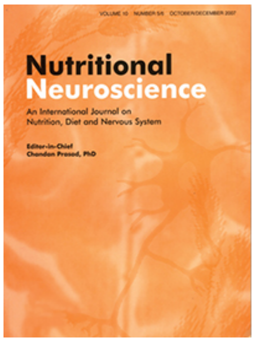

Nutritional Neuroscience

An International Journal on Nutrition, Diet and Nervous System

ISSN: 1028-415X (Print) 1476-8305 (Online) Journal homepage: http://www.tandfonline.com/loi/ynns20

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To cite this article: A.M. Knivsberg, K.L. Reichelt, T. HØien & M. NØdland (2002) A Randomised, Controlled Study of Dietary Intervention in Autistic Syndromes, Nutritional Neuroscience, 5:4, 251-261, DOI: 10.1080/10284150290028945

To link to this article: http://dx.doi.org/10.1080/10284150290028945

Nutritional Neuroscience, 2002 VOL. 5 (4), pp. 251–261

Taylor & Francis healthsciences

A Randomised, Controlled Study of Dietary Intervention in Autistic Syndromes

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(Received 14 January 2002; In final form 27 February 2002)

Impaired social interaction, communication and imaginative skills characterize autistic syndromes. In these syndromes urinary peptide abnormalities, derived from gluten, gliadin, and casein, are reported. They reflect processes with opioid effect. The aim of this single blind study was to evaluate effect of gluten and casein-free diet for children with autistic syndromes and urinary peptide abnormalities. A randomly selected diet and control group with 10 children in each group participated. Observations and tests were done before and after a period of 1 year. The development for the group of children on diet was significantly better than for the controls.

Keywords: Autism; Casein; Gluten; Diet; Peptides

INTRODUCTION

For children with autistic syndromes normal development is hampered by a combination of impaired social interaction, impaired communication and imaginative skills. The children also display a variety of routines and rituals and have a restricted number of interests. In addition they lack normal curiosity and interest in learning. In this single blind controlled study the 20 participants all had autistic syndromes, and they also had urinary peptide abnormalities.

Urinary peptide abnormalities, reflecting insufficient break down of the proteins gluten, gliadin and casein were first reported in autistic syndromes 20 years ago (Reichelt *et al.*, 1981). These abnormalities have been confirmed by further and more recent research (Israngkun *et al.*, 1986; Reichelt *et al.*, 1986; Shattock *et al.*, 1990; Cade *et al.*, 1999; Shanahan *et al.*, 2000). Gluten and gliadin are found in wheat and other common cereals and casein in milk and milk products. The question raised is whether a gluten and casein-free diet may enhance learning for children with autistic syndromes. Previously reported studies indicate that this may be the case (Reichelt *et al.*, 1990; Knivsberg *et al.*, 1990; 1995; 1998; 1999; Rimland, 1988; 2000; Lucarelli *et al.*, 1995; Shattock, 1995; Adams and Conn, 1997; Cade *et al.*, 1999; Whiteley *et al.*, 1999). The participants in our study were fpair wise matched and thereafter randomly selected to a diet group or a control group, the latter a non diet group. To our knowledge this is the first controlled study of its kind lasting 1 year.

When urinary peptide abnormalities in autism were first reported (Reichelt *et al.*, 1981), similarities in the behaviour of children with autism and the effects of opioid drugs in animals and people were also highlighted (Panksepp, 1979). The high level of peptides in the urines indicates that the origin of the peptides is of exogenous nature, i.e. from food containing gluten, gliadin and casein (Shattock and Savery, 1997). The basic hypothesis is that the urinary peptide abnormalities reflect processes that have opioid effect, the rationale for which will be further explained.

Proteins are largely broken down to amino acids and peptides, short chains of amino acids, by enzymes in the intestinal lumen. The amino acids are absorbed from the intestine, but also more effectively peptides and trace amounts of proteins are absorbed (Gardner, 1994). In autism increased

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ISSN 1028-415X print/ISSN 1476-8305 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/10284150290028945

intestinal permeability is reported (D'Eufemia *et al.*, 1996). Increased IgA antibodies to gluten and increased endomycial antibody are found in celiac disease, whereas increased IgA antibodies to gluten and casein without endomycial antibody increase are reported in autistic syndromes (Reichelt *et al.*, 1991; Lucarelli *et al.*, 1995; Cade *et al.*, 1999). This points to increased protein uptake. Peptides from casein are also found in plasma postprandially (Chabance *et al.*, 1998). One of the peptide groups from casein comprises the caseomorphines, which, as the name implies, have opioid-like effects (Brantl and Teschemacher, 1979). Opioids are also formed from gluten and gliadin; these are gluteomorphines (Fukudome and Yoshikawa, 1992) and gliadinmorphines (Graf *et al.*, 1987). Opioid-like peptides are found in autism both in urine, serum, and spinal fluid (Gillberg *et al.*, 1985; Israngkun *et al.*, 1986; Reichelt *et al.*, 1986; LeBoyer *et al.*, 1994; Cade *et al.*, 1999; Shanahan *et al.*, 2000). It has also been demonstrated that opioids from gluten and casein affect the CNS (Lindstrøm *et al.*, 1984; Reichelt and Reichelt, 1997; Cade *et al.*, 1999; Sun *et al.*, 1999), and opioid peptides inhibit CNS maturation (Zagon and McLaughlin, 1987). Casomorphin 1–7 induces FOS antigens in key nuclei of the brain (Sun *et al.*, 1999) pointing to trophic effects of this peptide. Opioids also cross the blood-brain barrier (Banks and Kastin, 1990; Ermisch *et al.*, 1993).

