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A gluten-free diet as an intervention for autism and associated spectrum disorders: preliminary findings

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abstract The opioid-excess hypothesis of autism suggests that autism is the consequence of the incomplete breakdown and excessive absorption of peptides with opioid activity (derived from foods which contain gluten and casein), causing disruption to biochemical and neuroregulatory processes. Biochemical evidence has indicated the presence of increased levels of peptides in the urine of people with autism, and previous behavioural studies have demonstrated a connection between the long term exclusion of gluten and casein from the diet and improvements in the behaviour of some children with autism. The introduction of a gluten-free diet to children with autism and associated spectrum disorders (n = 22) was monitored over a 5 month period using a battery of parental and teacher interview/questionnaire sessions, observation reports, psychometric tests and urinary profiling. Results suggested that participants on a gluten-free diet showed an improvement on a number of behavioural measures. However there was no significant decrease in specific urinary compounds excreted when compared with controls and a gluten challenge group.

keywords *autism; diet; gluten; peptides*

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Introduction

The lack of a consensus about the aetiology of autism and associated spectrum disorders, coupled with the diagnosis of autism by observed

symptoms alone, has led to the majority of interventions in this area of disorders to be based primarily around psychological and behavioural strategies aimed at treating symptoms. Although success has been noted with such tactics, the ideology behind such interventions does not address the fundamental causation of the specific deficits observed in autism. Accumulating evidence for a biochemical theory of autism, and in particular a metabolic rationale for the emergence of autism, has cast new light on how the syndrome is viewed, and presented the possibility of additional avenues of biological intervention in autism.

A metabolic hypothesis of autism

The 'opioid-excess' theory of autism (Panksepp, 1979; Reichelt et al., 1981; 1993; 1994; Shattock et al., 1990; Wakefield et al., 1998) suggests that autism is the consequence of the action of peptides[1] of exogenous origin affecting neurotransmission within the central nervous system (CNS). Peptides,[2] formed through the incomplete breakdown of foods[2] containing gluten (found in cereal produce) and casein (derived from dairy produce), exhibit direct opioid activity or form ligands for the peptidase enzymes which break down endogenous endorphins and enkephalins. The passage of elevated levels of peptides through to the CNS is further aided by gastrointestinal conditions indicative of an abnormally porous intestinal membrane (Waring and Ngong, 1993; Gardner, 1994; D'Eufemia et al., 1996; Wakefield et al., 1998). The resultant presence of intensified opioid activity disrupts a variety of systems within the CNS.

Urinary analysis

Urinary analysis has suggested the presence of elevated levels of peptides in the urine of people with autism (Reichelt et al., 1981; Shattock et al., 1990; Shattock and Savery, 1996; 1997). Urinary investigation using high performance liquid chromatography (HPLC) revealed specific food-derived peptides and demonstrated distinct urinary profiles for subsets of people with autism on the basis of dietary patterns (Reichelt et al., 1981; Shattock et al., 1990; Shattock and Savery, 1997). Urinary analysis has also indicated the presence of elevated levels of other compounds in the urine of people with autism. Principally among them is the compound *trans*-indolylacryloylglycine (IAG) (Shattock and Savery, 1997; Mills et al., 1998), a compound previously found in the urine of people with metabolic conditions such as Hartnup's disease (Milne et al., 1960). Although the exact relationship between autism and IAG is yet to be discovered, the compound could further support the metabolic theory of autism, through a possible involvement with membrane integrity (Shattock and Savery, 1997).[4]

Dietary intervention in autism

The removal of gluten and/or casein from the diet of people with autism has, for many years, been reported by numerous parents as being related to significant improvements in behaviours associated with autism. The results from dietary intervention with people with autism (Knivsberg et al., 1990; 1995) have shown significant improvements in the behavioural and cognitive functioning of participants involved on the gluten- and casein-free diet, with regression reported following the suspension of the diet. Similar changes in the pathological urine patterns and levels of peptides have also been demonstrated in children with autism on dietary intervention (Reichelt et al., 1991). Gluten may be specifically related to late onset pervasive developmental disorder because of the coincidence of increasing gluten intake at that time (Reichelt et al., 1986; Shattock and Savery, 1996). The aim of this pilot study was to provide a more substantial investigation into the short term effects of a gluten-free diet with children with autism and associated spectrum disorders.

Method

Subjects

Originally, 31 children, 23 males and 8 females, were involved with the gluten-free diet trial. Data were analysed from 22 children who completed the regime: 9 children diagnosed with autism labelled A, B, C, D, E, F, G, H, I (7 males and 2 females; mean age = 68.1 months), 4 children diagnosed with Asperger syndrome labelled J, K, L, M (all males; mean age = 79.9 months), 5 children diagnosed as having an autistic spectrum disorder (ASD) labelled N, O, P, Q, R (4 males and 1 female); (mean age = 40 months), 2 children diagnosed with semantic pragmatic disorder (SPD) labelled S and T (both male; mean age = 54.5 months), and 2 children diagnosed as having dyspraxia labelled U and V (both males; mean age = 102 months). Mean age of participants when diagnosed was 47.2 months.

In addition, 5 children, all males, ages ranging from 56 to 106 months (mean = 73 months), 4 diagnosed with autism labelled GC1, GC3, GC4, GC5 and 1 diagnosed with ASD and attention deficit hyperactivity disorder labelled GC2, volunteered for a challenge to their gluten-free diet which they had been following for over 6 months. A further 6 children, 5 males and 1 female, ages ranging from 42 to 93 months (mean = 64 months), all diagnosed with autism, formed a control group of children not involved with any dietary intervention.

