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DES ALIMENTS

**FRENCH FOOD
SAFETY AGENCY**

Efficacy and safety of gluten-free and casein-free diets proposed in children presenting with pervasive developmental disorders (autism and related syndromes)

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Chairmanship of the working group

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Five associations of patients with pervasive developmental disorders have been invited to participating in an exchange with the working group on 13 June 2006:

- Autism France;
- ARAPI (Association pour la recherche sur l'autisme et la prévention des inadaptations);
- Pro Aid Autism;
- SATEDI (Spectre autistique - troubles envahissants du développement - international);
- Fédération Sésame Autisme;
- UNAPEI (Union nationale des associations de parents et amis des personnes handicapées mentales);

LIST OF ABBREVIATIONS

ACE: angiotensin converting enzymes
ADI-R: autism diagnostic interview - R
ADOS: Autism diagnostic observation scale
AM: alternative management
ASD: autism spectrum disorders
BSE: behavioural summarized evaluation
CARS: children autistic rating scale
CIBD: chronic inflammatory bowel disease
CMC: circulating mononuclear cells
DIPAB: diagnosis of psychotic behaviour in children
DSM: diagnostic and statistical manual of mental disorders
EAB: evaluation of autistic behaviour
ECO: ecological communication orientation language sampling summary
GI: gastro-intestinal
HPLC: high performance liquid chromatography
IBSE: infant behavioural summarized evaluation
ICD: international classification of diseases
IDP: intolerance to dietary proteins
LC-MSMS: liquid chromatography – mass spectrometry in combination
LPS: bacterial lipopolysaccharide
MALDI-TOF: matrix-assisted laser desorption / ionisation of flight mass spectrometry
MNS: mirror neuron system
MRIf: functional magnetic resonance imaging
NLH: nodular lymphatic hyperplasia
PASS: parental satisfaction scales
PDD: pervasive developmental disorders
PET: positon emission tomography
RDA: Recommended Daily Allowances
TOMI: test of motor impairment

1 INTRODUCTION

1.1 Context of request

Afssa has received a request for a mission of evaluation of the efficacy and safety of gluten-free and casein-free diets offered to children presenting with pervasive developmental disorders (PDD or autism and related disorders), following a request from paediatricians in the French Group of Gastro-enterology and Paediatric Nutrition who have reported a high increase in the number of families with autistic children planning to use this diet.

1.2 Autism: definition, origin, practical implications

1.2.1 Definition of autism and related disorders

Childhood autism is a syndrome affecting the psychological and cognitive development of children, whose prevalence is estimated at 2/1,000 and for which the sex-ratio is 3 to 4 boys for one girl (Baghdadli, 2005).
It occurs at a very early age, with signs developing before two years of age in nearly 80% of cases (Rogers & DiLalla, 1990). Autism results in rapid and insidious disorganisation of methods of communication and interactions between the child and his family.

It is characterised by:
- an alteration of reciprocal social interactions;
- and alteration of verbal and non-verbal communication;
- a restricted, repetitive, stereotypical characteristic of behaviour and interests.

These abnormalities comprise the basis of the diagnosis. Therefore, they exist when the diagnosis is established but to variable degrees depending on the severity of symptoms. This triad is mentioned in the main international classification of disease *international classification of diseases 10* (ICD-10, World Health Organisation, http://www.who.int) or *diagnostic and statistical manual of mental disorders IV* (DSM-IV, American Psychiatric Association, http://www.psych.org).
Furthermore, abnormalities in cognitive and sensory function, language, motor function and adaptive capacity or epilepsy can be associated with it. The clinical polymorphism of autism is such that one no longer refers to autism but to autisms (Geschwind & Levitt, 2007). Childhood autism is classified with other related clinical entities (Asperger syndrome, atypical autism), under the heading “pervasive developmental disorders” (PDD).

For several years, the term “autistic spectrum” (autism spectrum disorders, ASD) has been used to describe all disorders related to autism depending on the continuous severity scale. This name has not yet been incorporated in the system of classification of diseases.

1.2.2 Origins of autism

The origin of autism remains unknown. Initially, psychodynamic theories put forth the environment, in particular, familial, as the exclusive cause of the disease. Research conducted over the last two decades in the fields of neurobiology and genetics have gradually led to the emergence of a more organic concept by revealing the existence of abnormalities in brain development (Polleux & Lauder, 2004; Baron-Cohen & Belmonte, 2005).

1.1.2.1 Neurobiological studies

Neurobiology seeks to correlate behavioural anomalies and neuronal dysfunction. For example, functional imaging (functional magnetic resonance imaging or MRIf; positon emission tomography-PET) and electro physiology provide access to real time analysis of the

functioning of precise areas in the central nervous system in response to specific tasks. These techniques have demonstrated the defective functioning of a specific neuronal system involved in complex cognitive processes. When a person observes a movement of the human body, the area of his/her own brain corresponding to visualised movement is activated. In a certain manner, the observer's brain executes a movement similar to the one observed. This cerebral "motor imitation" subsequent to perception of movement is the result of mirror neurons. It seems that the human mirror neuron system (MNS) is involved not only in the observation and execution of movement, but also in very elaborate processes such as learning by imitation and the development of social relationships, in particular through understanding of the other person's intentions and emotions.
The comparison of children with autism to children with normal development has demonstrated a marked reduction in the activity of MNS during observation or imitation of facial expressions in the former. MNS activity (measured by functional MRI) correlated with the severity of disorders: MNS activity is that much weaker when manifestations of autism are more severe (Iacoboni & Dapretto, 2006).

