

The ScanBrit randomised, controlled, single-blind study of a gluten- and casein-free dietary intervention for children with autism spectrum disorders

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There is increasing interest in the use of gluten- and casein-free diets for children with autism spectrum disorders (ASDs). We report results from a two-stage, 24-month, randomised, controlled trial incorporating an adaptive 'catch-up' design and interim analysis. Stage 1 of the trial saw 72 Danish children (aged 4 years to 10 years 11 months) assigned to diet (A) or non-diet (B) groups by stratified randomisation. Autism Diagnostic Observation Schedule (ADOS) and the Gilliam Autism Rating Scale (GARS) were used to assess core autism behaviours, Vineland Adaptive Behaviour Scales (VABS) to ascertain developmental level, and Attention-Deficit Hyperactivity Disorder – IV scale (ADHD-IV) to determine inattention and hyperactivity. Participants were tested at baseline, 8, and 12 months. Based on per protocol repeated measures analysis, data for 26 diet children and 29 controls were available at 12 months. At this point, there was a significant improvement to mean diet group scores (time*treatment interaction) on sub-domains of ADOS, GARS and ADHD-IV measures. Surpassing of predefined statistical thresholds as evidence of improvement in group A at 12 months sanctioned the re-assignment of group B participants to active dietary treatment. Stage 2 data for 18 group A and 17 group B participants were available at 24 months. Multiple scenario analysis based on inter- and intra-group comparisons showed some evidence of sustained clinical group improvements although possibly indicative of a plateau effect for intervention. Our results suggest that dietary intervention may positively affect developmental outcome for some children diagnosed with ASD. In the absence of a placebo condition to the current investigation, we are, however, unable to disqualify potential effects derived from intervention outside of dietary changes. Further studies are required to ascertain potential best- and non-responders to intervention. The study was registered with ClincialTrials.gov, number NCT00614198.

Keywords: autism spectrum disorder (ASD), diet; gluten, casein, randomised controlled trial, adaptive design

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Introduction

As a group of life-long conditions, pervasive developmental disorder (PDD), otherwise known as autism spectrum disorder (ASD; MIM 209850), describes a continuum of heterogeneous characteristics focused on core impairments in social and communicative abilities alongside the presence of repetitive behaviours.^{1,2} Diagnosis is derived exclusively from presented behavioural manifestation and scrutiny of developmental history. The diverse clinical picture of ASD is complicated by chronological age-related changes to symptoms and the variable presence of peripheral and co-morbid conditions including learning disability and epilepsy. Attention-deficit hyperactivity disorder (ADHD)³ and behavioural problems can also co-exist with ASD. Outside of the prescribed borders for the classification of ASD, evidence continues to emerge on the presence of a broader phenotype of autism based on the appearance of sub-clinical communicative and social features.⁴

The continued lack of a universally pertinent theory of aetiology and pathology combined with an absence of consistent genetic or biological correlates continues to constrain knowledge of the condition. There is an increasing recognition that the expression of ASD in some sub-groups may also include a number of additional elements beyond psychiatry that may be potentially relevant.⁵ The focus of some investigations has moved from an exclusively brain-based view towards a more systemic outlook for the condition.^{6,7} A possible increase in cases⁸ and the prospect of gene-environment interactions including pathogenic mechanisms analogous to phenylketonuria (PKU)⁹⁻¹¹ represent important conceptual developments in understanding ASD. Such factors have been contributory to the increased adoption of several measures attempting to moderate the early presentation of core and peripheral symptoms, influence developmental outcome and improve quality of life.¹² Aside from the personal and familial effects of the condition, estimates of significant economic costs attached to support and service structures for ASD continue to be reported.¹³

Of the myriad of non-educational interventions being used for ASD, few have received more scientific interest than the implementation of specific dietary interventions to ameliorate symptoms.¹⁴⁻¹⁶ Whilst multiple examples of a link between diet and physical health exist, the relationship between food and mental health is less well researched and understood. Coeliac disease and inborn errors of metabolism (such as PKU) represent archetypal examples of conditions where failure to implement dietary restrictions can cause permanent

disability or death. Recent reports on the relationship between food additives affecting childhood behaviour¹⁷ and the effects of a few foods' diet on symptoms of ADHD¹⁸ illustrate how diet may also influence behaviour and affect developmental course. Improved clinical outcome following the adoption of diets devoid of gluten (a protein found in wheat, barley and rye) and casein (derived from mammalian dairy produce) for other psychiatric conditions including schizophrenia have been documented.^{19,20} Espousal of such diets as potential ameliorative strategies for ASD continues to attract interest.

Open, non-randomised studies on the use of gluten- and/or casein-free diets for ASD²¹⁻²⁵ based largely on proposals for abnormalities in exogenously derived opioid peptide chemistry and adverse gastrointestinal pathology have shown efficacy.^{6,26,27} Primary areas of behavioural changes were in attention, communication, social interaction and motor skills. Such positive changes to symptoms have been observed experimentally for as long as 4 years on diet.²² A more uneven pattern of results have been reported from the few controlled trials of diet.^{28,29} Several weaknesses in previous studies have been identified based on study design, length of intervention period and clarity of diagnosis of participants.^{15,30} Related methodological issues including a lack of suitable control groups, randomisation and blinding measures, and inappropriateness of psychometric tools and outcome measures used are also frequently cited.

We report clinical results from a two-stage, 24-month, randomised, controlled study of the gluten- and casein-free diet with children diagnosed with ASD. The aim was to evaluate the effect of dietary intervention by comparing results from a diet and non-diet group using a comprehensive assessment battery. The experimental hypothesis was that children with ASD on dietary intervention would show a significantly improved group developmental outcome in the medium- and long-term with regard to core and/or secondary symptoms. The primary end-points were the change in scores of the diet group on one or more measures against predefined statistical thresholds as evidence of improvement, alongside changes to intra- and inter-group scores at study end.