Additional participants drawn from populations of children diagnosed with autism who had been on the diet for over 6 months (n = 8) and

children diagnosed with autism who were not embarking on any dietary intervention ($n = 6$) provided background information and urine samples for later analysis and comparison. Although all participants had received formal diagnoses from experienced clinicians using DSM-IV and/or ICD-10, resources did not allow for an independent confirmation of the child's diagnosis.

Tools

Background questionnaire An interview questionnaire was designed and constructed from author review of the files of parental information ($n = 96$) held at the Autism Research Unit. Behavioural and physiological characteristics common to reports were extracted and reduced to question items on the interview questionnaire. Pilot testing of the questionnaire was conducted with informant parents ($n = 15$) to ascertain the extent to which items were comprehensible to lay raters. Post-intervention questionnaires included qualitative data on parental views of the success of the intervention.

Observation schedule The Behaviour Summarized Evaluation (BSE) scale (Barthelemy et al., 1990) is a standardized observation schedule made up of a number of individual items relating to various aspects of autistic functioning and behaviour (Barthelemy et al., 1997). Ratings are scored on a five-point scale according to the frequency of observed behaviours (0 = never seen, 1 = sometimes, 2 = often, 3 = very often, 4 = always seen). Author adaptation made the schedule more comprehensible to parental and teacher raters. An additional six items relating to aspects of physiology were included with the schedule.

Psychometric tests The Kaufmann Assessment Battery for Children (K-ABC) (Kaufmann and Kaufmann, 1983) is a standardized battery of subtests designed to measure a broad range of cognitive functions. On account of the wide variation in levels of functioning and specific issues relating to the testing of children with autism, only six of the subtests were used: Magic Window, Face Recognition, Gestalt Closure, Matrix Analogies, Spatial Memory and Photo Series. Previous studies have shown the reliability of using the K-ABC in the assessment of children with autism (Stavrou and French, 1992).

Parental satisfaction scale The Parental Satisfaction Survey (PASS) (Panksepp et al., 1991) consists of a 30-item questionnaire, grouped into six categories corresponding to behaviours associated with autism. Ratings are

given on a nine-point scale to gauge the perceived changes in behaviour over the course of the study.

Urine analysis: materials Urinary analysis consisted of a first morning sample, taken mid-stream in a 30 ml tube (containing a small amount of the preservative thymol) from participants and stored frozen at −20 °C. Chromatographic equipment consists of a CR4A Chromatopak computing integrator, 2 Shimadzu LC10–AD solvent delivery pumps with a Shimadzu SP6–AD UV/VIS detector and a Pye Unicam UV detector. All Shimadzu equipment was supplied by Dyson Instruments Ltd, Houghton-Le-Spring, UK. The analytical column is a 25 cm × 4.6 mm ID Vydac C18 (5 μm) supplied by Phenomenex, Macclesfield, UK. Sample injections are made with SGE syringes via a Rheodyne 7125 valve (Anachem, Luton, UK). Bond Elut™ SPE cartridges (10 ml, 200 mg C18) were supplied by Phenomenex, Macclesfield, UK. Acetonitrile (HPLC grade) and TFA (trifluoroacetic acid) (all AR grade) were supplied by Sigma, Poole, UK. The research is carried out in accordance with the Declaration of Helsinki and with the authorization of the University of Sunderland Ethics Committee.

Urinary analysis: procedure The samples are subjected to a preliminary 'clean-up' process using a 10 ml Bond Elut SPE cartridge. Final urine collection is in 1 ml of 40 percent acetonitrile in 0.1 percent TFA; 10 μl of the sample is injected into the system. Initial mobile phase conditions are acetonitrile 0.1 percent v/v aqueous TFA (5:95 v/v) for 8 min, followed by a gradient of 5–50 percent v/v acetonitrile in 0.1 percent v/v aqueous TFA over 8–40 min, flow rate 2.0 ml min^{-1}. UV detection was at 215 nm and 326 nm. The products are detected as they emerge from the column and the results recorded in graphical and numerical form. Peptides with biological activity tend to appear in the region which lies between 17–18 and 30 minutes, with the vast majority of people with autism showing a major compound peak around 20–21 minutes on the graphs. This compound has been identified as *trans*-indolylacryloylglycine (IAG). Creatinine concentration was determined by Drug Development Ltd, Scotland.

Procedure

Gluten-free diets are traditionally used in mainstream medicine to treat gluten/wheat sensitivity or allergy and coeliac disease.[5] They exclude foods which contain grains such as wheat, oats, barley and rye. Parents were given information on the types of foods to avoid and details of alternative, gluten-free foods. Background details about the child's past and present development/symptomatology, and familial information

relevant to the study, were taken from all participants pre- and post-intervention, with parental and teacher observations taken at weekly intervals using the BSE observation scheme. Urine samples were taken pre- and post-dietary intervention. The selected subtests of the Kaufmann Assessment Battery for Children (K-ABC) were conducted pre- and post-intervention. The PASS follow-up questionnaires were administered post-intervention.

Results

Parental interviews

Data from parental interviews and parental and teacher observations demonstrated that a proportion of participants on the gluten-free diet were reported as showing some improvement in autistic behaviours, predominantly after 3 months on the diet. The possible significance of this 3 month mark is discussed later. Participants were described by parents as showing improvements in vocal and non-vocal communication (11/22), increased level of attention and concentration (10/22), decrease in episodes of hetero-aggressiveness (10/22) and auto-aggressiveness (9/22), increased affection and affection-seeking behaviours (8/22), improved physical coordination and motor skills (8/22), increased awareness of self and environment (7/22), calmer disposition (7/22), and improvements in sleeping patterns (7/22).