The opioid peptides in question modulate transmitter systems, especially monoamines (Shattock *et al.*, 1990). Unusual effects on transmitter systems may explain a variety of the behavioural traits in autism (Shattock *et al.*, 1990).

We assumed, in our study, that the participating children's attention and social indifference was disrupted due to opioid effects caused by peptides from gluten and casein. We hypothesised that problems with attention and socialising would have affected the social and emotional domain, communication, cognition, and sensory and motor development. On a gluten and casein-free diet the children's autistic traits would decrease, the children would be more attentive, and in a better position to use their abilities, which we hypothesised could be registered in all areas of development.

METHOD

Design

The study, approved by the regional committee for scientific ethics and the data inspectorate, was single blind and randomised. The single blind design was chosen because it has been reported that children with autism that had been on casein-free diet for 8 weeks deteriorated when they were given casein again for 2 weeks (Lucarelli *et al.*, 1995). Participation included only children with both the diagnosis of autism and abnormal urinary peptide patterns.

Information on autistic traits, cognitive level, language, and motor skills was obtained through standardised observations forms (Haracopos and Kelstrup, 1975) and tests (Gjessing *et al.*, 1975; Leiter, 1979; Hagtvet and Lillestølen, 1985; Henderson and Sugden, 1992) before the start of the experiment. Individual reports on each child's function with regard to attention, social and emotional aspects, communicative skills, cognitive skills, motor skills and sensitivity were written.

As autistic traits are differently expressed at different intellectual levels, and they may appear on a scale from very severe to mild, and also change with age, the children were pair wise matched on severity of autistic symptoms as well as age, and cognitive level. They were then randomly selected to a diet or a control group. After an experimental period of 1 year, observations and tests were again carried out, and each child's function and development thoroughly described.

Participants

Mean age for the 10 children randomly selected to use diet was at the start of the project 91 months (range 62–120), and for the 10 children in the control group 86 months (range 59–127). The individual child's age can be read from Tables I and II.

All the participants had autistic syndromes and also abnormal urinary peptide patterns.

Observation Scheme, Tests and Individual Reports

DIPAB (Haracopos and Kelstrup, 1975), a standardised Danish scheme, was utilised for registration of behaviour. The scheme is constructed as a tool to register and evaluate autistic behaviour and individual function of language, motor skills, communication and social contact. It has been standardised on 392 Danish children. The main part of the scheme, used in this study, consists of questions reflecting typical autistic traits. Each trait is explained with several examples, and is graded on a scale from 0 to 4, the latter reflecting the most severe degree of deviant behaviour. One group of traits consists of questions regarding communication and social interaction. The traits registered are verbal communication, non verbal communication, reaction when spoken to, behaviour in learning situations, sharing of emotions, reaction to physical contact, eye contact, and interaction with other children. Behaviour that is strange or difficult for others to understand forms another group of traits. Traits registered are repetitive and peculiar language, echolalia, adult dependency, unusual emotional expressions, unusual fear or anxiety, rigidity,

TABLE I The diet group. Each participant is given a code name/letter. Age before the experimental period, and standardised assessment done before and after the experimental period of 1 year regarding autistic traits (Haracopos and Kelstrup, 1975), non verbal cognitive level (Leiter, 1979), linguistic age (Gjessing *et al.*, 1975; Hagtvet and Lillestølen, 1985), and motor problems (Henderson and Sugden, 1992). One participant did not respond adequately to the cognitive and linguistic tasks. Reduction in autistic traits and motor problems indicate better or more normalised function, while increased scores in cognitive level indicate better use of cognitive skills

Code	Age in months	Number of autistic traits		Non verbal cognitive level		Linguistic age in months		Motor problems	
		Before	After	Before	After	Before	After	Before	After
A	62	15	5	111	123	27	33	37	22
B	67	10	8	–	–	–	–	40	40
C	71	16	9	56	56	24	30	37.5	29
D	78	10	2	100	98	57	75	11.5	5
E	82	10	6	53	62	36	54	37	32.5
F	102	12	5	41	44	39	51	36.5	34
G	107	12	3	116	118	99	120	2	10
H	109	12	5	133	151	88	108	22	23
I	116	13	4	35	43	27	30	38	38
J	120	15	9	84	85	102	98	31	29

peculiar handling of toys, attachment to particular items, and peculiar gait or movements. A combination of the registrations in these two main groups indicates to what degree a child's development is hampered by his or her autistic traits.

With regard to the children's cognitive skills, these were measured with Leiter International Performance Scale (Leiter, 1979). This non verbal intelligence test was originally designed for deaf children, but is a very useful assessment instrument also for children with language disorders, AD/HD, autism, and other developmental disorders. The test is standardised for the mental range from 2 to 18 years. The test material consists of approximately 200 wooden, one-inch cubical blocks, a response frame with slots, and paper stimulus strips. The test uses numbers, perceptual, and abstract reasoning tasks, but no verbal material at all. The tasks are presented on the wooden frame, and demonstrated by the test leader without words, and the child does not have to look directly at the test leader, and does not have to utter a word to answer. He or she only has to move blocks into their appropriate slots to demonstrate their understanding of the concept being measured. According to the Norwegian Psychological Association (2000) this test is a very useful instrument to measure effect of intervention programs.