Subjects and methods

Participants

The study was conducted between April 2006 and October 2008 at the Center for Autisme, Denmark (see *Note) under the remit of a Scandinavian-British

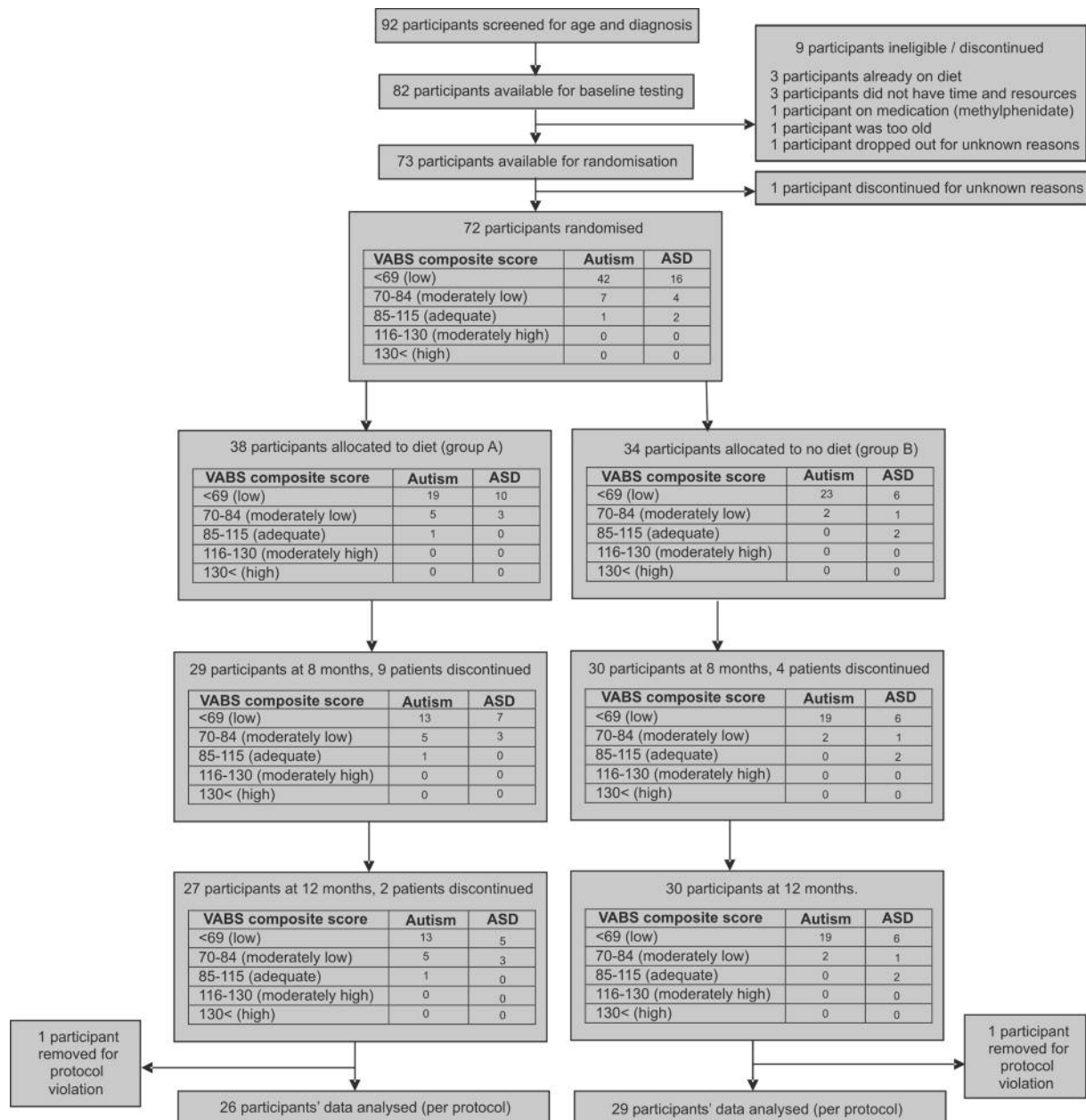


Figure 1 Trial profile for the first 12 months (stage 1). Number of children participating before trial started, after 8 months and after 12 months. Participants are shown per group according to composite scores within the five functional levels of the Vineland Adaptive Behaviour Scale (VABS)

(ScanBrit) collaborative research group. Danish children aged between 4 years and 10 years 11 months formally diagnosed with PDD [ICD-10 code F84] at the Center for Autisme or other child psychiatric clinics were enrolled in the study. Exclusion criteria were co-morbid diagnoses of epilepsy, fragile-X syndrome, tuberous sclerosis or a developmental age below 24 months. All but one of the participants were free from psychotropic pharmacotherapy. (This child was in receipt of phenothiazine antipsychotic medication [Nozinan™]. Based on our protocol [PP]

analysis, this participant was subsequently removed from 12-month data examination following protocol violation.)

Study information was disseminated through advertisement in the Danish national autism society newsletter, pamphlets at a national conference on ASD, advertisement on web-pages, and contact with parents of children diagnosed at the Center for Autisme and schools catering for pupils with ASD.

Interested parents were provided with verbal and written information about the study. Parents who gave