Parental interviews of children participating in the gluten challenge suggested that while many aspects of behaviour remained unchanged following the reintroduction of dietary gluten, a worsening of behaviours was noted in areas of hyperactive and impulsive behaviours (3/5), an increase in the number of stereotyped behaviours and aggression (3/5), and a slight worsening of language and communication skills (3/5). These regressions were observed to be a gradual process over the course of the study.

Parental reports also described several other key features of their child's experiences on the gluten-free diet. First, many parents reported participants to experience a period of initial behavioural regression when the gluten-free diet was first put in place (16/22). This was characterized by behaviours such as crying for no particular reason, clinginess, mood swings and evidence of discomfort. This period was reported to last between 7 and 21 days. Similar behavioural regressions were reported by parents following temporary lapses to the diet (accidental gluten ingestion). Although there were no evident episodes of epilepsy with any of the children involved with the diet in the current study, there was a single

account of a child coming off the gluten-free diet at the conclusion of the study and suffering a seizure within a few days of gluten reintroduction.

Behaviour Summarized Evaluation (BSE) observation scores

Individual parental and teacher scores for each participant were combined to form monthly score blocks in seven key behavioural areas (illustrated in Table 1) over the course of the study.

Parental observations

Completed results were taken from 22 of the 31 participants on the gluten-free diet. Of the remaining 9 participants, 1 subject was taken off the gluten-free diet after 10 weeks (showing no discernible change in behaviour from pre-diet), 4 participants failed to implement a totally gluten-free diet, and the remaining 4 subjects were excluded because of incomplete data.

Table 2 shows the changes in parental observation scores for all 22 participants in each behaviour category of the BSE schedule between testing sessions at baseline and 5 months after commencement of the gluten-free diet. Changes to mean global rating scores (GRS) are also shown (a negative difference suggestive of improvement). Here 16 of the 22 participants were rated by parents as showing some degree of global

Table 1 **The grouped item categories of the Behaviour Summarized Evaluation (BSE) scale (including additional physiological groupings)**

I	*Autistic isolation*
1	Is eager for aloneness
2	Ignores people
3	Poor social interaction
4	Abnormal eye contact
II	*Impairment of verbal and non-verbal communication*
5	Does not make an effort to communicate using voice and/or words
6	Lack of appropriate facial expressions and gestures
7	Stereotyped vocal and voice utterances, echolalia
III	*Bizarre responses to the environment*
8	Lack of initiative, poor activity
9	Inappropriate relating to inanimate objects
10	Resistance to change
IV	*Motor disturbances*
11	Stereotyped sensorimotor activity
12	Agitation, restlessness
13	Bizarre posture and gait
V	*Inappropriate emotional/affective responses*
14	Auto-aggressiveness
15	Hetero-aggressiveness
16	Soft anxiety signs
17	Mood difficulties
VI	*Primary instinctual disturbances*
18	Disturbances of feeding behaviour
VII	*Disturbances in attention, perception and intellectual functions*
19	Unstable attention
20	Bizarre responses to auditory stimuli

Physiological groupings: VIII ear infections; IX problems with allergies; X irregular bowel movements; XI irregular urination patterns; XII signs of skin disorder; XIII irregular sleeping patterns.

improvement at the end of the dietary period as compared with the beginning. Subjects C, G, H and I showed the most consistent marked changes in mean GRS when compared with other dietary participants (indicative of improvement). Overall analysis of all 22 participants on the gluten-free diet using a two-way paired *t*-test showed that there was a significant change in parental observation scores between baseline and post-dietary assessment for the behaviour groupings relating to motor disturbances ($t = 2.13$; $p = 0.04$), primary instinctual disturbances ($t = 2.83$; $p = 0.01$) and disturbances in attention, perception and intellectual functions ($t = 2.46$; $p = 0.02$).

Table 2 **Changes to parental observation ratings on each of the behavioural groupings of the BSE for individual participants on a gluten-free diet. The mean GRS (global rating score) provides a summary of the changes in ratings of observed autistic behaviours from all the behavioural groupings analysed, between baseline and post-dietary assessment. Minus (−) scores denote improvement in observation ratings**

Diagnosis	*Subjects*	*I Autistic isolation*	*II V/NV commun.*	*III Envir. responses*	*IV Motor*	*V Emotion/ affective*	*VI Primary instinct*	*VII Attention*	*Mean GRS*
Autism	A	1.0	0.2	0.3	−0.6	0.4	−1.0	0.5	0.1
	B	0.3	0.6	−0.1	−0.3	0.0	0.5	0.0	0.1
	C	−1.0	−0.1	−1.3	0.2	−0.3	0.3	−1.0	**−0.5**
	D	−0.1	0.0	1.2	0.1	0.3	0.0	0.5	0.3
	E	0.1	−0.7	−0.6	−0.6	−0.5	0.0	0.2	**−0.3**
	F	−0.5	−0.2	−0.8	0.1	0.0	−0.7	−0.3	**−0.3**
	G	−0.9	−0.1	0.0	0.2	−0.2	−1.0	−0.6	**−0.4**
	H	0.0	−1.0	0.7	−0.4	−1.1	−2.0	−1.5	**−0.8**
	I	−1.0	−1.5	−1.2	−1.6	−0.6	−1.0	−1.0	**−1.1**
Asperger syndrome	J	0.4	−0.3	−0.1	−0.1	−0.6	−0.3	0.4	**−0.1**
	K	−0.4	−0.2	0.1	0.0	0.5	−0.2	0.0	0.0
	L	−0.2	0.5	−0.3	−0.6	0.3	−0.5	−1.9	**−0.4**
	M	−0.2	−0.5	−0.5	0.3	0.1	0.0	−1.2	**−0.3**
ASD	N	0.6	0.9	0.9	0.7	0.6	−0.5	0.0	0.5
	O	−0.1	0.4	−0.3	−0.6	−0.7	0.0	0.3	**−0.1**
	P	0.1	−0.5	−1.0	−0.3	0.1	−0.2	−1.5	**−0.1**
	Q	0.1	−0.2	0.0	0.0	0.0	0.0	0.0	0.0
	R	−0.3	0.3	0.0	−0.4	0.0	0.0	−0.1	**−0.1**
SPD	S	−0.3	0.1	−0.6	−0.2	−0.4	0.0	0.0	**−0.2**
	T	0.0	−0.2	0.0	−0.1	−0.2	0.0	−0.1	**−0.1**
Dyspraxia	U	0.7	0.7	−0.5	−0.4	−0.4	−0.3	−0.8	**−0.2**
	V	−0.6	−0.6	0.0	0.1	0.2	0.0	0.0	**−0.1**

