

# **Is there evidence to support the hypothesis that there is a “leaky gut” in Autistic Spectrum Disorder (ASD)?**

A major project submitted in partial fulfilment for the award of the degree Master of Science (Nutrition and Dietetics) University of Wollongong

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# Abstract

**Introduction:** ASD encompasses a number of developmental disorders and for children with ASD normal development is hampered by a triad of marker characteristics including impaired social interaction, communication and imaginative skills. The role of diet in ASD has been in question since abnormalities in urinary peptide excretion were first investigated in the early 1980's. These peptides derived from the insufficient breakdown of dietary proteins gluten and casein are hypothesised to enter the blood through a "leaky gut" and cross the blood brain barrier to act on opioid receptors causing neurological abnormalities seen in ASD. Consequently dietary intervention in the form of a gluten and casein free diet is used in the management of ASD with clinical improvements reported. However absence of these dietary proteins cannot be conclusively said to be the cause of these improvements, as removing these foods from the diet is also likely to result in an altered intake of other food chemicals.

**Aim:** To determine whether children with ASD have a "leaky gut".

**Method:** Questionnaires, 4 day food records and 24 hour urine specimens were collected from sub populations of children with and without ASD aged between 3 and 10 years. The 4 day food record was kept over the time the urine was collected, in order to validate dietary intake with excretion, of particular interest being the opioid peptides. Questionnaires were also used to examine issues such as behaviour and food related symptoms potentially related to food intolerance.

**Results:** Data was analysed from 44 participants. No significant correlations were established between gluten intake and gluten excretion and also casein intake and casein excretion for the ASD and non-ASD groups. From an intolerance point of view, no correlations could be made with behaviour and intakes of gluten, casein, salicylates and amines for ASD subjects. However a relationship was observed for the non-ASD group, where increasing intakes of total casein, amines and salicylates were associated with increasing incidence of bowel problems.

**Conclusion:** It is not possible to ascertain whether an opioid peptide effect or "leaky gut" exists in this population, as in general, everyone excretes peptides and the excretory pattern is extremely individual. A relationship between gluten and casein in the diet and the excretion of peptides could not be found. It is essential that other potential gut mechanisms be investigated to determine whether they are related to peptide excretion. Food intolerance issues also need to be explored further to truly establish whether ASD children are sensitive to food chemicals and whether clinical improvements reported are attributed to reducing the load of these chemicals.

# Introduction

ASD encompasses a number of Pervasive Developmental Disorders (PDD), with the two most common being Autistic Disorder and Asperger's Syndrome (American Psychiatric Association 2000; Arnold *et al* 2003). As the name implies, ASD corresponds to a spectrum of common characteristics, each ranging in degrees of severity (Arnold *et al* 2003; Cohen 2003). Autism disorder however is a disability that profoundly affects the way a child relates and communicates with people around them (Cornish 2002; Arnold *et al* 2003). For children with autistic disorders normal development is hampered by a triad of marker characteristics including impaired social interaction, impaired or absent communication and impaired development of imaginative skills (Cornish 2002; Knivsberg *et al* 2002).

ASD is the most common developmental disorder and current prevalence is estimated at 1 in 100 children in Australia, with epidemiological studies finding that autism is identified four times more commonly in males than females (Fombonne 2003; Arnold *et al* 2003). Autism was first reported medically only 60 years ago and whilst the specific aetiology is still unknown, genetic, infectious, metabolic, immunologic and possible environmental influences have been implicated as possible causes or triggers (Kidd 2003; Knivsberg *et al* 2003). ASD often presents unexpectedly following a normal developmental period from birth to between approximately 12 to 18 months of age in which developmental regression becomes apparent (Levy & Hyman 2002). As there are no medical tests for diagnosing autism, an accurate diagnosis must be based on a detailed history and clinical observations of the child's communication, behaviour and developmental levels as specified by the diagnostic criteria DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) (Gail Williams *et al* 2000; Jyonouchi *et al* 2002).

An association between behavioural disorders and dietary intake was made as early as the mid sixties and has been associated with significant improvements in behaviour (Gillberg and Wing 1999; Green *et al* 2002). The theory and rationale behind the dietary intervention is that autistic spectrum characteristics can sometimes be affected by natural opioids produced by incomplete digestion of certain foods, namely protein (Levy and Hyman 2002).