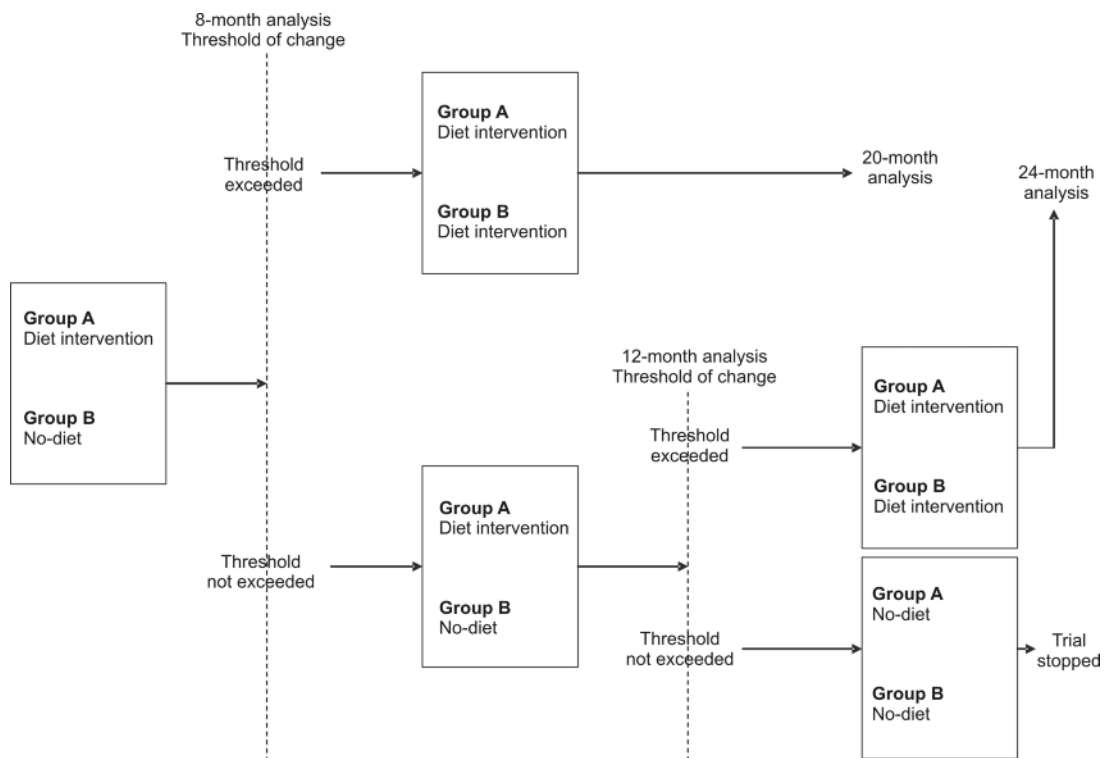


Figure 2 Trial adaptive design with interim analysis

written informed consent for their child’s participation were asked to complete a questionnaire regarding diagnosis and other pertinent information. No financial inducements or economic support were provided to participants during the study.

Procedures

The study protocol was approved by the Danish National Committee on Biomedical Research Ethics (reference no. KA0503g). The study was registered with ClinicalTrials.gov, number NCT00614198. This two-stage, randomised, controlled trial testing the efficacy of a gluten- and casein-free diet with children with ASD incorporated an adaptive ‘catch-up’ design³¹ with interim analysis. Figure 1 shows the trial profile for the first stage up to 12 months. This was followed by a comparative inter- and intra-group repeated measures strategy for an additional 12 months (stage 2).

Duplicate urine samples were collected from all prospective participants by parents, frozen and returned to the Center for Autisme. Each of two independent laboratories received one sample. Specimens were prepared and analysed blind and independently by high-performance liquid chromatography (HPLC) with UV diode-array detection (DAD) according to established protocols.^{32–35} Analysis on urine fractions was executed for compounds co-eluting with exogenous opioid peptide standards and/or *trans*-indolyl-3-acryloylglycine (IAG).

These compounds have been previously associated with dietary efficacy. A positive result from one or both laboratories as represented by a significant UV correlation ($\geq 95\%$) with external IAG or peptide standards confirmed study eligibility. This criterion was met for all participants.

Children invited to join the study ($n = 73$), received dietary assessment by study nutritionists. Accompanied by their parents, participants underwent a comprehensive behavioural and psychometric assessment at the Center for Autisme forming baseline period scores. Schedules requiring certification were carried out by trained personnel. Core autism behaviours were assessed by the Autism Diagnostic Observation Schedule (ADOS)³⁶ and the Gilliam Autism Rating Scale (GARS).³⁷ ADOS comprises four modules apposite to different levels of language capability. The schedule elicits and scores target behaviours via a number of prompts. An algorithm score is generated for core areas (communication, social interaction, repetitive behaviours) based on standardised cut-off points. We report scores for each of the ADOS sub-domains. GARS is a 56-item Likert scale questionnaire consisting of four scales measuring the symptoms of ASD – social interaction, communication, stereotyped behaviours and developmental disturbances. Scores for items in each scale are summed and converted to standard scores based on a reference sample. For the

purposes of our study, only current behaviours (for the first three scales) were analysed.

Developmental ability as determined by adaptive behaviour was assessed by the Vineland Adaptive Behaviour Scale (VABS).³⁸ Based on multiple domains of communication, socialisation and daily living skills, VABS is a parent-report questionnaire providing a composite estimate of a child's adaptive developmental age.

Inattention and hyperactivity were assessed using the Attention-Deficit Hyperactivity Disorder – IV rating scale (ADHD-IV).³⁹ Based on the DSM-IV¹ criteria for ADHD, ADHD-IV is a parent report schedule providing normative data on inattention and hyperactivity that characterise a diagnosis of ADHD.

Prior to randomisation, one participant dropped out of the study. A statistician, not involved in the study, generated a random allocation, stratified for age and VABS composite scores ($n = 72$). Participants were allocated to gluten- and casein-free diet (group A; $n = 38$) or no diet intervention (group B; $n = 34$). Aside from study nutritionists, all study members were blinded. Parents could not be blinded because they had to oversee the child's food intake and consequently knew whether the child was following an exclusion diet.

Figure 2 illustrates the trial adaptive design with interim analysis. The first stage of the trial saw group A introduce a strict gluten- and casein-free diet over the course of 2 weeks, for an initial period of 8 months. Group B were at the same time instructed to continue with their existing diet. Nutritionists monitored experimental participants over the course of the intervention to ensure dietary compliance and nutritional intake. Dietary participants were advised to take a multi-vitamin supplement including calcium during the trial to compensate for any nutritional deficiency during intervention. Height and weight were regularly assessed during the study by the participant's own physicians to ensure independently that adequate nutritional intake was being maintained for all participants.

At 8 months, all participants were re-tested by blinded investigators with baseline measures. This included repeat urine samples in order to study any potential changes to urinary excretions during our investigation. We recognise and appreciate the importance of comparing the results of urine analysis with the behavioural and psychometric assessments. These results will be reported separately. Following completion of assessments, a stop-go committee made up of an external researcher and the study statistician, not otherwise involved in psychometric

testing, broke participant codes in order to ascertain any significant changes to behavioural or psychometric scores for group A compared to baseline. The primary outcome at this point was a change in scores of the diet group (A) from baseline on one or more measures against pre-defined evidence of improvement thresholds at 8 months ($P < 0.01$) and 12 months ($P < 0.05$). If the thresholds were surpassed at 8 or 12 months, the second stage of the trial would see group B participants re-assigned to dietary intervention for 12 months. The original experimental diet group (A) would at the same time also continue on dietary intervention for a further 12 months. At 20 or 24 months, all participants would be re-tested with baseline measures and intra- and inter-group analyses performed. If the threshold was not exceeded at 12 months, the trial would be stopped. ADOS was not included as part of the assessment schedule at 12 months due to the short intervening period with testing at 8 months.