Teacher observations

Table 3 shows the results of teacher observations. Complete data were extracted from teacher observations for 13 of the 22 children. Changes to mean GRS indicative of improvement were found for 7 of the 13 children for whom completed data were obtained.

Interrater GRS correlations between the 13 subjects rated by both parent and teacher averaged $r = 0.167$. Mean GRS improvements found by parental ratings were confirmed for 4 participants, rated by teachers as showing global improvement (subjects C, G, H, L). The small numbers of participants in each diagnostic subgroup prohibited the statistical analysis of changes in observation scores in these groups. The overall analysis of all 13 subjects observed by teachers showed an overall improvement in teacher observation scores between baseline and post-dietary assessment, although this was not significant using a two-way paired *t*-test.

Examination of parental and teacher scores for all dietary intervention participants (where gluten was removed) on the individual behavioural items of the BSE found correlations between the two sets of scores showing improvement for the items: abnormal eye contact ($r = 0.53$), lack of effort in communicating ($r = 0.53$), lack of appropriate facial gestures ($r = 0.86$), lack of initiative/poor activity ($r = 0.64$), inap-

Table 3 **Changes to teacher observation ratings on the behavioural groupings of the BSE for participants on a gluten-free diet. Minus (−) scores denote improvement in observation ratings**

Diagnosis	*Subjects*	*I Autistic isolation*	*II V/NV commun.*	*III Envir. responses*	*IV Motor*	*V Emotion/ affective*	*VI Primary instinct*	*VII Attention*	*Mean GRS*
Autism	A	0.1	0.4	0.4	0.2	1.1	1.0	0.3	0.5
	C	−0.9	−0.2	−1.2	−1.2	−1.0	0.0	−0.2	**−0.7**
	D	−0.7	−0.4	0.2	0.3	0.3	−0.5	0.0	**−0.1**
	G	−0.3	−0.3	0.3	0.0	−0.3	−1.8	−1.7	**−0.6**
	H	−0.6	−0.1	−0.7	0.5	−0.6	0.8	−0.9	**−0.2**
	I	0.7	0.3	0.0	−0.1	0.0	0.0	0.0	0.1
Asperger syndrome	J	1.0	0.5	0.8	0.1	0.3	0.0	0.4	0.4
	L	−0.5	−0.3	−1.2	0.0	0.4	−0.5	−0.9	**−0.4**
ASD	N	−0.5	−0.6	−0.7	0.1	−0.1	0.0	0.0	**−0.3**
	Q	0.1	−0.5	−0.2	−0.3	−0.1	0.0	−1.1	**−0.3**
SPD	T	0.2	0.5	0.0	0.4	−0.1	−0.5	0.3	0.1
Dyspraxia	U	0.0	0.0	0.3	0.0	0.5	0.4	0.4	0.2
	V	−0.1	0.1	0.1	–	−0.1	0.7	0.7	0.2

propriate relating to objects ($r = 0.68$) and stereotyped sensorimotor behaviours ($r = 0.63$). Figure 1 shows the profile of mean changes in scores given by parents and teachers between pre- and post-dietary testing sessions.

Gluten challenge group

Changes in parental ratings for the gluten challenge group showed individual accounts of improvements in some behaviour groupings of observed autistic behaviours throughout the course of the study. There was, however, a mean percentage change indicative of a group worsening of behaviours relating to primary instinctual disturbances (7.5 percent increase in the frequency of observed behaviours) and disturbances in attention, perception and intellectual functions (5 percent increase in observed behaviours) when the gluten was re-introduced into the diet. Analysis of teacher ratings was not possible owing to the lack of completed observation schedules. Percentage changes in parental and teacher ratings for the control group illustrate a discordant picture of change, reporting improvement in some behaviours and deterioration in others. The only agreement found between parent and teacher ratings was for a worsening of problems with verbal and non-verbal communication.

Kaufmann Assessment Battery for Children (K-ABC)

Six children completing the diet (subjects A, C, G, H, K, S) were tested on both pre- and post-diet sessions using six of the subtests from the K-ABC (Kaufmann and Kaufmann, 1983). Four children from the gluten challenge group (GC1, GC2, GC3, GC4) and four children from the control group completed the tests. Subjects A and C (autism diagnosed) did not score on either testing occasion. Subject H (autism diagnosed) showed an increase in scores on three of the six subtests, as did subject K (Asperger syndrome diagnosed). Subject S (semantic pragmatic disorder diagnosed) showed an improvement on four of the six subtests. As a whole, the gluten-free group showed a significant increase in scores between the two sessions for three of the six subtests using a one-tailed paired *t*-test (Magic Window, $t = -2.26$, $p = 0.02$; Face Recognition, $t = -1.92$, $p = 0.04$; Gestalt Closure: $t = -2.02$, $p = 0.04$). The gluten challenge group and the control group showed no significant change in scores between the two testing sessions (although the gluten challenge group did show a slight non-significant worsening of scores for the Face Recognition subtest). There was no apparent correlation between the global scores of the six children tested on the K-ABC and parental or teacher observation GRS for the six children (parents $r = -0.05$; teachers $r = 0.06$).