Linguistic abilities were measured with two different tests in accordance with age and functional level of the participants. ITPA, Illinois Test of Psycholinguistic Abilities, (Gjessing *et al.*, 1975) is standardised for Norwegian children between 4 and 10 years of age. It may also be used with older children as long as their psycholinguistic age does not exceed 10. It consists of sub tests assessing various aspects of the language, both verbal and non verbal ones. It covers receptive processes, associative processes, and expressive processes. The material consists of pictures, orally presented questions or sentences, and toys and bricks to be handled. The second test, Reynells språktest (Hagtvet and Lillestølen, 1985) is standardised for Norwegian children between one-and-a-half and six years. It may also be used for older children with linguistic

TABLE II The control group. Each participant is given a code name/letter. Age before the experimental period and standardised assessment done before and after the experimental period of 1 year regarding autistic traits (Haracopos and Kelstrup, 1975), non verbal cognitive level (Leiter, 1979), linguistic age (Gjessing *et al.*, 1975; Hagtvet and Lillestølen, 1985), and motor problems (Henderson and Sugden, 1992). One participant did not respond adequately to the cognitive and linguistic tasks. Reduction in autistic traits and motor problems indicate better or more normalised function, while increased scores in cognitive level indicate better use of cognitive skills

Code	Age in months	Number of autistic traits		Non verbal cognitive level		Linguistic age in months		Motor problems	
		Before	After	Before	After	Before	After	Before	After
K	59	15	10	88	63	27	33	35.5	26
L	66	10	14	–	–	–	–	40	40
M	70	15	12	44	44	27	42	40	40
N	84	16	14	112	107	61	87	4.5	13.5
O	62	13	16	60	66	24	21	33	32
P	107	8	14	73	53	30	33	32.5	40
Q	101	6	7	127	92	78	81	4.5	10.5
R	100	9	7	144	132	78	79	8	10.5
S	88	16	17	37	35	33	33	32	36
T	127	7	1	76	75	72	92	16.5	29

age in the same age band. It consists of various sub tests covering both impressive and expressive language. The sub tests regarding comprehension consist of toys to be manipulated or pointed on. The sub tests on the expressive language consist of toys, pictures and orally presented words.

Motor abilities were assessed with Movement Assessment Battery for Children (Henderson and Sugden, 1992). The assessment battery consists of a checklist and a test. The checklist is developed for classroom assessment and screening of children that may have movement difficulties. The test, utilised in the project, is a revised and restandardised version of the Test of Motor Impairment (TOMI). It has been standardised in the United States for children between 4 and 12. It is divided into four age groups or age bands with increasingly difficult tasks that compensate for maturation. The movement competence registered with the test is manual dexterity, ball skills, and static and dynamic balance. By adding the registrations in these various areas a child's total impairment score is found.

Procedure

Information on the project was distributed through journals and through the school psychological service. Interested parents were given oral and written information. Participation was based on the parents' voluntary and written consent, and they could withdraw from the project whenever they wanted. Each child had been or was diagnosed by professionals working with child psychiatry/child neurology. The urine samples from the children were blind analysed. Urines were analysed by HPLC using reverse phase c-18 columns. Application volume was urine equivalent to 250 nmol creatine. Column elution was monitored at 215 and 280 nm, and their ratio and elution position used to estimate purity and nature of each peak. Cochromatography with standards with peak augmentation was regularly used. (Further details of this HPLC-method are given in Reichelt and Reichelt, 1997).

Structured interviews regarding autistic traits were done with the parents in their homes. As children with autistic syndromes habituate very slowly to people, settings, and tasks (Bernal and Miller, 1970), formal testing of skills and abilities were done in the school or in the kindergarten the child attended. The testing was carried out in the same room and at the same time of the day that the child usually had special one to one training. The special educator or the child's special assistant was asked to be present during the testing.

Each pair of parents of the children in the diet group was given individual written and oral information on the gluten and casein-free diet by a dietician. All parents had telephone contact on regular basis with special educators familiar with the project.

Tests and interviews were repeated after the experimental period of 1 year. The individual reports on attention, social and emotional aspects, communicative skills, cognitive skills, motor skills and sensitivity, that had been written after the *first* information had been collected, were discussed with each pair of parents. They evaluated original problem items, and if changes had been registered, the parents rated them as minor or remarkable.

The project leader did not know which child belonged to which group until the formal retesting and interviews were done.

Statistics

Non parametric tests were chosen because of the small number of participants and because we could not anticipate the measures to be normally distributed. For the repeated measures within each of the two groups Wilcoxon two-tailed paired test was utilised to evaluate the development. As the children in the two groups might differ from each other, in spite of the fact that they had been pair wised matched, Mann–Whitney *U*-test was chosen to compare the development between the two groups.

RESULTS

Aloofness is a characteristic symptom in the behaviour of many people with autistic syndromes, reflected in the expression "the child in the glass bulb". The child is physically close, but yet impossible to reach. All the participants showed this trait prior to the experimental period, which can be seen in Fig. 1.