Statistical analysis

Following assessment of statistical assumptions, continuous parameters for the first stage of the trial including baseline, 8- and 12-month data were compared using a repeated measures model by means of the PROC MIXED function (SAS statistical software, v.9.2) for assessment measures of ADOS, VABS, ADHD-IV and GARS scores. Besides treatment, the model included baseline, age, time and the interaction between time and treatment (time*treatment) as explanatory variables. Time*treatment refers to the variation of treatment effects over the course of the study.⁴⁰ Terms that were not significant were taken out of the model to allow for a more parsimonious model and P -values for the final, reduced model reported. No adjustment for multiplicity was carried out, although study direction at 8 months was reliant on at least two measures with a significance of $P < 0.01$ in effect corresponding to a Bonferroni correction. A per protocol (PP) model formed the basis for participant inclusion in the data analysis in contrast to an intention to treat (ITT) standard. Only observed values were included for repeated measures analyses, which took partially completed profiles into account.

Assuming successful re-assignment of all participants to active treatment at or before 12 months, three scenarios for data analysis were included for the second stage of the study.

Scenario 1 This used data from the first 12-month group A (diet) scores and the final 12-month group B scores (no diet). Pooled results from both groups were used to ascertain any developmental change

between baseline and 12 months on diet. Age was also included as an explanatory factor in the statistical model taking into account the chronological age differences as a function of the time elapsed between the groups starting intervention. A significant change over time would indicate improved developmental outcome following intervention. A significant change in time*group interaction would indicate that scores between the groups did not show the same effect at all combinations of time and treatment. Thus, meaning that profiles over time were not the same for both groups.

Scenario 2 This used data from group B alone using a cross-over design (paired analysis) comparing 12 months of no dietary intervention with 12 months of dietary intervention.

Scenario 3 This used data from group A alone comparing 24-month data on diet compared with baseline. Analysis was conducted for time alone as no time*group interaction term is possible for this model.

All continuous parameters were evaluated using a repeated measures analysis (repeated measures ANCOVA) adjusting for respective baselines between the groups. Discrete parameters in the behaviour tests were analysed using the Cochran–Mantel–Haenszel

test and stratifying by gender. Analysis was again based on observed cases rather than last observation carried forward (LOCF). Additional analyses based on aggregated ADOS scores as a function of revised algorithms were also carried out.

Results

Seventy-two participants started the trial. All subjects fulfilled the criteria for PDD/ASD either through diagnostic procedures at the Center for Autism based on previously completed ADOS and ADI-R schedules, or at a child psychiatric clinic. Figure 1 shows baseline VABS composite scores. The majority of study participants were categorised in the low or moderately low category of functioning according to VABS composite scores. A current spoken language rating as obtained through ADOS module selection showed that 46 children (64%) had adequate spoken language, 19 (26%) had some spoken language and seven (10%) had little or no spoken language. Aside from a larger proportion of females in the dietary group, there were no obvious differences in group demographics or scores at baseline.

Stage 1 results for the first 12-month period

Fifteen children (21%) dropped out during the 12-month period (group A, *n* = 11; group B, *n* = 4; Fig. 1). From the non-diet group, one child was withdrawn

Table 1 Mean group scores and significance values (*P*) following per protocol repeated measures analysis at 8 and 12 months for extant participants in Group A (diet group) (*n* = 26; males = 21; females = 5; mean age = 94.2 months; IQR = 76.6–118.0) and Group B (non-diet group) (*n* = 29; males = 28; females = 1; mean age = 96.4 months; IQR = 76.3–120.3)

	Diet group			Non-diet group			Treatment	P-value		
	Base-line	8 mths	12 mths	Base-line	8 mths	12 mths		Time	Age	Time* treatment interaction
ADOS										
ADOS-communication	1.13	0.87	–	1.00	1.07	–	NS	NS	NS	0.0022
ADOS-social	1.18	1.09	–	1.22	1.30	–	NS	NS	NS	NS
ADOS-repetitive	0.46	0.35	–	0.36	0.34	–	NS	NS	0.0471	NS
GARS										
GARS-social	6.96	5.88	5.38	6.41	6.34	6.00	NS	0.0001	NS	0.0001
GARS-communication	7.23	6.00	5.74	7.28	6.76	5.81	NS	0.0001	NS	NS
GARS-stereotyped	6.81	5.85	5.35	6.76	5.97	5.37	NS	0.0001	NS	NS
VABS										
VABS-communication	66.46	66.69	67.38	62.45	63.46	64.07	NS	NS	NS	NS
VABS-social	64.58	66.12	60.42	64.03	65.39	61.21	NS	NS	0.0154	NS
VABS-daily living	59.88	62.92	63.35	55.55	55.18	53.96	NS	NS	NS	0.0208
ADHD										
ADHD-inattention	11.96	9.81	9.77	11.21	11.52	11.11	NS	0.0484	NS	0.0007
ADHD-hyperactivity	10.31	8.27	8.62	10.79	10.62	10.00	NS	0.0138	NS	0.0188

NS, not significant.

Significant items are marked in bold.

Decreasing scores on ADOS, GARS and ADHD-IV are indicative of improvement in functioning. Increasing scores on VABS indicate improved developmental functioning.

Table 2 Test sub-domain significance values per statistical analysis scenario for all psychometric parameters at 24 months. Data are based on per protocol analyses on remaining participants at study end. Scenario 1 includes analyses based on both groups. Scenario 2 includes data from group B alone. Scenario 3 includes data from group A alone

	Scenarios		
	1	2	3
ADOS			
ADOS-communication			
Time	NS ^a	NS	NS
Time*group	0.0021	NS	N/R
ADOS-social			
Time	NS	NS	NS
Time*group	NS	NS	N/R
ADOS-repetitive			
Time	NS	NS	NS
Time*group	0.0212	NS	N/R
GARS			
GARS-social			
Time	0.0012	NS	0.0025
Time*group	0.0003	NS	N/R
GARS-communication			
Time	NS	0.0269	0.0019
Time*group	< 0.0001	NS	N/R
GARS-stereo			
Time	0.037	0.0174	NS
Time*group	< 0.0001	NS	N/R
VABS			
VABS-communication			
Time	NS	NS	N/R
Time*group	NS	NS	N/R
VABS-social			
Time	NS	NS	NS
Time*group	0.0248	NS	N/R
VABS-daily living			
Time	NS	NS	NS
Time*group	NS	NS	N/R
ADHD			
ADHD-inattention			
Time	0.0003	NS	NS
Time*group	NS	NS	N/R
ADHD-hyperactivity			
Time	0.0047	0.0148	NS
Time*group	NS	NS	N/R

NS, not significant at $p < 0.05$.

N/R, analysis not required.

Significant items are marked in bold.