Parental Satisfaction Survey (PASS)

Analysis of parental responses on PASS (Panksepp et al., 1991) on the evaluation of the diet suggests that the introduction of the gluten-free diet coincided with improvements in many aspects of autistic behaviour. Mean parental responses suggested that all areas examined either improved or at least stayed the same when the gluten-free diet had been in place. The most marked improvements (means between slight and clear improvement) for the gluten-free group are shown in Figure 2 with underlines, and included: increased desire to interact, increased curiosity/interest, an increased number of 'good' days, increases in smiling, eye contact, and play behaviour, increased attempts to communicate and an increased number of initiations of interactions. Mean PASS ratings by parents of children on the gluten challenge suggested that, although improvements were noted in many areas, these tended to be rated as less dramatic improvements, and on some items, participants tended to show a slight regression in behaviour, for example, a worsening of sound tolerance, anger and aggression, concern with the welfare of others and an increased tendency to be self-centred.

Parents were asked to rate the overall direct effect of the gluten-free diet on their child's behaviour, as they perceived it. First, 67 percent of parents of children on dietary intervention rated the introduction of a gluten-free diet for 5 months as leading to clear or substantial improvement in their child's autistic behaviours. Furthermore, 94 percent of parents confirmed that their child would continue on the gluten-free diet when the study concluded. Conversely, 60 percent of parents of children involved with gluten challenge suggested that the reintroduction of gluten back into the diet was correlated with a slight worsening of autistic behaviours.

Urinary analysis

Urinary analysis (including creatinine levels) was completed on six participants who completed the gluten-free diet (subjects A, C, G, H, K, S), four participants on the gluten challenge (subjects GC1, GC3, GC4, GC5) and three participants of the control group.[6] Examination of the urine samples of participants on the gluten-free diet showed a mean reduction in urinary IAG excretion between baseline samples and post-diet samples (−44.24 percent), compared with increases of 1.93 percent in the gluten challenge group and 25.33 percent in the control group (see Figure 3a). None of these changes to urinary IAG excretion was significant using a two-tailed paired *t*-test. Five of the six gluten-free urinary profiles showed a decrease in IAG excretion, with only subject S (SPD diagnosed) showing an increase (see Figure 3b).

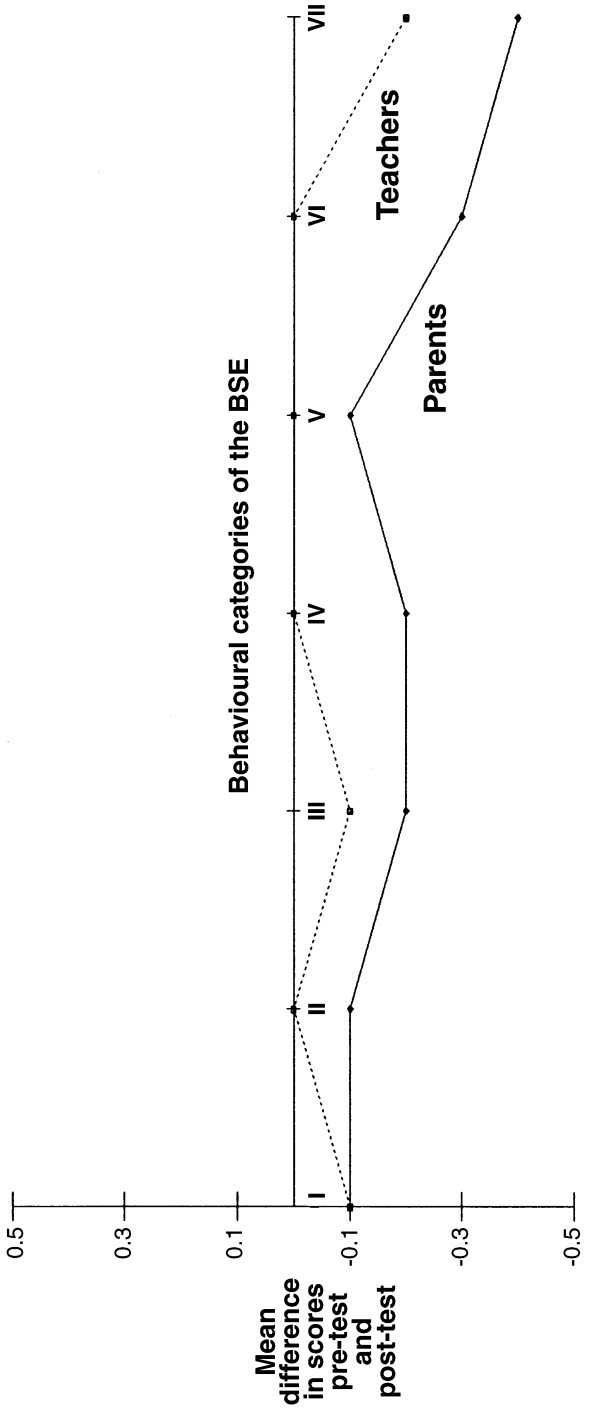

Figure 1 **Comparison of mean parental and teacher observation scores for the behavioural categories of the BSE pre- and post-dietary intervention. Negative values indicate an improvement in mean scores**