Changes were observed in both groups of children, the most remarkable ones for the children on diet. The first two bars in the each of the two diagrams in the figure also illustrate that although both small and more remarkable changes were observed after the experimental period, aloofness as a problem persisted for some of the children, more in the control than the diet group. Distraction was also a problem for some, a problem that continued in both groups, for the dieting children to a smaller degree. Rapid adjustment to new situations is difficult for children with autistic syndromes, and many of them display a variety of routines and rituals, so also most of the children in this study. The reduction of these traits was clearly more marked for the children on diet than the controls. Resistance to learning, from pushing the teacher or the material away, closing the eyes and covering the ears and turning away from the teacher, or plain indifference was also more reduced in the diet than the control group. For the children in the diet

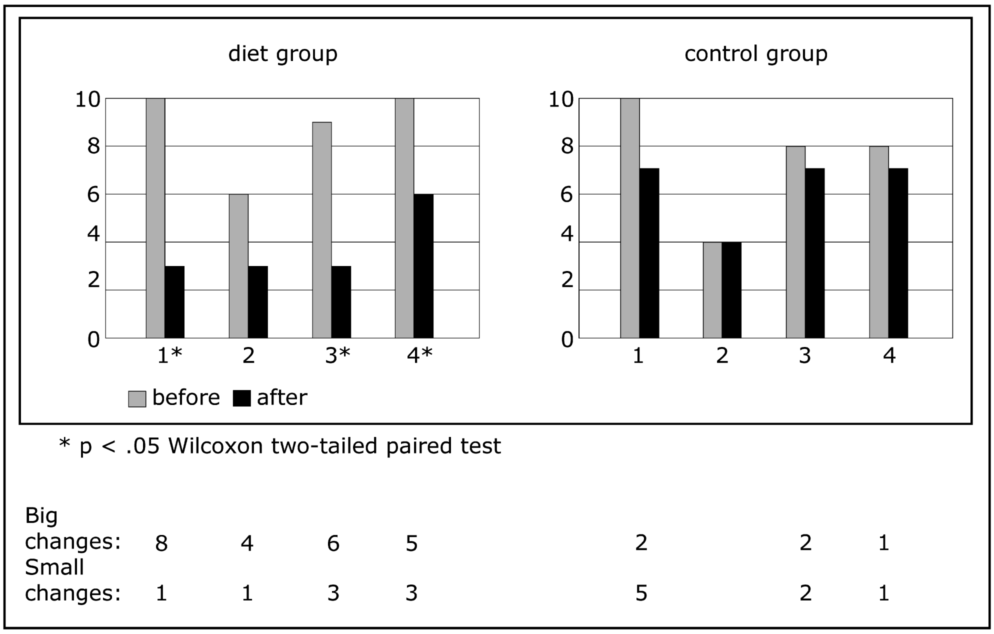

FIGURE 1 Attention. Number of children in the diet and the control group observed to have attention problems before (grey) and after (black) the experimental period of 1 year. The numbers on the horizontal line refer to whether the child at times could be aloof (1), was easily distracted (2), needed routines and rituals (3), and how the child responded to teaching (4). Significant change for each trait is marked as *. The total number of children, in the two groups, showing the specific traits of attention problems before and after the experimental period has been compared, using the non parametric, two-tailed Mann–Whitney *U*-test, $p < 0.028$. Underneath the diagrams is information on the observed changes, whether they were remarkable or small.

group significant changes were found with regard to aloofness ($p < 0.008$), routines and rituals ($p < 0.014$), and responses to learning ($p < 0.046$). For the children in the control group no significant changes were found.

The area in which the development within and between the two groups was most markedly different was the social and emotional one, shown in Fig. 2.

All the participants initially shared a most common trait in autistic syndromes, lack of peer relationships. Some ignored other children, while others tried to make contact, but did not know how to interact. Some also had abnormal temper tantrums. Paradoxical and strange emotional expressions were displayed by some, like laughing when other people cried, abnormal fear when watching an insect, or extreme joy caused by flushing toilets or watching a moving rocking chair. Extreme anxiety could in some of the children be caused by the sound of the school bell, noises from a lawn mover, sleeping in a new bed, or entering a room with many people. These unusual emotions were drastically reduced in the diet group, but not in the control group. Inability to take other people's perspective and lack of empathy are also common traits in autistic syndromes. Some of the children could suddenly hit or bite others, or they could make negative comments on people's appearance or classmates' schoolwork. Progress was made regarding development of empathy in the diet, but not in the control group. Some children also disliked and rejected physical contact even from their parents. This was no longer a problem in the diet group after the experimental period was over. While none of the changes were significant in the control group, significant changes were registered in the diet group regarding peer relationship ($p < 0.008$), anxiety ($p < 0.025$), empathy ($p < 0.025$), and physical contact ($p < 0.046$).

With regard to communication the development in the diet group was also more positive than in the control group, which is displayed in Fig. 3.

Children with autistic syndromes have few facial expressions. They often avoid eye contact, or they may stare too hard or too long for what most people find comfortable. They do not communicate with their eyes. Quite often they do not respond when spoken to in an ordinary way. These traits were found in the participating children. Language peculiarities, like echolalia, high pitch, and stilted language were also registered. So were peculiar interests, like timetables or attachment to strings or hard plastic toys. In this area the changes in the control group were relatively small, and as for the other areas, not significant. In the diet group the changes were significant for non verbal communication ($p < 0.046$), eye contact ($p < 0.046$), reaction when spoken to ($p < 0.005$), and language peculiarities ($p < 0.046$).

Small, but insignificant changes were also registered in the control group regarding use of cognition, displayed in Fig. 4.