^aScenario 1 time significance values marginally exceeded 0.05 significance level ($P = 0.0657$).

because of a desire to start diet intervention, families of two children lacked the time and resources to commit to the project, and the remaining participant listed no reason. From the diet group, three children did not want to be on the diet and the families of four children lacked time and resources to commit to the project. The remaining four children were withdrawn because of no effect of intervention (1 at 1 month; 2 at 7 months; 1 after 11 months). In line with the per protocol (PP) model of data analysis adopted, data from a further two participants (one from each group)

were excluded from the 12-month analysis on the grounds of protocol violation (non-adherence to groupings).

Table 1 shows the 8- and 12-month group data for standardised psychometric parameters. Following our per protocol (PP) approach to data analysis based on observed values over time, no significant ($P < 0.01$) changes in diet group scores on any measure were identified at 8 months in time for interim analysis judgement. Data from ADOS subsequently showed a significant change at this point on the time*treatment interaction for the communication measure ($P = 0.0022$) in favour of the dietary group – 2 children from the dietary group changed from module 2 to module 3 between baseline and 8 months. (Due to reliability testing, this data was not available in time for the stop committee at 8 months). Based on the data for completing participants, children in the diet group also showed a significant improvement at 12 months in social interaction (GARS; time*treatment, $P = 0.0001$), inattention (ADHD-IV; time*treatment, $P = 0.0007$), and hyperactivity (ADHD-IV; time*treatment, $P = 0.0188$). VABS scores for daily living skills (time*treatment, $P = 0.0208$) showed a significant effect for the control group at 12 months compared with baseline. Changes in test administration to VABS (face-to-face to telephone interview and variation of parental respondent) cannot be ruled out as a potential confounder for this variable.

In addition to the statistically significant differences noted at 12 months, several test scores showed a downward trend in favour of improvement for the diet group compared with baseline. With the exception of some reported behavioural episodes during the introduction and early stages of dietary intervention, we recorded no significant adverse effects for any participant. A small number of experimental participants reported initial problems with food acceptance following dietary implementation. These were not long-term problems and were resolved early in the study. Aside from the one participant in the dietary group removed from the final analysis, reports of dietary infractions amongst the experimental group were low.

Stage 2 results for 12–24-month period

At the end of study (24 months), 35 participants (group A, $n = 18$; group B, $n = 17$) remained in the project. Mean group ages at study conclusion showed no obvious differences (group A 114.0 months vs group B 116.4 months). Per protocol analysis was conducted for surviving participants. All subsequent tables show values based on these participants only. Table 2 summarises all test sub-domain significance

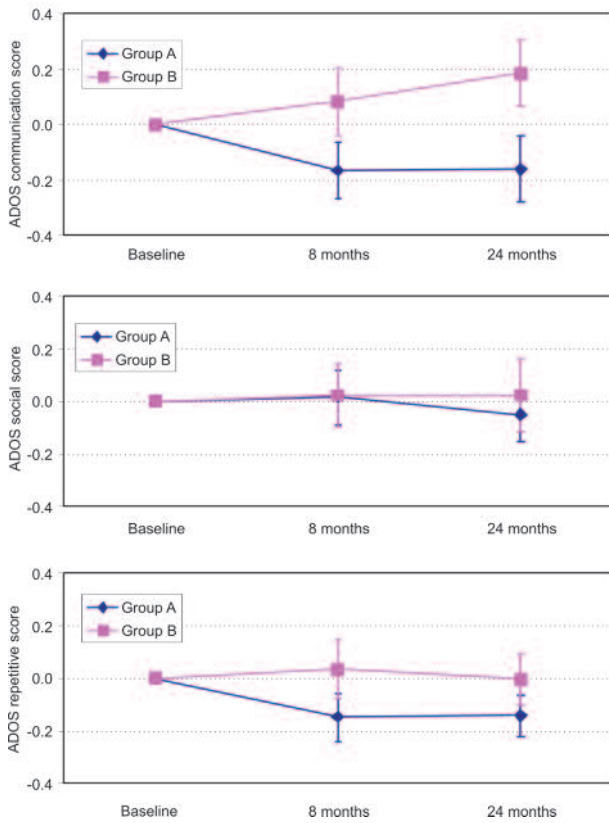


Figure 3 Group plots of ADOS sub-domain mean scores at all testing occasions. Decreasing scores on ADOS sub-domains are indicative of improvement in functioning

values for the three statistical analysis scenarios previously detailed above. Figures 3–6 show plotted mean group scores for psychometric parameters between baseline and the various testing occasions with error bars (SE). Where baseline scores equal zero, group scores at subsequent testing sessions show differences from baseline. Aside from VABS, decreasing scores from baseline on all measures are indicative of improvement. Group A was on diet for the entire 24 months whilst group B started diet at 12 months.

ADOS

Figure 3 shows ADOS scores per grouping across the trial period. No ADOS testing session was completed at 12 months due to the risk of practice effects. Both the communication and repetitive behaviour sub-domain measures of ADOS showed significant time*group effects following repeated measures analysis for Scenario 1. This implied that group scores were not the same for both groups at all combinations of time and treatment. No other significant longitudinal effects were found for this outcome measure.

Figure 3 illustrates the various trends according to

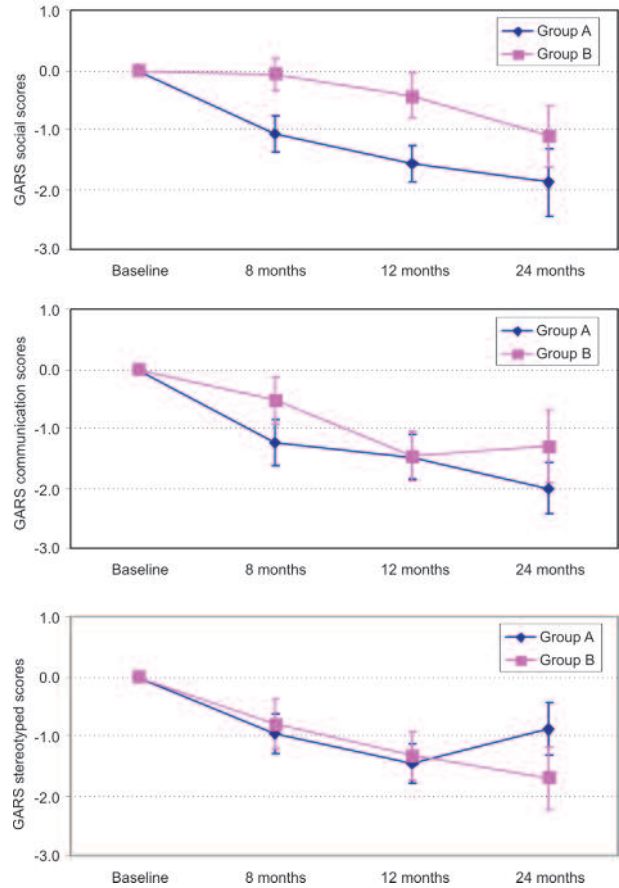


Figure 4 Group plots of GARS sub-domain mean scores at all testing occasions. Decreasing scores on GARS sub-domains are indicative of improvement in functioning