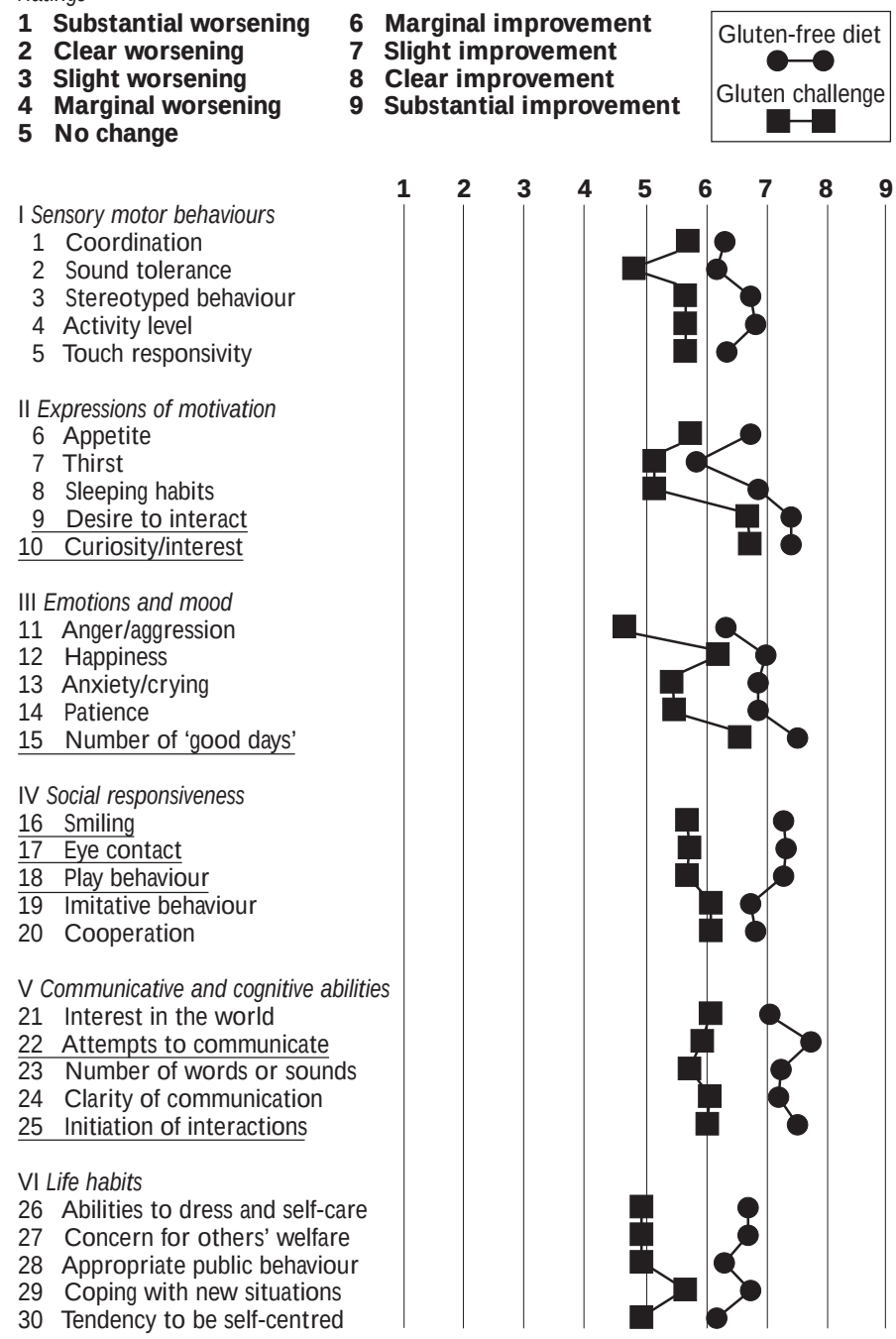

Figure 2 **Mean results from parental ratings of children on the gluten-free diet and children on the gluten challenge**

Relationship between urinary IAG and behavioural data

Analysis showed that there was no discernible correlation between the change in the amount of IAG excreted between pre- and post-dietary intervention and either changes to global scores on the K-ABC ($r = -0.233$) psychometric test or parental observation GRS ($r = 0.452$) or teacher observation GRS ($r = -0.27$). Although IAG levels showed a decrease in gluten-free participants compared with an increase in levels in other participants, it is not possible in this study to establish a direct relationship between IAG levels and behaviour. For example, subject S showed an increase in IAG excretion (+55.96 percent) but also demonstrated an improvement in performance on four of the subtests of the K-ABC tests. Conversely, subject A showed a substantial decrease in the IAG excretion (−88.26 percent), but showed no change in performance on both psychometric testing sessions (not scoring on either occasion).

Discussion

The methodological and ethical problems inherent in this kind of research need to be borne in mind when interpreting the results. Firstly of course, parents were not blind to the intervention and this may well have influenced their perceptions of children's behaviour. Secondly, this was a pilot study conducted on a very small sample of children. Excluding one of the major foodstuffs of modern Western society from the diet of children whose circumstances often allow little or no informed consent to be obtained also raises problems. Previous research in this field has focused on the exclusion of both gluten and casein products from the diet of children with autism (Knivsberg et al., 1990), because of the similarity in breakdown products of the two foodstuffs. The deliberate exclusion of only gluten products from the diet was in part to assess the relative impact of a gluten-free diet with children with autism, but also because of the added difficulty which would have been present with the exclusion of other products, such as casein, from the diet. It is not possible to ascertain, therefore, the behavioural effects of products such as casein, or other interfering foodstuffs which have been implicated. Studies of milk-free diets with children with autism have revealed improvements in similar areas to the current study (Lucarelli et al., 1995).

