3 (11.54%)	

APPENDIX 3. Age at Onset of Behavioural and GI Symptoms

Age at Onset of Behavioural and GI Symptoms (number of ASD subjects within a time period)

	From Birth	1 – 3 months	3 – 6 months	6 – 9 months	9 – 12 months	12 – 18 months	18 – 24 months	After 24 months	Total
Abnormal Behaviour	0	0	2	0	4	7	2	1	**16**
Other Behaviour	4	4	1	2	1	2	0	NA	**15**
GI Symptoms	5	1	1	1	0	2	0	NA	**12**

NA = no data available for this time period, for these symptoms

APPENDIX 4 – Current Behavioural and GI Symptoms

Current Symptoms – Percentage of Each Group Affected by Individual Behavioural and GI Symptoms

	ASD (n = 16)	MI (n = 21)	Control (n = 26)
Hyperactive	68.75%	23.81%	14.29%
Irritable	50.00%	38.10%	14.29%
Abdominal Distension/Pain	12.50%	42.86%	0.00%
Reflux/Swallowing Difficulty	6.25%	19.05%	0.00%
Nausea/Vomiting	0.00%	14.29%	0.00%
Diarrhoea	31.25%	38.10%	7.69%
Constipation	12.50%	9.52%	0.00%

APPENDIX 5 – Children's Sleep Disturbance Scale

Oneway ANOVA Results – Children's Sleep Disturbance Scale

Mean Scores, Standard Deviations and Minimum and Maximum Values

		N	Mean	Std. Deviation	Minimum	Maximum
Disorders of Initiating & Maintaining Sleep	ASD	13	4.692	2.8978	.0	11.0
	MI	20	6.400	5.1442	.0	18.0
	Control	26	3.885	3.5477	.0	13.0
	Total	59	4.915	4.1327	.0	18.0
Sleep Breathing DIsorders	ASD	15	.87	2.031	0	8
	MI	20	1.15	1.599	0	6
	Control	26	.69	1.050	0	3
	Total	61	.89	1.507	0	8
Disorders of Arousal	ASD	15	.27	0.458	0	1
	MI	20	1.25	1.446	0	4
	Control	25	.48	0.918	0	4
	Total	60	.68	1.112	0	4
Sleep-Wake Transition Disorders	ASD	14	2.07	2.200	0	7
	MI	20	4.75	3.059	0	9
	Control	25	2.40	2.121	0	7
	Total	59	3.12	2.723	0	9
Excessive Somnolence	ASD	15	1.20	2.007	0	7
	MI	20	1.45	1.905	0	6
	Control	25	1.16	1.748	0	6
	Total	60	1.27	1.840	0	7
Sleep Hyperhydrosis	ASD	15	.73	0.961	0	3
	MI	20	.75	1.118	0	3
	Control	26	.46	0.647	0	2
	Total	61	.62	0.897	0	3
Total Score	ASD	12	8.00	5.343	0	21
	MI	20	15.75	9.547	3	34
	Control	24	9.00	7.253	1	29
	Total	56	11.20	8.430	0	34

Scheffe Post-hoc test; Comparison of Mean Scores between the ASD and Milk Intolerant (MI) and the ASD and Control Groups

SCALE	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Sig.
Disorders of Initiating & Maintaining Sleep	ASD	MI	-1.708	0.501
		Control	0.808	0.842
Sleep Breathing DIsorders	ASD	MI	-0.28	0.862
		Control	0.17	0.939
Disorders of Arousal	ASD	MI	-0.98(*)	0.029
		Control	-0.21	0.825
Sleep-Wake Transition Disorders	ASD	MI	-2.68(*)	0.012
		Control	-0.33	0.925
Excessive Somnolence	ASD	MI	-0.25	0.926
		Control	0.04	0.998
Sleep Hyperhydrosis	ASD	MI	-0.02	0.999
		Control	0.27	0.651
Total Score	ASD	MI	-7.75(*)	0.032
		Control	-1.00	0.937

* The mean difference is significant at the .05 level.