testing occasions where ADOS communication scores show an improvement for Group A from baseline to 8 months followed by a slight worsening from 8–24 months. Group B scores show continued worsening of communication throughout the trial. For the ADOS social scores, Group A show a similar pattern as that illustrated in the communication domain. Group B scores show a worsening between baseline and 8 months and a slight improvement between 8–24 months. For the ADOS repetitive behaviour scores, Group A show an improvement between baseline and 8 months followed by *status quo* between 8–24 months. Group B scores show a similar trend to Group B ADOS social scores.

Further analysis was undertaken on aggregated ADOS scores related to actual clinical outcome for each participant as a function of the introduction of revised ADOS algorithms⁴¹ published after our study had commenced. Social affect (incorporating both communication and reciprocal social interaction domains) and repetitive behaviour sub-domain scores

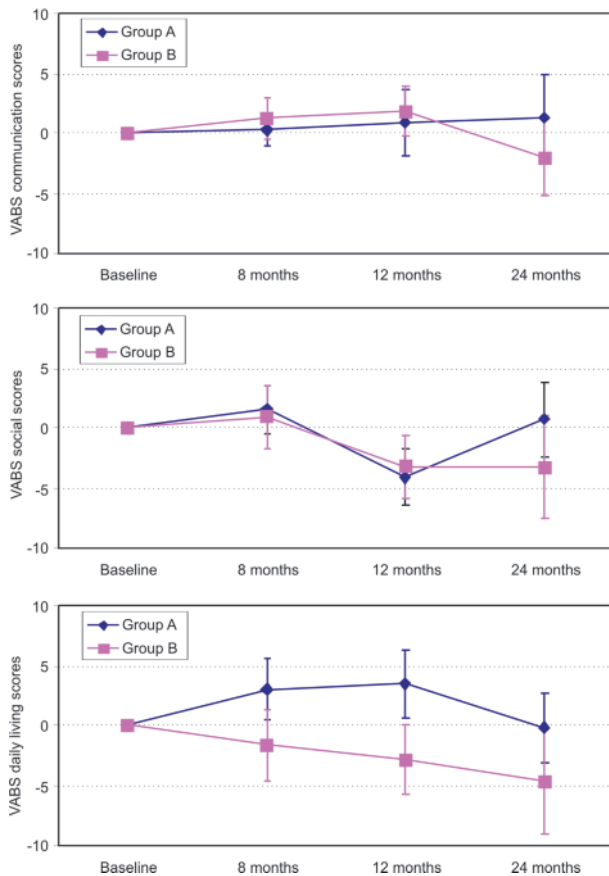


Figure 5 Group plots of VABS sub-domain mean scores at all testing occasions. Increasing scores on VABS sub-domains are indicative of improved developmental outcome

were combined into a total score. Data from 8 or 24 month summed ADOS scores were subtracted from baseline to discern any differences. These were defined as: negative difference (≥ 3), no change ($-2,0,2$) and positive difference (≤ -3). A Fisher's Exact test was used to analyse various comparisons including: (i) 0–8 months Group A on diet versus Group B controls; (ii) difference from 0–8 months for Group A versus 8–24 months for Group B; (iii) difference from 0–8 months for Group B only versus 8–24 months; and (iv) 8–24 months Group A and Group B on diet for 12 months. No significant effects ($P < 0.05$) were found for aggregated scores on any comparative measure.

GARS

All three sub-domain measures of the GARS showed significant effects for each of the statistical scenarios analysed indicative of improvement. As shown in Figure 4, scores for both groups on all sub-domains showed a general downward trend throughout the study irrespective of group allocation. This effect, however, tended to be more pronounced for group A

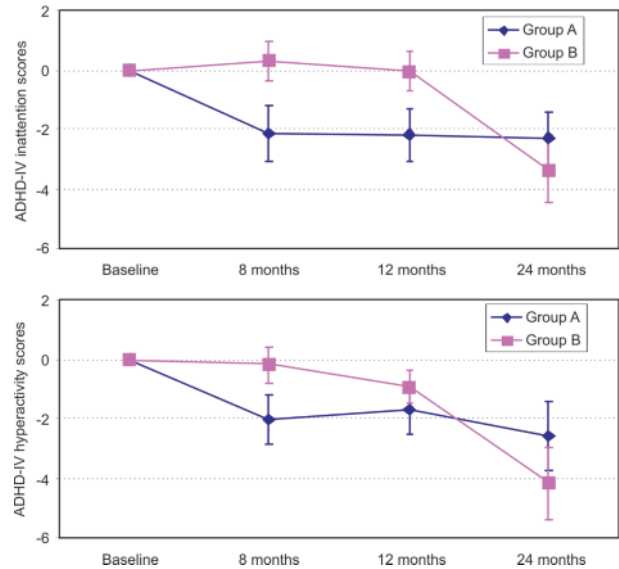


Figure 6 Group plots of ADHD-IV sub-domain mean scores at all testing occasions. Decreasing scores on ADHD-IV sub-domains are indicative of improvement in functioning

at all study points for the social interaction and communication sub-domains. The degree of improvement whilst on diet was not, however, consistent between the groups as demonstrated by the significant results for all time*group interactions for Scenario 1 across the sub-domains.

VABS

VABS scores over the entire period of intervention showed large variations in scores at the various testing occasions, in some part due to the changes in test administration throughout the study (Fig. 5). In contrast to the other schedules used during our study, an increase in scores on VABS is indicative of improved developmental functioning. There was only one single significant effect noted for the socialisation sub-domain suggesting that profiles were not parallel between the groups over the period of study in this area.

ADHD-IV

Both sub-domains of the ADHD-IV showed trends towards a reduction of behaviours as a consequence of dietary intervention for both groups. Significant effects were found for time in Scenario 1 suggestive of improvement in both inattention and hyperactivity. This effect was also parallel across the groups in the first 12 months of dietary intervention as a consequence of no significant time*group interaction for this scenario. A significant time effect for Scenario 2 for the hyperactivity sub-domain complimented this finding when comparing no diet with dietary

intervention. As illustrated in Figure 6, group A showed a significant improvement in the first 8 and 12 months of dietary intervention for the inattention sub-domain which then seemed to plateau throughout the rest of the investigation. A lack of data for group B after 12 months on intervention did not allow us to explore this trend further.