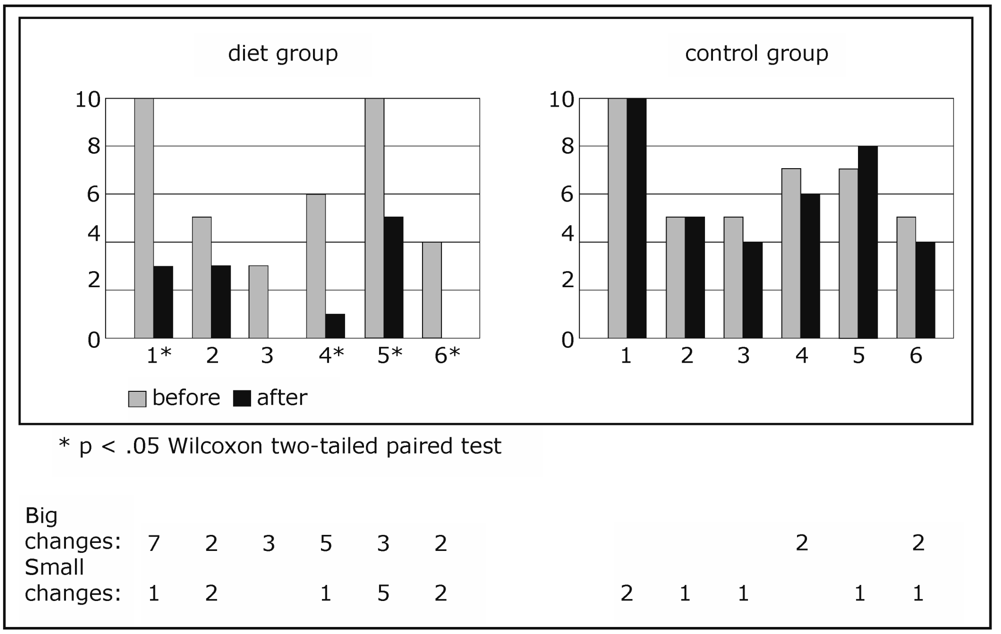

FIGURE 2 Social and emotional factors. Number of children in the diet and the control group observed to have social and emotional problems before (grey) and after (black) the experimental period of 1 year. The numbers on the horizontal line refer to whether the child had problems with peer relationships (1), had temper tantrums (2), had paradoxical emotional expressions (3), showed anxiety (4), expressed empathy (5), and rejected physical contact (6). Significant change for each trait is marked as *. The total number of children, in the two groups, showing the specific traits of social and emotional problems before and after the experimental period has been compared, using the non parametric, two-tailed Mann–Whitney *U*-test, $p < 0.004$. Underneath the diagrams is information on the observed changes, whether they were remarkable or small.

Both groups of children initially found it difficult to understand the pattern of action and reaction. It was difficult for them to evaluate danger and realise, for example that they might fall into the water from the edge of a quay, that an approaching car might hurt them, or that jumping of the roof might have negative consequences. They also had little imaginative play. Toy cars were lined up in the same

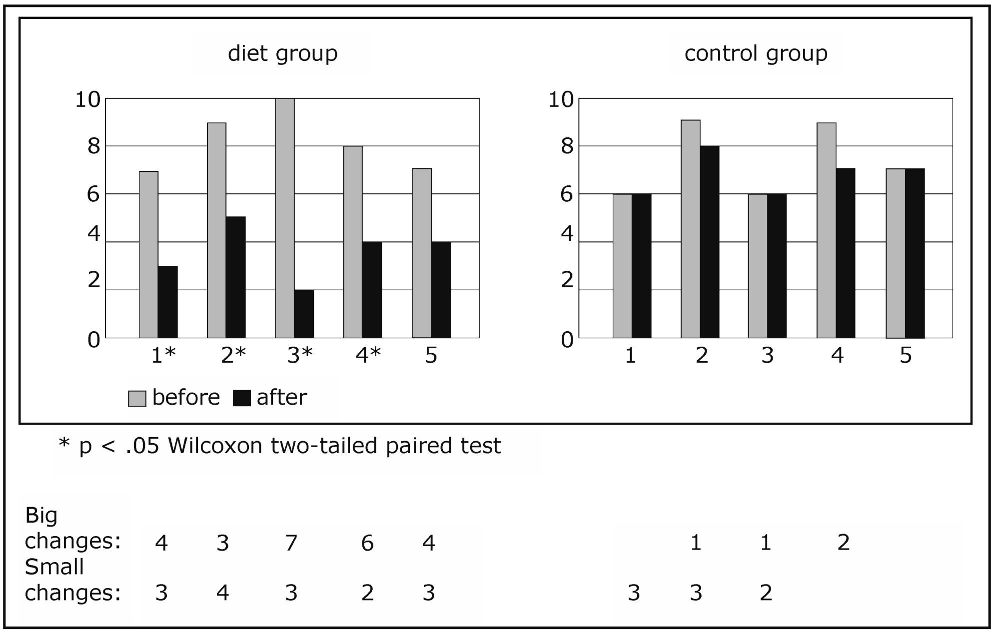

FIGURE 3 Communicative factors. Number of children in the diet and the control group observed to have communicative problems before (grey) and after (black) the experimental period of 1 year. The numbers on the horizontal line refer to whether the child had problems with non verbal communication (1), had abnormal eye contact (2), reacted when spoken to (3), had language peculiarities (4), had peculiar interests (5). Significant change for each trait is marked as *. The total number of children, in the two groups, showing the specific traits of communicate problems before and after the experimental period has been compared, using the non parametric, two-tailed Mann–Whitney *U*-test, $p < 0.007$. Underneath the diagrams is information on the observed changes, whether they were remarkable or small.