Urinary peptide abnormalities, entailing abnormal patterns and elevated levels of peptides, reflect insufficient break down of the proteins, gluten and casein and according to Knivsberg *et al* (2002) were first reported in ASD 20 years ago. These abnormalities have been confirmed by further and more recent research (Shattock 1995; Cade *et al* 1999; Reichelt and Knivsberg 2003; Alcorn *et al*

2004) with the exception of one known contemporary study which failed to replicate the detected urinary peptide abnormalities in autistic children (Hunter *et al* 2003). Nonetheless it has been proposed if digestion is incomplete, short chains of amino acids or peptides will result and if not excreted in the urine, that opioid by-products of these dietary proteins will enter the blood through a “leaky gut” (Knivsberg *et al* 2002; Arnold *et al* 2003; Cornish 2002). Generally proteins from food pass through the cells lining the gut wall, and are altered during the process to become harmless (Steel Reid 2004). In “leaky gut” syndrome the proteins pass between the cells (Knivsberg *et al* 2002; Cade *et al* 1999) and into the bloodstream where they would cross the blood-brain barrier and interfere with transmission in such a way that normal activity is altered or disrupted (Knivsberg *et al* 2002). Consequently intensified opioid activity would result, effectively cutting down transmission in all neurological systems (Shattock 1995) inevitably causing the abnormalities of perception, cognition, emotions and behaviours that are observed in autism (Cornish 2002).

If the opioid excess hypothesis is correct, intervention is based on the principle that raised levels of opioid peptides in the central nervous system are the problem and the strategy to be adopted is eliminating gluten, casein and other potential sources of opioid peptides from the diet (Shattock 1995; Whiteley *et al* 1999). This approach has been implemented with positive results from dietary intervention ie reduction in autistic behaviours and increase of communicative skills, being reported from studies (Shattock 1995; Knivsberg *et al* 2002; Cade *et al* 1999; Whiteley *et al* 1999) and case studies (Knivsberg *et al* 2002; Blade 2000).

Food intolerances are predominantly associated with modified and adverse behaviours, gastrointestinal symptoms and skin rashes due to the naturally occurring salicylates, amines and glutamates found in food in addition to MSG, colours and preservatives (Loblay and Swain 1986). For that reason the role of food intolerance in ASD needs to be largely examined as gastrointestinal symptoms such as diarrhoea, constipation, bloating and reflux are frequently reported in approximately 40 to 60% of children diagnosed with ASD (Page 2000; Black *et al* 2002). Change to the diet resulting from removal of foods containing casein and gluten is not limited to these proteins. It will also result in changes to the overall intake of a range of natural and added chemicals found in food. Therefore if the only dietary changes are made through the removal of casein and/or gluten, it is unfeasible to ascertain whether these proteins alone are the source of the problem. The only way to definitively manifest which dietary components an individual is sensitive to, is to carry out a full elimination diet, followed by challenges. This diet minimises food intolerance chemicals, as well as casein and gluten and once adverse symptoms have diminished, challenges with

individual components of foods help clarify which chemicals the individual is vulnerable to (Swain *et al* 1997; Loblay and Swain 1986). Subsequently it appears food intolerance chemicals are a possible cause of symptoms in children with ASD and that the diet may impact unfavourably on ASD behaviour indirectly through food intolerance reactions.

It is apparent that the roles of both diet and gastrointestinal symptoms in ASD need to further investigated. The aim of this study is to determine whether the “leaky gut” theory is valid. In other words whether behavioural improvements reported in ASD children following a casein and gluten free diet, are related specifically to the removal of these proteins from the diet, or whether the food intolerance chemicals are the more likely cause and the behavioural improvements reported are attributed to the reduced intake of these naturally occurring chemicals.