As per the findings from stage 1 of the investigation, we recorded no significant adverse effects for any participant on diet intervention during this stage of the trial.

Discussion

In this two-stage, randomised, controlled study of gluten- and casein-free diet of children with ASD, significant group improvements in core autistic and related behaviours were present after 8 and 12 months on diet. As part of our adaptive study, the attainment of predefined evidence of improvement beyond statistical thresholds for the dietary group sanctioned the re-assignment of control participants to active treatment for the following 12 months. Although the first stage of the study continued to 12 months before the control group switched to intervention, the significant change in scores on ADOS at 8 months would have approved introduction of diet for control participants at this earlier time.

Based on results from a reduced participant sample for the second stage of the study, a sustained trend towards improvements in areas of social interactive abilities and stereotyped and repetitive behaviours for the initial experimental group after 24 months on diet was partially observed. Comparisons of the two groups at similar chronological times on intervention did not, however, show a wholly consistent pattern of development coupled to dietary change. Only improvements in scores related to the inattention and hyperactivity sub-domains of ADHD-IV reliably indicated parity as a function of intervention. Variations in scores throughout the study indicate some intra-group differences in the level of positive response to dietary change.

Although sourced from a Danish paediatric cohort, our results show concordance with other medium- and long-term dietary studies conducted in other countries. Several key features have been consistently reported from previous dietary investigations. Amelioration of problems in the core domains of communicative and social functioning represent the most common changes reported, alongside improvements in areas of attention and concentration.¹⁴

Significant changes to ADOS, GARS and ADHD-IV sub-domain scores for the diet group replicate some of these findings. Parallel group changes of reduced inattention and hyperactivity on ADHD-IV following dietary intervention would imply an improvement in the capability to learn. This would have obvious effects for developmental outcome.

ADOS results for the first stage of the study in particular provide compelling evidence for the medium-term efficacy of the intervention as a function of their standardised objectivity. In addition to the statistically significant item, all core areas showed a reduction in scores (improvement) for the diet group between baseline and 8-month testing occasions. This is contrasted with a worsening of group scores for the non-diet group during the same period.

Pooled longitudinal results on ADOS for remaining participants between 8–24 months showed a less dramatic trajectory in terms of improvement for the original diet group although all domains showed improvement from baseline testing. The control group did not show the same pattern of results based on group ADOS scores. The additional analyses undertaken based on the revised ADOS algorithms described by Gotham *et al.*⁴¹ using summed scores of actual clinical outcome did not indicate any significant effect. These findings combined with other instrument data particularly that derived from ADHD-IV could be reflective of a plateau effect for dietary intervention after 8 or 12 months in our study. Other studies have shown clinical improvement after 4 years of dietary intervention.²²

Several features of the present study were intended to overcome limitations of previous research.¹⁶ The sample size represents, to our knowledge, the largest group studied to date using this type of intervention. Power analysis was not done prior to participant recruitment due to limited previous controlled research undertaken in this area. Although not best practice to calculate sample size after investigation, the statistical effects reported in our study would validate the participant numbers included. A larger sample size in a study with the same attrition rate may have provided a clearer picture at 24 months. Stratified randomisation by random allocation based on age and development (VABS) by an external statistician provided suitable sequence generation with little risk of bias. The use of an adaptive (catch-up) design with fixed interval significance thresholds represents, to our knowledge, the first time such a methodology has been applied to dietary intervention research in autism. The strategy was chosen in order to balance various ethical

and scientific considerations that such a demanding intervention entails against an often severely incapacitating condition. In the event that goals were not achieved, control participants would not have to engage in intervention. If a significance level was exceeded, control participants would be assigned to treatment with the prospect of improved outcomes. This type of approach has in previous years become more wide-spread based on its flexibility and responsiveness to real-life scenarios.³¹ The use of more stringent significance thresholds in the first interim analysis at 8 months ($P < 0.01$) reduced the potential for a type-1 error (rejecting the null hypothesis when it is true). The attrition rate reported in the first 12 months of our study was smaller than has been typically reported in autism diet intervention studies.¹⁵ This may be reflective of the extensive nutritional support afforded to participants during this study period. The drop-out rate at 24 months is more pronounced despite equal support provided. The precise reasons for this trend are unclear, although the prolonged study period used may be contributory. Low participant numbers at 24 months may also have affected reported results for the second stage of the study.

Based on previous results from dietary studies, we implemented an extensive assessment battery inclusive of autism-specific and more generalised schedules in order to best explore any potential dietary effects. This included ADOS as a standardised, objective measure of core autism symptoms. The testing intervals we employed were designed to provide data on longer-term outcome and also reflected the need for appropriate intervals between testing sessions to preclude practice effects.

Exclusion of participants diagnosed with epilepsy or seizure-type disorder eliminated any influence of co-morbidity and/or anti-epileptic medication use on results. Other pharmacotherapeutic effects were minimised throughout the study. The long experimental period reduced any extraneous effects associated with wash-out periods or individual temporary episodes of dietary non-compliance described by other investigators.²⁸

Controlling for chronological age of participants was an important feature of our study. According to the International Classification of Diseases 10th revision (ICD-10), a diagnosis of autism should only be received where there is evidence of impairment present before 3 years of age. Studies of actual age at diagnosis, however, have shown a mean age of between 5.3–5.9 years to be more common in clinical practice in Denmark and other developed countries.^{42,43} In this respect, any reduction of the age

limit used during study would have presented problems in recruiting suitably diagnosed children. More recent studies on diagnostic stability have also emphasised the problem of changing symptom severity for some children diagnosed at an early age. Van Daalen *et al.*⁴⁴ found that a proportion of diagnoses made at 23 months in their preschool sample altered by 42 months as a consequence of symptom improvement. For any intervention study such an issue could potentially lead to a type-1 error. The ceiling age limit was designed to capture a paediatric group, who would not traditionally be involved in any specific pharmacotherapy for the management of symptoms, nor were undergoing any changes in behaviour influenced by pubertal onset.⁴⁵ Given the length of time our study continued, it is possible that results for some older participants, particularly towards the latter stages of the investigation, may have been affected by such pubertal changes. The wide age distribution of participants means, however, that such an effect was unlikely to have significantly changed the group outcomes.