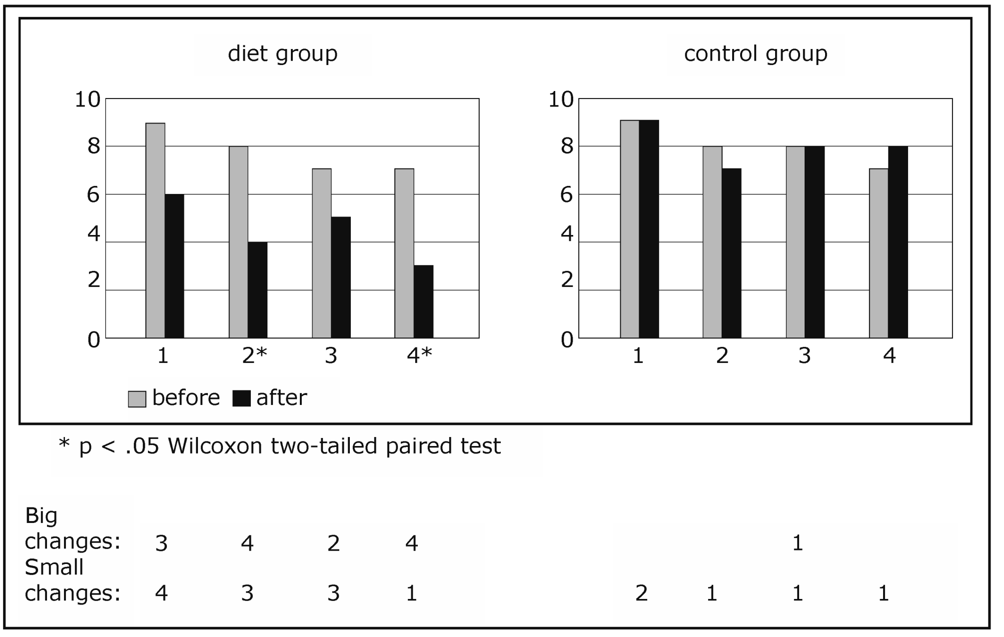

FIGURE 4 Cognitive factors. Number of children in the diet and the control group observed to have cognitive problems before (grey) and after (black) the experimental period of 1 year. The numbers on the horizontal line refer to whether the child had problems with causal relations (1), could judge dangerous situations (2), would express imagination (3), had a restricted number of interests (4). Significant change for each trait is marked as *. The total number of children, in the two groups, showing the specific traits of cognitive problems before and after the experimental period has been compared, using the non parametric, two-tailed Mann–Whitney *U*-test, $p < 0.019$. Underneath the diagrams is information on the observed changes, whether they were remarkable or small.

pattern every time they played. Dolls could be dressed and undressed, mechanically. They found it difficult to understand stories or books describing social relations, but enjoyed books with plain facts. Their number of interests was limited. Also in this area the development for the children on diet was more positive than for the controls with significant changes regarding judgement of dangerous situations ($p < 0.046$) and regarding number of interests ($p < 0.046$).

Motor problems and extraordinary sensitivity are also common in autistic disorders. Many of the children are clumsy, and have poor motor control. They seem to tolerate more pain, heat or cold than other people do, but are also more sensitive than others to sounds, lights, colours, and touches. They may find shadows fascinating, particular colours overwhelming, and the fabric of new clothes painful. While they may experience a mild stroke or hug as extremely uncomfortable, they may on the other hand enjoy rough play and tumbling. Furthermore strange movements like tiptoe walking, rocking of the body, and flapping of arms are common.

All these various traits were registered amongst the children. Reduced abnormalities were registered for the children on diet after the experimental period, shown in Fig. 5, significant with regard to extraordinary restlessness or passiveness in the diet group ($p < 0.046$). No significant changes were found in the control group.

The development in the five areas described and illustrated in the Figs. 1–5, was also compared between the two groups, using the two tailed, non parametric Mann–Whitney *U*-test. The total number of children with these traits in each of the groups, before and after the experimental period was compared, and significance of differences was registered in the first four of the five areas (attention $p < 0.028$, social and emotional factors $p < 0.004$; communicative factors $p < 0.007$; cognitive factors $p < 0.019$, sensory/motor factors $p < 0.083$).

Results of observation of behaviour and testing of skills before and after the experimental period are given in Tables I and II. The development *within* the two groups and *between* the two groups is given in Table III.

Regarding autistic behaviour (Haracopos and Kelstrup, 1975) the total impairment score for each of the participants, before and after the experimental period, is given in Tables I and II. The children in the diet group were slightly more impaired than the children in the control group before the experiment started with a mean of 12.5 vs. 11.5. All the children in the diet group had a reduction of autistic behaviour, some more than others. In the control group a reduction of autistic behaviour was registered for five, but an increase in the other five. As can be seen from Table III the reduction of autistic behaviour was significant in the diet group, but not in the control group. A significant difference was also