# Methods

## Participants and Participant Recruitment

Two well-defined groups of volunteer children were recruited and consented to participate in the study. The first cohort consisted of 28 ASD children (26 males & 2 females, mean age  $8.6\pm 2.6$ ) aged between 3 and 10 years previously seen by the Paediatrician at either the Royal Prince Alfred Hospital (RPAH) Allergy Clinic or the Kogarah Developmental Assessment Service with a diagnosis of ASD according to the DSM-IV. The second cohort comprised of 16 children (11 males & 5 females, mean age  $7.7\pm 2.2$ ) aged between 3 and 10 years without a diagnosis of ASD from either the childcare centres and primary schools in the CSAHS Region or siblings of the ASD children. This formed an age matched control group considered “healthy” in that the subjects had not been diagnosed with ASD or food intolerance.

Parents interested in participating in the research project were sent a package containing an expression of interest form, which formalised their consent to study participation and a parent information sheet elucidating the purpose and methods of the study (Appendix 1 & 2). A coded questionnaire booklet and food intake diary (distinguishing cohort and identification number) were also included along with a urinary collection kit (outlined under “Part Three: Urine”) and reply paid envelopes for the return of the questionnaires, expression of interest form and urine.

## Ethics Approval

As this study was a follow on study based on preliminary findings from the first phase, ethics approval had previously been sought and granted by the Central Sydney Area Health Service (CSAHS) Ethics Review Committee (RPAH Zone). However minor modifications were made prior to commencement of the project.

## Study Design

The study design for the project consisted of 3 parts, which included:

Part One: Questionnaires;

Part Two: 4 Day Food Records; and

Part Three: Urine

## **Part One: Questionnaires**

The previously developed questionnaire booklet, “Dietary Issues in Children with and without Autistic Spectrum Disorder”, was administered to participant’s parents and consisted of nine questionnaires, four of which were used for this study. These questionnaires were utilised to gather information on the child’s development history, in particular health problems in the first 2 years (“Background Questionnaire”), food related symptoms (“General Health and Behaviour Checklist”) and overall behaviour in particular regards to conduct, emotion, anxiety, and hyperactivity (“Conner’s Rating Scale”). A diet history in the form of a food frequency questionnaire (“Your Child’s Eating and Drinking Preferences”) was also used (Appendix 3, 4, 5 & 6). These questionnaires were specifically chosen for this study as they allowed issues relating to food intolerance to be identified such as eating behaviours, food related symptoms and the overall load of food chemicals in the child’s diet, which could then be compared with intakes of gluten, casein, salicylates and amines. The food frequency questionnaire was also used to account for any gluten or dairy containing foods that the child had eaten in the past 3 months to establish a more complete and comprehensive insight into the child’s habitual intake.

## **Part Two: 4 Day Food Records**

The previously developed “Children’s Food and Drink Intake Diary” (Appendix 7) was completed by the participant’s parents which involved recording the child’s intake for 4 consecutive days including 3 weekdays and 1 weekend day. Participant’s parents were required to initiate the food record three days prior to the 24-hour urine collection in order to assist in determining whether any relationship exists between urinary excretion of peptide compounds from casomorphin and gluten exorphin and dietary intake.

## **Part Three: Urine**

### **Sample Collection**

As a 24-hour urine collection was prescribed for the analysis, a graduated 3-litre vacutainer with approximately 1 teaspoon of ascorbic acid for preservation purposes, a styrofoam box containing an ice brick and 3 specimen jars and instructions regarding urine collection (Appendix 8) were sent to participant’s in the initial study pack. Participants were required to collect urine over a 24-hour period in the 3-litre vacutainer after the first urine of the day was discarded when the child woke. This was then transferred to the 3 specimen jars where participants were requested to record the total volume of urine output, the date the urine was collected on the stickers surrounding the jars (Appendix 9) and freeze the urine until delivery or postage of the urine back to clinic was possible.

### Sample Preparation

When specimen jars containing urine were returned to the clinic, the pH and presence of proteins were measured using a multistix (reagent strips for urinalysis) and recorded in conjunction with total output volume and date of collection (Appendix 10). Two vials each containing 1.5ml of sample urine were labelled, filtered and stored for direct insertion into the HPLC. One of the specimen jars were reserved to assess creatinine levels, which was measured by the standard clinical chemical method, based on the Jaffe reaction as described by Popper *et al*, Seeligand and Wust and modified by Bartels, by the Department of Biochemistry, RPAH. The remaining specimen jars were labelled according to their identifier and stored for future considerations.