Behavioural data obtained from parents and teachers of children on the gluten-free diet also suggested that changes were not totally universal in their type and extent. Moreover, it was not possible to rule out the relative influences of age, gender, development and education as providing an alternative platform for the interpretation of improvements in the behaviour of gluten-free participants, because of the heterogeneity of the children

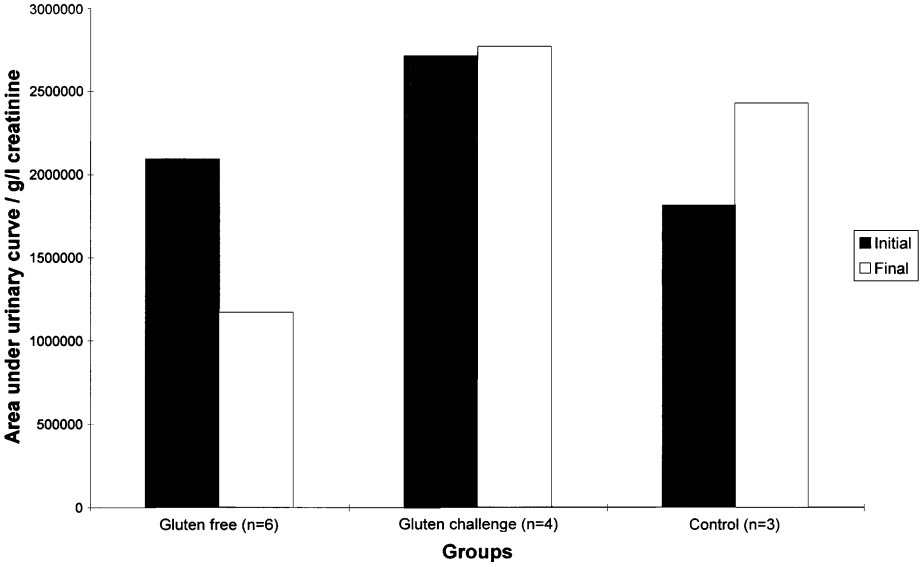

Figure 3a **The mean amount of trans-indolylacryloylglycine (IAG) excreted in the urine of participants pre- and post-intervention**

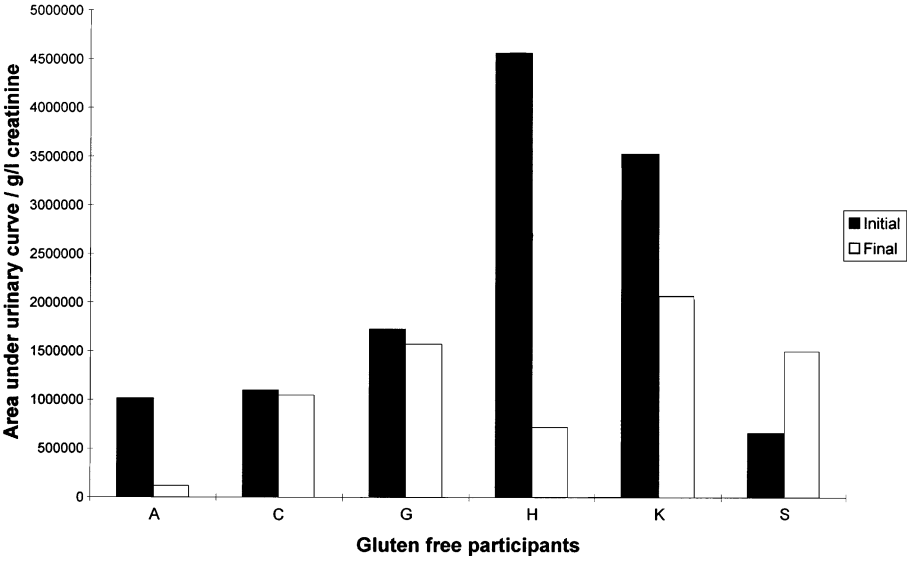

Figure 3b **The amount of IAG excreted in the urine of gluten free participants pre- and post-dietary intervention**

involved in the study. Knivsberg et al. (1995) discussed the value of the results gained from their study, suggesting that although uneven developmental profiles epitomize the autistic syndrome, the marked effects accompanying the diets are difficult to explain solely in terms of natural development. The short time scale of the present study also suggests that results cannot wholly be put down to developmental issues or educational strategies, although clearly these influences cannot be discounted. Neither can the role of other environmental factors cannot be dismissed as an influence on results. Seasonal changes have been implicated as being a mediating factor in certain behaviours of children with autism and other psychiatric conditions (Fossey and Shapiro, 1992). Biological changes associated with circadian rhythms have also been related to behaviour and cognitive functioning in people with autism (Shattock and Lowdon, 1991).

Inference can be made that gluten exclusion from the diet may be affecting one or several components in the proposed abnormal biological functioning of people with autism; the variability of success on the diet is possibly reflective of different biological mechanisms associated with different subsets of people with autism. The short time scale of the study and suggestions about the long term nature of the diet being reflective of true changes (Knivsberg et al., 1990; 1995) might account for the lack of significant change noted in, for example, the levels of urinary compound excretion. However, the reports of significant improvement in aspects of autistic behaviours following the 3 month dietary threshold suggests the effects of dietary change may be more immediate than previously reported.

Open and double-blind trials of opioid-blocking agents, although inconsistent, have suggested that drugs such as Naltrexone exert a stimulant activity on aspects of behaviour such as communication and sociability, and produce a regressive action on behaviours such as hyperactivity and self-injurious behaviour (SIB) (Panksepp et al., 1991). These results would seem to be compatible with the current study findings. The theoretical explanation for the 'withdrawal period' reported by many parents might also be accounted for by this relationship. Since the suggested compounds derived from the incomplete breakdown of gluten are opioid in nature, this could suggest the initial withdrawal period is comparable with the withdrawal behaviours exhibited by opioid addicts on the removal of opioids (Reynolds, 1993). However, although feasible, this explanation cannot be confirmed in the study. It also fails to take account of the psychological effects of the exclusion of an important part of the child's diet and subsequent disruption to eating patterns and routines. Nevertheless, the reduction of SIB reported in several participants

following the introduction of the diet (and subsequent re-emergence when gluten was put back in the diet) support theories of an opioid–SIB relationship, through a possible reorganization of abnormal functioning of the pain receptors, governed by the presence of elevated levels of opioid peptides (Shattock and Lowdon, 1991).