APPENDIX 6 – Food/Food Chemical Reactions of the ASD Group

i) Foods/Food Chemicals Found to Affect Child's Behaviour or Well-being – Parent Reported (ASD Group, n = 16)

	Number	Percentage
Dairy	10	62.5%
Wheat/Gluten	8	50.00%
Soy	3	18.75%
Eggs	4	25.00%
Nuts	1	6.25%
Salicylates	7	43.75%
Amines	6	37.50%
Gluatamate	3	18.75%
Preservatives		
- Proprionate	3	18.75%
- Sulphites	1	6.25%
- Antioxidants	1	6.25%
Colours	2	12.50%
Sugar	1	6.25%

ii) Foods Causing Adverse Reactions in the First Year of Life – Parent Reported (ASD Group, n = 16)

Food	Number	Percentage
Dairy Foods	8	50.00%
Soy Milk/Soy-based Formula	3	18.75%
Foods Containing Wheat/gluten	2	12.50%
Eggs	2	12.50%
Nuts	1	6.25%
Meat/fish	2	12.50%
Sesame	1	6.25%
Foods containing Salicylates	5	31.25%
Foods containing Amines	6	37.50%
Foods containing Glutamates	5	31.25%

iii) Foods/Food Chemicals Affecting ASD Subjects

Subject's Study ID	Foods Reported by Parents	
2003	1. CaProprionate	2. Amines
	3. Salicylates	4. Dairy
2004	None identified	
2005	1. Dairy	2. MSG
	3. Salicylate	4. Amines
2006	1. Lactose	
2009	1. Gluten	2. Colours
	3. Soy	4. Dairy
	5. Salicylate	6. nuts
	7. Eggs	
2011	1. Dairy	
	2. Sugar/colours	
2013	1. Wheat	2. Milk
	3. Salicylates	4. Amines
	5. Glutamate	6. Soy
	7. Eggs	
2014	1. Wheat	
2016	1. Dairy	
2017	1. Dairy	
	2. Wheat	
2019	1. Salicylates	2. Amines
	3. Antioxidants	
2020	1. Amines	
	2. Preservatives (not specified)	
	3. Salicylates	4. Sulphites
	5. Wheat	6. Dairy
	7. Soy	
2021	1. Gluten/Wheat	2. Dairy
	3. Egg	4. Salicylates
	5. Amines	6. MSG
2022	None Identified	
2023	1. Eggs (allergy)	2. Milk (allergy)
	3. Gluten	4. Amines
	5. Salicylates	6. Nuts (allergy)
2024	None Identified	

iv) Numbers of Foods/Food Chemicals Affecting Individual ASD Subjects (n = 16)

	None determined	1 food	2 foods	3 foods	4 foods	5 foods	6 foods	7 foods
number of subjects	3	3	2	1	2	0	2	3

APPENDIX 7 – Impact on Family Scale

Oneway ANOVA Results – Impact on Family Scale

Mean Scores, Standard Deviations and Minimum and Maximum Values

SCALE	GROUP	N	Mean	Std. Deviation	Minimum	Maximum
Financial	ASD	15	5.93	3.751	1	12
	MI	21	3.48	3.683	0	9
	Control	26	.62	0.983	0	3
	Total	62	2.87	3.560	0	12
Familial Social	ASD	16	13.63	5.572	5	27
	MI	21	7.86	7.806	0	20
	Control	26	.04	0.196	0	1
	Total	63	6.10	7.632	0	27
Personal Strain	ASD	16	10.88	4.064	3	18
	MI	20	5.25	6.155	0	17
	Control	26	.27	0.724	0	3
	Total	62	4.61	5.877	0	18
Mastery	ASD	16	8.94	3.356	2	15
	MI	21	7.76	3.632	0	14
	Control	26	2.19	3.567	0	12
	Total	63	5.76	4.627	0	15
Total Score	ASD	15	39.40	11.915	14	63
	MI	20	24.75	16.914	0	51
	Control	26	3.12	4.208	0	13
	Total	61	19.13	18.854	0	63

Scheffe Post-hoc test; Comparison of Mean Scores between the ASD and Milk Intolerant (MI) and the ASD and Control Groups

SCALE	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Sig.
Financial	ASD	MI	2.46(*)	.049
		Control	5.32(*)	.000
Familial/Social	ASD	MI	5.77(*)	.007
		Control	13.59(*)	.000
Personal Strain	ASD	MI	5.63(*)	.001
		Control	10.61(*)	.000
Mastery	ASD	MI	1.18	.608
		Control	6.75(*)	.000
Total Score	ASD	MI	14.65(*)	.002
		Control	36.28(*)	.000