### Standards

Standards for thymol, ascorbic acid, and the two opioid peptides  $\beta$ -casomorphin (H-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-OH) and gluten exorphin A5 (H-Gly-Tyr-Tyr-Pro-Thr-OH) were prepared and run. The  $\beta$ -casomorphin and gluten exorphin A5 were run as concentrate and dilutions (1:10).

### Buffers

Two buffers were prepared according to Reichelt *et al* (1999) method and applied utilising the same gradient. Buffer A consisted of 0.1% by volume of trifluoroacetic acid (TFA) and buffer B 0.1% TFA in 95% by volume acetonitrile with 4.9% water.

### High Performance Liquid Chromatography (HPLC) Analysis

A HPLC system (Hewlett Packard 1050 series), with automatic sample application and 250mm by 4.6mm C18 reversed phase columns (Alltech, Apollo C18 series) were used. The columns were run at room temperature with an automatic gradient control and a flow rate of 0.5ml/min. Standard injection was 0.1ml and absorbance was set at 215nm and 280nm to identify peptide bonds and aromatic compounds respectively with a slope sensitivity of 0.2. Total run time was set at 100 minutes per sample and details of the gradient used are outlined in Table 1.

**Table 1: Gradient used in HPLC for urine analysis**

	<b>Buffer A</b>	<b>Buffer B</b>	<b>Minutes</b>
1	99%	1%	0 – 15
2	Linear decrease to 60%	Linear increase to 40%	15 – 75
3	Linear decrease to 60%	Linear increase to 60%	75 – 80
4	40%	60%	80 – 89%
5	Linear increase to 99%	Linear decrease to 1%	89 – 95
6	Reequilibration		95 – 100

## **Data Analysis**

### **Questionnaires**

Identification coded data from questionnaires were entered using Microsoft Access 2002, and processed via Microsoft SQL Query Analyser 2000 (Microsoft Corp., USA) and Microsoft Excel 2002. When responses to questionnaires were being recorded only definitive answers were incorporated and blank replies were treated as a null response.

Once all data from the questionnaires was entered onto the spreadsheet, independently the total gluten, casein, amine and salicylate intakes were sorted in descending order and manually compared with each of the various behaviours and symptoms to determine if any association was evident.

### **4 Day Food Records**

Analyses of the 4 day food records were completed via SERVE, a food evaluation program developed by Mike and Hazel Williams for specific use in the RPAH Allergy Unit. In order to show consistency with the current Dietary Guidelines for Children and Adolescents in Australia, serving sizes of gluten and casein were calculated manually according to these standards. Serving sizes of salicylates and amines were also manually determined, according to RPAH Allergy Unit food chemical calculation spreadsheet, as the SERVE program did not account for these particular components in its analysis.

Once all data from the 4 day food records and urine analysis was entered on a spreadsheet (Appendix 11) further evaluation was carried out in the form of paired samples t-tests, independent samples t-tests and Pearson's correlations using SPSS (Statistical Package for the Social Sciences) for Windows, version 12 (SPSS, Inc., Chicago, IL, USA) to establish the degree of variance within and between the groups.

# Results

## Urine

### HPLC Analysis

Elution times for the gluten exorphin and casomorphin standards were 45.053 minutes and 65.506 minutes respectively. Figure 1 shows the urinary peptide trace pattern of a subject with ASD whilst Figure 2 illustrates the urinary peptide trace pattern of a subject without ASD. Elution times for peptides for all study participants are located in Appendix 11.

**Figure 1: HPLC trace patterns for a subject with ASD**

**Figure 2: HPLC trace patterns for a non-ASD subject**

## Urine and 4 Day Food Records

### Gluten Intake and Gluten Exorphin Excretion

Figure 3: Gluten exorphin excretion versus gluten intake in ASD and non-ASD subjects