Our investigation examined behavioural effects based on the combinatorial use of a gluten- and casein-free diet. Other studies have indicated positive changes following adoption of individual diets with differences in the time-scale of reported change.^{23,24} Outside of our fixed testing occasions, we are unable to provide any exact details as to when dietary intervention began to affect responding participants' behaviour.

A double-blind or placebo element was not carried out due to uncertainties related to reliable measures of total gluten and casein intake for participants, tailoring appropriate individual dosages and the excessive financial burden relating to the extended study period used. Reports detailing clinical regression following the re-introduction of excluded foods^{22–24} add a further ethical dimension⁴⁶ to any fixed cross-over design. Caregiver expectations of dietary effects, as a function of a lack of blinding, may, therefore, have influenced the parental report measures employed. The use of the professional rater-scored ADOS at baseline and other testing occasions did not carry such bias.

All participants on dietary intervention received advice on supplementation to ensure adequate vitamin and mineral intake and minimise the physical risks associated with such restricted diets.⁴⁷ Control participants were not formally supplemented during the first stage of the study given the thorough dietary assessment delivered prior to study commencement. The supplementation regimen for many participants

irrespective of initial group assignment already included essential fatty acids before study enrolment in light of positive behavioural results having been described,⁴⁸ although not replicated for ASD.⁴⁹ Any such effects in our study are unlikely to explain the observed differences between the control and dietary participants.

We did not record parental socio-economic status and are, therefore, unable to rule out any influence on results or attrition rates. Aside from the exclusion of participants with epilepsy, fragile-X syndrome or tuberous sclerosis, we did not undertake any further testing for genetic, chromosomal or other medical diagnoses which may influence results. This includes any potential effects or changes related to the presence of abnormal bowel habits or associated gastrointestinal conditions.

As per our *a priori* assumptions, the majority of participants who ceased intervention before 12 months primarily did so either because children did not want to engage in a dietary change or parents found the diet too difficult to maintain. This relates, in part, to the concept of a cost–benefit ratio, whereby cost (use of diet) was deemed greater than benefit (effect of diet). A per protocol (PP) model of statistical analysis on the basis of using data from surviving participants adhering to the experimental protocol was, therefore, chosen over an intention to treat (ITT) model. The reasons for this reflect a need to assess efficacy of intervention; that is, does diet lead to improved developmental outcome, alongside continuing debate on the suitability of ITT where data on performance and conformity are available to researchers. Whilst data missing completely at random (MCAR) is the preferred scenario, Feinman⁵⁰ illustrates that use of ITT specifically in dietary research can clearly be problematic as a function of the inclusion of non-participating data. Likewise, due to the potential risks associated with unwanted effects,^{51,52} LOCF was also not included as part of the statistical plan. Given the small number of testing occasions employed and the use of a repeated measures model taking all measurements into account, we assume any bias based on non-inclusion of LOCF is minimal.

The method by which dietary intervention affects developmental outcome remains uncertain. Whilst no participant presented with a diagnosis of coeliac disease or lactose intolerance in their medical history, we did not undertake independent screening for these conditions in view of the potential invasiveness of such testing. Likewise, we are unable to rule out any potential effect based on the presence of classical IgE-mediated allergy notwithstanding a general lack of evidence for such a mechanism.^{53,54} The original assertion detailing the

toxicological effect of food-derived opioid peptides either directly or acting peripherally on the immune system⁵⁵ remains a possibility. This theory has added weight following reports on the potential effectiveness of appropriate dosages of the opioid receptor antagonist Naltrexone™ (Revia) for some cases of ASD⁵⁶ and immune-mediated inflammatory conditions.⁵⁷ Urine samples from participants were collected at specific intervals throughout the study. We recognise the importance of comparing urinary results with behavioural assessment data. Results will be reported separately and may help inform discussions on such possible pathogenic mechanisms being involved. Also recognising the fact that the two laboratories involved in the study used slightly different methods of urine analysis, we will address the issue of urine results as a function of each laboratory combined with behavioural and psychometric data in later reports.

Medium- and long-term experimental investigation of any intervention or management strategy for ASD symptoms is fraught with difficulties. Pervasiveness, fluidity and heterogeneity of symptoms combined with a lack of suitable markers outside of observed behaviour are all major methodological hurdles to overcome. The additional debate as to whether it is ethical to implement such a restricted dietary regimen where no life-threatening risk has yet been identified continues. The question is whether, as is incontrovertible in PKU, benefits in quality of life for a proportion of children with ASD may outweigh natural reticence to implement significant dietary restriction. ASD can be a highly disruptive developmental condition with serious life-long ramifications that may, in some cases, even approach levels seen in PKU. This issue also forms part of a wider moral question on how far any intervention should proceed for ASD, where perceived amelioration of ‘disability’ and improved developmental outcome is balanced against existing strengths, abilities and fundamental patient rights.^{58,59}

Conclusions

Introducing a gluten- and casein-free diet had a significant beneficial group effect at 8, 12 and 24 months of intervention on core autistic and related behaviours of prepubescent children diagnosed with ASD and pathological urinary results. The results showed a less dramatic change in group scores between 8–24 months, possibly reflective of a plateau effect during this period. Our results suggest that dietary intervention may positively affect developmental outcome for some children diagnosed with

ASD. Additional investigations are required in order to identify phenotypes based on best- and non-responders to dietary modifications and probe any biological correlates including anthropometric measures.⁶⁰ Due to the complexity and potential for nutritional deficiency as a result of long-term dietary exclusion, appropriate clinical and dietetic support should be utilised during any attempt to make such dietary changes. The lack of life-span data on any long-term health risks associated with such dietary intervention warrant further safety studies.

*Note

The Center for Autisme is a non-profit organisation delivering diagnostic and related services to people with ASD. It is part of the International Molecular Genetic Study of Autism Consortium (IMGSAC). Centre members are accredited users and trainers of various gold-standard diagnostic and assessment instruments.

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Conflict of interest statement

PW and PS undertook commercial analysis of urine samples from people with ASD and related conditions as part of the University of Sunderland Enterprises Ltd whilst involved in the study. AMK and KLR are members of the Norwegian Protein Intolerance Association. KLR is a consultant to TipoGen, a

biotechnology company involved in profiling psychiatric diseases. PS and JJ are parents of children with autism. SP has a sibling with autism. Other authors (DH, ARS, MS, LP) declare they have no conflicts of interest.

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