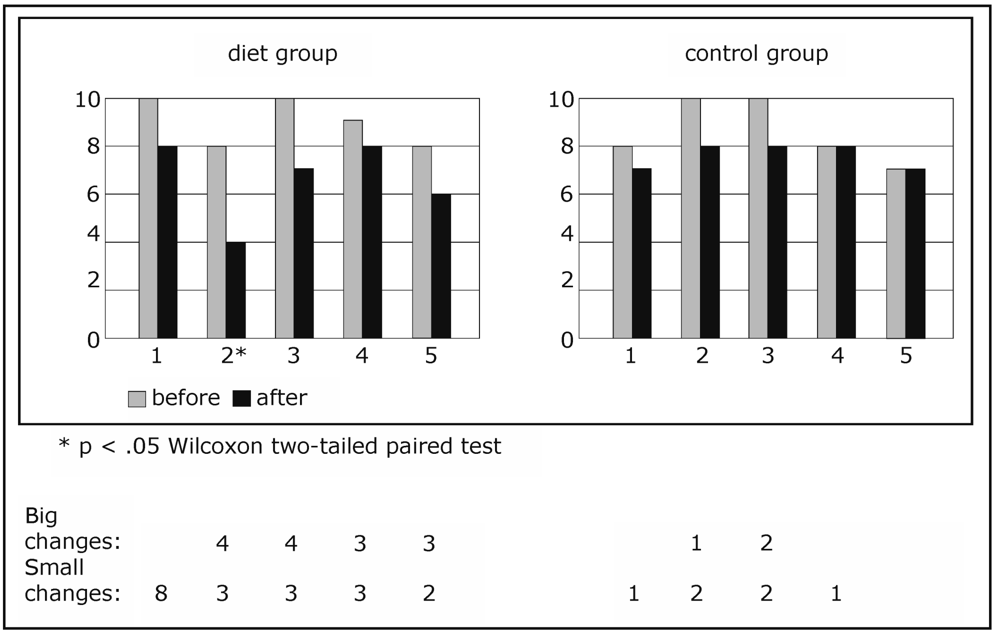

FIGURE 5 Sensory/motor factors. Number of children in the diet and the control group observed to have sensory/motor problems before (grey) and after (black) the experimental period of 1 year. The numbers on the horizontal line refer to whether the child was extremely sensitive (1), was extraordinary restless or passive (2), had strange movements (3), had problems with gross motor skills (4), had problems with fine motor skills (5). Significant change for each trait is marked as *. The total number of children, in the two groups, showing the specific traits of sensory and motor problems before and after the experimental period has been compared, using the non parametric, two-tailed Mann–Whitney *U*-test, $p < 0.083$. Underneath the diagrams is information on the observed changes, whether they were small or remarkable.

found between the two groups with regard to autistic behaviour before and after the experimental period.

The test results of the non verbal cognitive testing (Leiter, 1979) are also given in the tables. Significant differences were found for both groups of children, but while the children in the diet group had an increased mean of nearly seven points, the children in the control group had a decreased mean of 10 points. The variations within the groups were more marked in the control than in the diet group, which can be seen in Tables I and II.

With regard to the development of the linguistic skills as registered with the tests standardised for Norwegian children (Gjessing *et al.*, 1975; Hagtvet and Lillestølen, 1985) the linguistic age in the diet group had a mean increase of 11 months and the control group a mean increase of 8 months. These changes were for both groups significant. When comparing the development between the two groups, no significant difference was found as can be seen from Table III.

The motor competence in the two groups was also tested (Henderson and Sugden, 1992), and details are, as for the other tests, given in the tables. It should be noted that on this test, a decrease in total score indicate an improvement, the child demonstrates fewer motor problems. The children on diet showed a slight improvement with a mean decrease of 3 points, while the control group had a little more problems after 1 year. The mean had an increase of 3 points. While none of the changes within the groups were significant, the development between the two groups was.

DISCUSSION

The results from the observations and tests illustrate that the children on diet prospered more than the children in the control group, supporting our hypothesis that when a child's attention is disrupted it negatively affects all developmental areas. The autistic traits diminished, which we also hypothesised.

Support for the rationale behind the intervention, opioid effect on the transmitter system are further strengthened by the fact that arousal, anxiety, affect, learning, memory, thermoregulation, motor control, pain modulation, and aggression may all be connected to abnormal serotonergic function (Wilkinson and Dourish, 1991), and abnormal levels of serotonin in whole blood, platelets and serum are reported in autism (Gillberg and Coleman, 2000). A peptide that stimulates serotonin uptake has also recently been found (Reichelt *et al.*, 1994; Pedersen *et al.*, 1999). Furthermore dopamine influences motor control as well as attention and hyperactivity, while noradrenaline is linked to functions like sleep, arousal, emotions, and selective attention (Ciaranello *et al.*, 1995), and the opioids found increase

TABLE III The development during the experimental period of 1 year within the groups and between the groups with regard to autistic behaviour (Haracopos and Kelstrup, 1975), non verbal cognitive level (Leiter, 1979), linguistic age (Gjessing *et al.*, 1975; Hagtvet and Lillestølen, 1985), and motor problems (Henderson and Sugden, 1992)

	Diet group			Control group			Diet and control group
	Mean before	Mean after		Mean before	Mean after	Significance of difference*	Significance of differences†
Autistic traits ($n = 10$)	12.5 (SD 2.2)	5.6 (SD 2.4)	0.005	11.5 (SD 3.9)	11.2 (SD 5.0)	0.798	0.001
Non verbal cognitive level ($n = 9$)	81.0 (SD 35.9)	86.7 (SD 38.5)	0.03	84.6 (SD 36.6)	74.3 (SD 31.4)	0.5	0.004
Linguistic age ($n = 9$)	55.4 (SD 32.3)	66.6 (SD 35.1)	0.015	47.8 (SD 23.9)	55.7 (SD 28.3)	0.035	0.375
Motor problems ($n = 10$)	29.3 (SD 13.1)	26.3 (SD 11.5)	0.161	24.7 (SD 14.6)	27.8 (SD 12.2)	0.123	0.040

*Non parametric two-tailed Wilcoxon test. †Non parametric two-tailed Mann–Whitney *U*.

dopamine activity (Hole *et al.*, 1979). Opioids are also linked to responses to stress, affective processes and reward mechanisms (Ciaranello *et al.*, 1995).