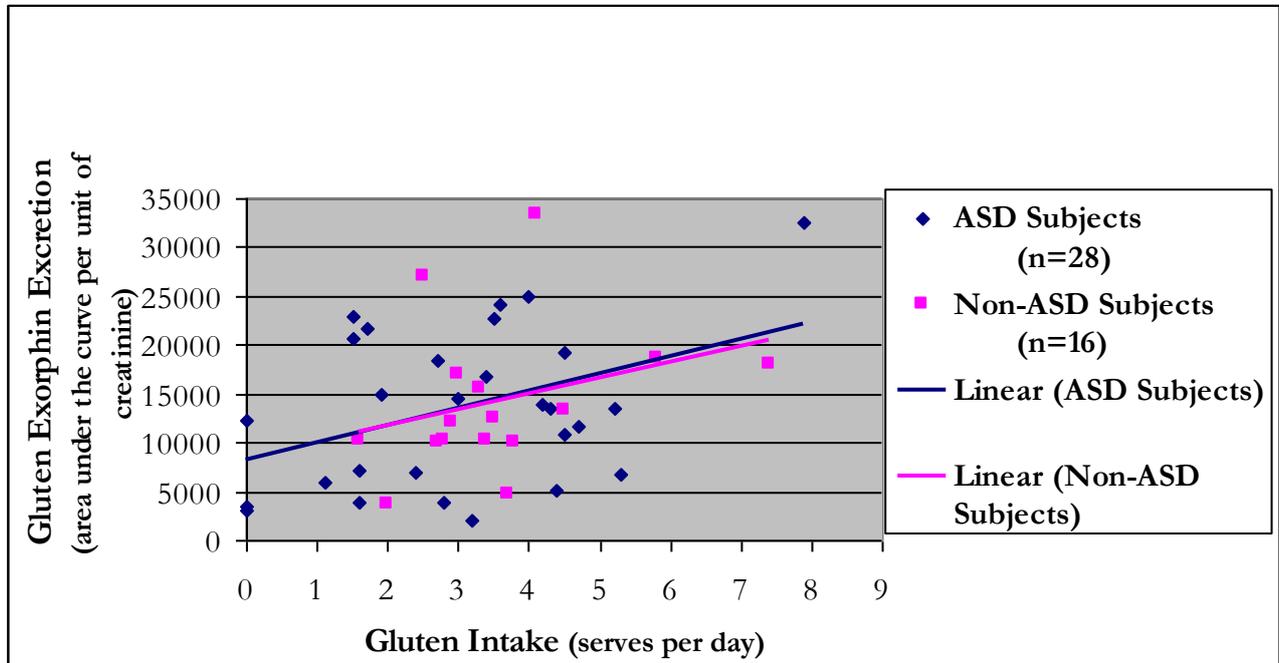


Figure 3 shows the serves per day of gluten for the ASD and non-ASD subjects as determined by the 4 day food records, against the amount of gluten exorphin excreted in their urine as determined by HPLC. From the graph it can be seen, for both ASD and non-ASD subjects, that there is a significant difference between the gluten intake and gluten exorphin excretion ( $p < 0.05$ ). That is to say for both groups, that there is no correlation between gluten consumed and gluten exorphin excreted in the urine.

**Table 2: Statistical association for gluten intake and gluten exorphin excretion between ASD and non-ASD groups**

	<b>Mean</b>	<b>Independent sample T Test (p value)</b>	<b>Pearson's Correlation (p value)</b>
<b>Gluten Intake (serves per day)</b>			
ASD	3.1	0.313	0.316
Non-ASD	3.6		0.304
<b>Gluten Exorphin Excretion (area under the curve per unit of creatinine)</b>			
ASD	13493.4	0.784	0.316
Non-ASD	14177.8		0.304

The mean scores for gluten intake and gluten exorphin excretion for the ASD and non-ASD group are shown in Table 2. The mean scores for gluten intake and gluten exorphin excretion were highest for the non-ASD group, however the 2 group's means were not significantly different ( $p > 0.05$ ). Correlations of gluten intake and gluten exorphin excretion for the ASD and non-ASD group were not statistically significant. Therefore there is no identifiable relationship between gluten intake and gluten exorphin excretion for the ASD and non-ASD groups.