Martineau et al. (1987) suggested that problems with attention and perception were primary symptoms of autism, stressing a relationship between these functions and regulation by the dopaminergic system. The theory suggests that a dysfunction in this system could be involved with the problems of 'sensory overload' noted by several people with autism (e.g. Williams, 1996). Improvements were noted on attentional and perceptual functions, for children involved on the current study, offering support for the dopaminergic system's involvement and possible reorganization during the course of the diet.

Tests for food allergy or coeliac disease were not conducted during the course of this research, although discussions with parents did reveal that a proportion of the children examined did have some possible problems in this area. This was suggested by the many physiological and behavioural reactions observed by parents and teachers when certain foods were eaten. The speed with which behaviour changed as a result of accidental gluten ingestion by the children on the gluten-free diet was dramatic and noticed by many parents. One possible assumption is that the subsequent shock to the body when small amounts of gluten were being ingested was indicative of an allergic response (participants sensitized to the reintroduction of small amounts of dietary gluten, at least in the short term). The overall failure of the gluten challenge to show any initial behavioural regressions when larger amounts of gluten were being ingested could be due to several factors: for example, the small numbers of participants in this group, the long period of non-exposure to dietary gluten, or the lack of response to larger amounts of dietary gluten.

The relationship between autism and coeliac disease has been discussed previously in the literature (e.g. Coleman et al., 1976). Shattock and Savery (1996) commented that only a small percentage of children with autism are suggested to have an underlying coeliac condition, although figures suggest this is a disproportionate number when compared with the general population. The underlying histological lesions indicative of an excessively porous intestinal wall associated with coeliac disease have been linked to similar problems in autism (Shattock and Savery, 1996). Problems with intestinal permeability have been found in a proportion of children with autism (D'Eufemia et al., 1996). Similarly, the gastrointestinal findings following measles-mumps-rubella immunization (MMR) reported by Wakefield et al. (1998) suggest impaired intestinal

function and increased permeability. The increased levels of IAG[3] and other, as yet unidentified, compounds found in the urine of people with tenuous autism also provides support for the existence of an abnormally porous intestinal membrane. Previous research has suggested that the use of gluten-free diets with patients with coeliac disease may, to a degree, improve the problems of intestinal permeability associated with the condition (Cummins et al., 1991). If similar mechanisms apply in people with autism, would suggest an amelioration of this symptomatology when a gluten-free diet is used. Recent research suggestive of a link between improvement in autistic behaviour and administration of the gastro-intestinal hormone secretin further serves to underpin the association between abnormal gastrointestinal conditions and behaviour in people with autism (Horvarth et al., 1998).

The proposed mechanism and the consequences of the study findings suggest that the behavioural improvements observed with participants on the gluten-free diet may have their origins in biochemistry and neurology rather than in a psychological-educational setting. This is not to say that the contributions from the psychological and educational fields do not represent a substantial role in some of the improvements noted. However, it may be that the initial positive impact of the diet on the primary biological dysfunction disrupting attentional and perceptual processes subsequently facilitated improvements in the participant's receptivity to education and learning strategies. These, in turn may have led to the increases in verbal and non-verbal communication which were noted in many children on the diet. Clearly, a complex interplay of factors is at work. Finally, the reliance upon parental and teacher subjective reports and interpretation, although allowing a valuable insight into the behavioural changes of participants during the course of the study, has many experimental flaws, and cannot be considered a conclusive empirical summation of the effectiveness of the diet. The use of clinical double-blind trials, similar to those used with studies of phenylketonuria (PKU) (Clarke et al., 1987) would provide a mechanism for more rigorous testing of the diet. However, if gluten exposure has negative effects on children with autism and associated spectrum disorders this suggests that the ethical value of double-blind trials would have to be weighed against the proposed benefits of such research.

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Notes

1. Proteins consist of long chains of smaller units called amino acids. Proteins are normally digested by enzymes in the intestines and broken down into these smaller units. However, if digestion is incomplete, short chains of amino acids are formed called peptides.
2. Increased levels of peptides in the gut are thought to be due to impairment in the peptidase system which is usually responsible for their breakdown (for example, genetically determined deficiencies, shortage of cofactors such as vitamins and minerals required for enzyme function, or inappropriate intestinal conditions such as gut acidity).
3. The presence and structure of IAG was confirmed on three separate analytical systems (Mills et al., 1998).
4. The assumption is that IAG may represent the detoxified version of a parent acid compound indole acrylic acid (IAcrA) which could, as one of its effects, have profound influences on the permeability of membranes (including the intestinal membrane) throughout the body. The presence of the detoxified version (IAG) suggests that the parent compound IAcrA would exist in the body in its original active form.
5. Coeliac disease is a lifelong gluten-sensitive disorder, characterized by malabsorption and typical small-bowel mucosal atrophy. Classic signs of the disease include diarrhoea, weight loss and weakness, although milder symptoms include indigestion in adults and recurrent abdominal pain in children. Other neurological symptoms include intellectual deterioration and brain atrophy, ataxia and epilepsy. Diagnosis of coeliac disease is made through serum gliadin antibody tests (IgG and IgA antigliadin antibodies), IgA antiendomysium antibody test, and intestinal biopsy. Treatment is the implementation of a gluten-free diet.
6. Because of the cost and limited resources for urinary analysis and creatinine concentration determination, only a proportion of samples were investigated at this time. Further analysis of all samples is planned for future research.

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