With regard to design it might be argued that a double blind, cross over study might have been ideal. With all children on diet, gluten and casein could have been administered, for example in capsules during specific altering periods. Parents and caretakers would then have been blind to who was on diet and who was on ordinary nutrition. From an ethical viewpoint we found this approach difficult as regression is reported in autism on reintroduction of avoided proteins (Lucarelli *et al.*, 1995), and altered EEG abnormalities are found nearly a year after gluten provocation in children with celiac disease (Paul *et al.*, 1985; Kozlowska, 1991). The approach we decided on, a single blind study, has previously been successfully used in dietary intervention over a year in rheumatoid arthritis (Kjeldsen-Kragh *et al.*, 1991).

A mixed factorial experiment, like this study, comparing repeated measures within groups and between groups, is common. With a larger number of participants or a normal distribution of the measures, analyses of covariance might have been the preferred statistical method. For our study, with the small number of participants, we consider the non parametric tests, often regarded as more conservative and strict, to be the more warranted.

Regarding number of participants in a study of this kind a larger number of participants might have strengthened the results. On the other hand significant changes in small groups is a strong indication of the effect of dietary intervention. We did not divide the groups according to gender as specific information related to gender rarely is reported in autistic syndromes.

According to Gillberg (1995) most mothers are very good informants on their children's normal and abnormal behaviour and development. We chose to collect information both from observations done by the parents and from testing. The materials gave us good descriptions of each child and of the two groups. It should be noted, though, that testing of children with autistic syndromes is difficult. The test results therefore have to be interpreted with care. They reflect what the child managed to do at a specific time at a specific day. A cold, a bad night's sleep and a whole lot of other uncontrollable influences may affect test results. As children with autistic syndromes habituate slowly (Bernal and Miller, 1970), we tried to avoid difficult situations for the children. They were tested at the time of the day that they would normally have a one-to-one training session. The testing was carried out in the same room this session normally took place, and the child's teacher or assistant was present. This also gave us the opportunity to have the teacher's or assistant's opinion on the results, and they confirmed that the results reflected what the child would normally be expected to manage.

Both groups of parents had telephone contact on regular basis with special educators familiar with the project during the experimental period. The parents of the children on diet in addition had contact with a dietician. It might be argued that the expectations of a positive effect of diet have influenced the daily contact and communication with the children, that placebo effect can explain the results in the diet group. We consider it unlikely that such an effect might have lasted for a whole year. In addition the results corresponds with what has previously been reported from shorter and longer studies (Reichelt *et al.*, 1990; Knivsberg *et al.*, 1990; 1995; 1998; 1999; Lucarelli *et al.*, 1995; Shattock, 1995; Adams and Conn, 1997; Rimland, 1988; 2000; Cade *et al.*, 1999; Whiteley *et al.*, 1999).

The children on diet all had a positive development, some more than others. They had fewer autistic traits after 1 year, which we consider to be a direct result of the dietary intervention. The development in the control group was also to be expected, as it is reported that the autistic behaviour normally fluctuate (Shattock and Savery, 1997), good periods are followed by periods with more abnormal behaviour and vice versa. One child in the control group was a positive exception. According to his parents the child's autistic traits had disappeared during the experimental period. Full recovery from autism is uncommon.

We assume that the increased mean in the diet group on non verbal cognitive assessment is a result of the children's increased attention. They were probable in a better position to use their abilities as a result of decreased opioid activity and influence. In the control group we had expected to find nearly the same mean result before and after the experimental period, and have no explanation for the decrease registered. As far as we know, this has not previously been reported for children with autistic syndromes.

Regarding the linguistic assessment it is an unfortunate fact that only two linguistic tests are standardized for Norwegian children, one for the age group 1.5–6 (Hagtvet and Lillestølen, 1985), and the other for the age group 4–10 (Gjessing *et al.*, 1975). The latter can be used with older children as long as the psycholinguistic age does not exceed 10 years. The test results from the two tests cannot be directly compared, we therefore decided to use the linguistic age that can be deducted from the tests results. For both groups of children there was a positive development, which might have been expected as language and communication training is an essential part of remedial programs for children with autistic syndromes. The development in the diet group was more significant than in the control group, and we can only speculate if a significant difference between the groups would have emerged if the groups had been followed for a couple of more years.

Strange motor behaviour or motor impairments are common in autistic syndromes. The test results indicate severe motor impairments in many of the participants, this may however additionally reflect that it is difficult for the children too imitate motor behaviour (Heimann *et al.*, 1995). The improvement in the diet group may consequently reflect a combination of improved skills and improved ability to imitate, to learn. The slight decrease in the control group cannot logically be explained, but undoubtedly the development between the two groups was significantly different.

When evaluating the results, we find the development in the children on diet promising. Replications are, however, needed, and hopefully future research may bring more detailed and refined answers to how much can be gained by dietary intervention by whom, whether one subgroup of children with autistic syndromes may for example, respond better than another.

Acknowledgements

We want to express our gratitude to the parents of the participating children, and our gratitude for financial funding from The county council of Rogaland, Sigval Bergesen d.y. og hustru Nanki's almennyttige stiftelse, and Seim Family Foundation.

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