## Casein Intake and Casomorphin Excretion

Figure 4: Casomorphin excretion versus casein intake in ASD and non-ASD subjects

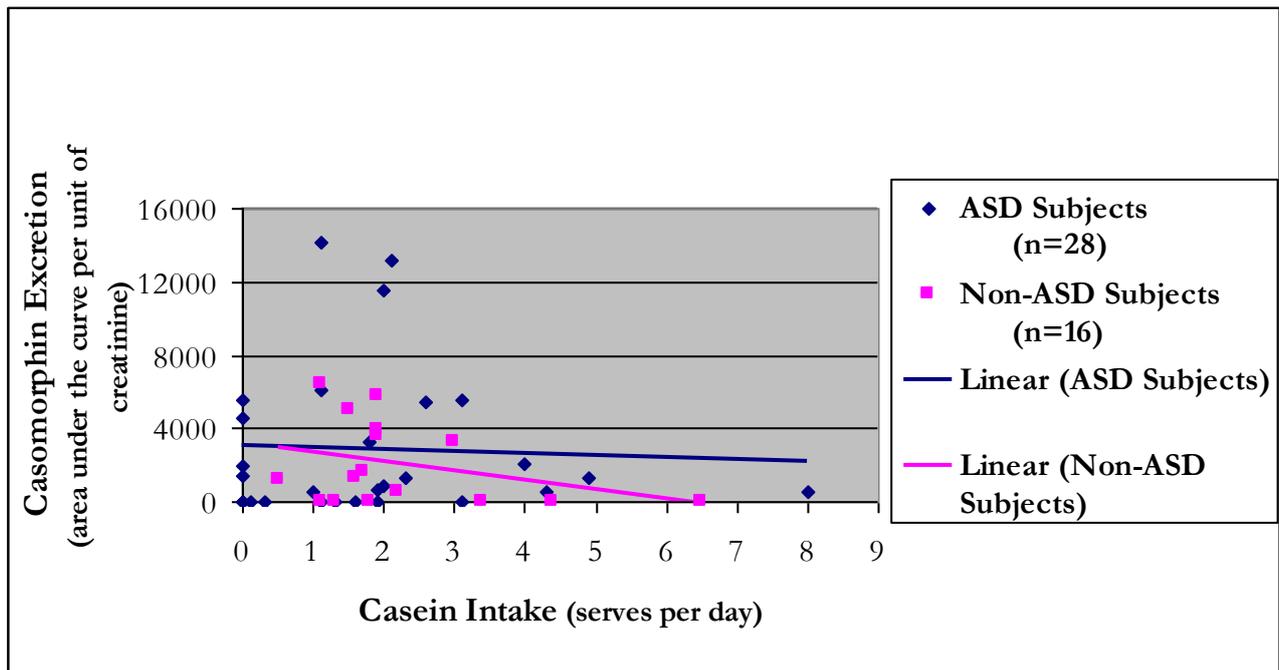


Figure 4 shows the serves per day of casein for the ASD and non-ASD subjects as determined by the 4 day food records, against the amount of casomorphin excreted in their urine as assessed by HPLC. The graph indicates that for the ASD subjects, there is a significant difference between their casein intake and casomorphin excretion ( $p < 0.05$ ). For the non-ASD subjects this trend was also observed ( $p < 0.05$ ). Effectively for both groups casein which is being consumed does not correspond with the casomorphin that is being excreted in the urine.

**Table 3: Statistical association for casein intake and casomorphin excretion between ASD and non-ASD groups**

	<b>Mean</b>	<b>Independent sample T Test (p value)</b>	<b>Pearson's Correlation (p value)</b>
<b>Casein Intake (serves per day)</b>			
ASD	1.8	0.467	-0.049
Non-ASD	1.5		-0.332
<b>Casein Excretion (area under the curve per unit of creatinine)</b>			
ASD	2881.9	0.453	-0.049
Non-ASD	2039.8		-0.332

The mean scores for casein intake and casomorphin excretion for ASD and non-ASD groups are shown in Table 3. The mean scores for casein intake and casomorphin excretion were highest for the ASD group, however the 2 group's means were not significantly different ( $p > 0.05$ ). Correlation of casein intake and casomorphin excretion for the ASD and non-ASD groups both show a negative correlation, indicating as the intake of casein decreases, the excretion of casomorphin increases. However both values were not significant, therefore there is no perceivable relationship between casein intake and casomorphin excretion for the ASD and non-ASD groups.

## Questionnaires

Issues relating to food intolerance were assessed with the "Background Questionnaire", "General Health and Behaviour Checklist", "Conner's Rating Scale" and the food frequency questionnaire. It was established from the questionnaires that 44% of ASD subjects were currently experiencing gastrointestinal symptoms which, in comparison to non-ASD subject, was higher as only 25% of non-ASD were currently encountering gastrointestinal symptoms. When total gluten, casein, amine and salicylate intakes were compared with the scores from the various questionnaires which, overall looked at early and current behaviour and food related gut and behavioural symptoms, no association could be established within the ASD group (Appendix 12). However for the non-ASD group, relationships were observed between casein, salicylate and amine intakes and bowel problems such as constipation and frequently loose stools. As the total casein, amine and salicylate intakes increased so did the incidence of bowel problems (Appendix 12).

# Discussion

Foods containing casein and gluten are suspected to contribute to ASD and various controlled studies have reported reduction in autistic behaviours following strict dietary exclusion of these proteins (Knivsberg *et al* 2003; Shattock & Whiteley 2001), prompting the notion that this dietary upgrade may be an essential prerequisite for improvement in ASD. The question raised in this study was whether children with autism have a “leaky gut” hence improvements documented in behaviour are related strictly to removing dietary proteins or whether clinical improvements reported are attributed to reducing the naturally occurring chemicals found in these dietary proteins. Observations of the HPLC trace patterns indicate that it is not possible to establish whether an opioid peptide excess hence a “leaky gut” exists in ASD children. It is evident that each person excretes peptides, but the concentration of the peptide excretion is purely on an individual basis. Analogous findings by one known contemporary study (Hunter *et al* 2003) also failed to detect the presence of opioid peptides in the urine of autistic subjects. Gluten exorphin A5 and  $\beta$ -casomorphine (bovine), the standards used in this study, were eluted at very specific times. These peptide excretion times were not replicated in any of the subject’s samples. It therefore proceeds that peptide excretion is highly characteristic and that an excess or even existence of a specific opioid peptide is more complicated to explain than the methods, which have been replicated here, suggest.

The extensive array of excretory products in an individual’s urine is highly reliant on foods consumed and the manner in which they are metabolised, that is the exogenous and endogenous peptides produced. The method replicated in this study was one portrayed by Reichelt *et al* 1999 and employed the use of chromatography methods to determine the amount of peptides in subject’s urine. It is our suggestion that due to the highly individual nature of the content of urine, HPLC methods of assessing this content are inaccurate and provide no more than a means to compare patterns of urine excretion. No definitive evidence in regards to opioid peptide excess can be determined by HPLC peaks. This study in conjunction with others highlights the complexity of low molecular weight compound and peptide content of urine (Hunter *et al* 2003) and methods to validate food diaries against peptide content of urine are yet to be accurately portrayed.

To our knowledge, this is one of the first studies, which has concurrently investigated both dietary intake and excretion of the proteins, gluten exorphin and casomorphin. Hyperpeptiduria has been reported in numerous studies (Knivsberg *et al* 2003; Reichelt *et al* 2002), however, validation involving specific dietary intake during the course of the study has not been documented making

previously reported findings, to a certain degree ambiguous. From this study it was not possible to identify a relationship between both ASD and non-ASD subjects gluten and casein intake and their gluten exorphin and casomorphin peptide excretion thereby suggesting that dietary intake may not entirely be a function of peptide excretion. Research has shown that there is continuous process by which the cells of the body break down a proportion of the proteins they are producing where peptides are formed (Martini *et al* 2001; Coffee *et al* 1998). These peptides generated by endogenously produced proteins therefore may be excreted in the urine and account for the peptides, which are shown to be present.

The HPLC method uses absorbance to indicate how much of the molecule is in the sample. The fact that the gluten and casein intake did show any association with gluten exorphin and casomorphin excretion may also be due to the products produced by protein breakdown within the body, which have a similar amino acid sequence to the peptides which are being examined. Therefore the peptides being measured in HPLC may not be a true indication of the peptides of reference.

The fact that there was no significant correlation may also be accounted for due to recording of food intake. Accurate measurements of food intake are described as one of the most difficult tasks to be undertaken by health professionals (Wahlqvist 2002). Records of food intake is undoubtedly the most demanding method of measuring food intake and this along with the possibility that the subjects may have consumed foods without the parents knowledge have to be taken into account especially in regards to the idiosyncratic outlying measurements. However this is a limitation that has to be taken into account in any study. It seems questionable, though, as several outlying and peculiar measurements were noted with the subject's intake and excretion, advocating the issue that intake is not necessarily equivalent with excretion and that benefits of dietary intervention in regards to gluten and casein are somewhat subjective and based on anecdotal findings.

The implementation of gluten and casein free diets in children with ASD have been associated with marked improvements in behaviour (Knivsberg *et al* 2003) and whilst links have been made between dietary modification through the removal of dietary proteins and clinical improvements, no links with food intolerance have been proposed. The gastrointestinal and behavioural symptoms which are so commonly reported (Levy *et al* 2001), are almost considered to be a part of ASD itself, rather than having a role in their own right. The evidence put forward by this study indicates that the role of diet in the aetiology of symptoms in ASD may involve more than just casein and gluten, due to the high occurrence of current gastrointestinal symptoms in ASD children, which are potentially related to food intolerance.

Whilst no particular correlation was noted between intake of dietary proteins and foods chemicals with behaviour or food related symptoms in ASD children, it is not to say that food intolerance chemicals do not play a role or that children with ASD are not sensitive to a range of these chemicals. The only way to accurately determine if food chemicals are a problem in children is to initiate the elimination diet followed by a series of challenges and as most of the ASD participants in this study were on regular diets, sensitivity was not able to be truly established. Whilst no correlation was found in ASD children, this was not the case with non-ASD children. Patterns were found between increasing intakes of casein, salicylates and amines and increasing incidence of bowel problems, which suggests that food intolerance may also be an unsuspecting problem for individuals not diagnosed with ASD.

## Limitations and Future Research

This study has several areas, which need to be taken into account for future research. Firstly the sample size. This study only comprised of 28 ASD and 16 non-ASD subjects. Increased number of study participants would assist by enhancing the findings through providing more conclusive outcomes. Secondly ASD subjects taking part in the study were not strictly on gluten and casein free diets. This makes it difficult to determine whether an opioid peptide excess is present. Future research needs to be undertaken looking at ASD children who are strictly on a gluten and casein free diet to better determine if an opioid peptide excess exists and whether the “leaky gut” theory is valid. It is also suggested that the amino acids in the gluteomorphin standard used in HPLC be sequenced in order to look for sequences that correspond with food, which is being consumed. Peptides, which are being excreted, could then with certainty be matched with foods being consumed, ensuring results obtained are as accurate as possible. Finally in order to establish the degree of food intolerance in ASD children more accurately, and determine its specific role in ASD, it would be necessary to implement elimination diets followed by a series of challenges, as data reported in regards to food intolerance was subjective according to parents reports.

# Conclusion

The present study demonstrated that it is not possible to determine whether children with ASD have a “leaky gut”. It is evident that each person excretes peptides, but the concentration of the peptide excretion is highly individual which indicates that opioid peptide excess can exist without ASD. Whilst no association was found between gluten and casein intake and excretion, it may be necessary to examine other possible gut mechanisms and more accurate methods which validate food intake with excretion. Overall further studies are required to expand and validate these findings so as to determine the exact role dietary proteins play, with more emphasis being placed on the potential role of food intolerance in ASD.

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# Appendix 1: Expression of Interest

# Appendix 2: Parent Information Sheet

## Appendix 3: Background Questionnaire

# Appendix 4: General Health and Behaviour Questionnaire

# Appendix 5: Conner's Rating Scale

# Appendix 6: Your Child's Eating and Drinking Preferences

# Appendix 7: Children's Food and Drink Intake Diary

## Appendix 8: Urine Instructions

## Appendix 9: Sticker

# Appendix 10: Urine Analysis Spreadsheet

# Appendix 11: Final Data Spreadsheet

# Appendix 12: Questionnaire Correlations

# Appendix 13: Statistical Analysis