The analysis of visual perception of human movement (carried out by quantified electroencephalogram) also indicates that the "motor contagion" process is altered in autistic children. Motor brain imitation subsequent to perception of movement exists in normal children, but is much reduced or absent in children with autism. Functional alterations of MNS may contribute to disorders of communication and social relations (Iacoboni & Dapreto, 2006).
This example is not isolated: data have been accumulating in support of the existence of neurophysiologic anomalies affecting different areas of the brain, among which lateralisation of language (Kleinhans et al., 2008), image processing in the visual areas (Vandenbroucke et al., 2008) and anatomical and functional anomalies involving social tasks in the superior temporal sulcus (Zilbovicius et al., 2006).

1.1.2.2 Genetic studies

The association of cytogenetic anomalies or monogenic disorders with some autistic syndromes has long attracted attention to the possible intervention of genetic factors.
Studies of autistic twins have demonstrated a very high agreement (60 to 92%) between identical twins, while it is only 0 to 10% between same sex fraternal twins (Bailey et al., 1995). Heritability calculated based on these results is greater than 90%. Therefore, autism is the childhood psychiatric disease which carries the highest heritability, a result which makes it possible to believe that, in contrast, the environment may play a relatively limited role in its initial phenotypic expression (Veenstra-VanderWeele & Cook, 2004; Freitag, 2007). Studies of twins also suggest that disorders of communication and social interaction on one hand, and repetitive and stereotypical behaviour on the other hand, depend on different genetic systems.
Studies of families indicate a high incidence of recurrence (about 5%). As in studies of twins, the studies of families suggest that disorders of communication and social interaction on one hand, and repetitive behaviours on the other hand, correspond to different genetic influences. In spite of high heritability, different strategies of molecular genetics have identified only a small number of candidate genes, potentially involved in the occurrence of childhood autism (Szatmari et al., 2007). These results provide a preliminary model involving a limited number of genes whose different variants may interact in defining the phenotypic expression of the disease (Abrahams & Geschwind, 2008).

Therefore, increasing, concurring scientific data indicate the existence of complex and early anomalies in the development of basic neurophysiologic functions in autism. It also appears that genetic factors may play a very important part in the aetiopathogenesis of this disorder.

1.2.3 Practical implications

The diagnosis of autism is a clinical diagnosis, based on observation of behaviour (ICD-10; DSM-IV).

There is no specific biological or genetic marker (Baghdadli, 2005).

The diversity of developmental disorders, the variability of their combinations, severity and age of onset underline the wide heterogeneity of the population of children grouped under the generic term autism. Yet, research on the mechanisms of the disease can advance only by investigating homogeneous groups of subjects whose phenotype is perfectly characterised.

The same requirement prevails for the management of autism (see 1.3). In the absence of a defined aetiology, additionally, the term "treatment" should be used with caution: the work of health care professionals consists of aiding those with autism to better adjust to their environment, to communicate and to interact with others.

In all cases, two prerequisites must be met to judge the efficacy of intervention in autism:

- reducing, insofar as possible, inter-individual variance, therefore, basing one's findings on groups whose characteristics are homogeneous;
- choosing tools whose sensitivity is appropriate to detection of the effect sought.

Detailed methodological recommendations have been formulated on this subject by a group of experts, including the evaluation of quality of life and the cost-benefit ratio (Charman et al., 2003).

1.3 Tools for diagnosis and evaluation of the course of autistic children

Different methods exist (rating scales, behavioural inventories) to identify or to assess the course of pervasive developmental disorders (PDD), but it is necessary to differentiate two principle types of instruments: diagnostic tools and instruments to measure behavioural traits (rating scales, measuring specific and non-specific signs of the autistic syndrome).

1.3.1 Diagnostic instruments

1.2.1.1 Autism Diagnostic Interview - R (ADI - R) (Lord et al, 1994)

This standardised diagnostic instrument allows the diagnosis of autism to be established based on diagnostic criteria of the ICD-10 and DSM-IV. The diagnostic interview is conducted with the child's parents and allows the child's capacities and behaviour to be evaluated. It investigates the three principle dimensions of the autistic syndrome (social disorders, disorders of communication and stereotypical behaviour). Each dimension is scored as subscores using specific items in the dimension investigator. The diagnosis is established when the score obtained in each dimension meets the threshold value.

1.2.1.2 ADOS (autism diagnostic observational schedule, Lord et al., 2000)

This is an observation scale for the diagnosis of autism under codified conditions. It does not involve evaluation of cognitive capacities, but rather it places the person in a social situation in which he has to interact. The activities proposed make it possible to evaluate communication, reciprocity of social interactions, play and/or imaginative use of a material, stereotypical behaviour, restricted interest and other abnormal behaviours according to age (Annexe I).

The ADOS makes it possible to establish the diagnosis of autistic spectrum disorders with reference to DSM-IV and to ICD-10 with a threshold. As with ADI-R, it is necessary to

acquire specific training and to obtain validation prior to the use of ADOS. The ADI-R and ADOS currently comprise the diagnostic instruments of reference.

1.2.1.3 Children Autistic Rating Scale (CARS) (Schopler et al., 1980)

CARS is an autistic symptom evaluation scale based on observation of the child in his usual environment. It contains 15 items scored 1 to 4, with possible intermediate scoring. Based on the score obtained, it is possible to classify the child according to severity of symptoms.

ADI-R, ADOS and CARS are perfectly validated for the diagnosis of autism, but are not sufficiently sensitive to reveal a variation in a therapeutic trial. It appears preferable to use scales which measure behavioural traits to be able to demonstrate a precise change in the setting of a controlled study.

1.3.2 Scales measuring behavioural traits

Two behavioural scales principally are used in interventional evaluation in autism:

1.2.2.1 BSE - Behavioural Summarized Evaluation scale

This purpose of this scale is to evaluate symptoms of children with PDD (Lelord & Barthélémy, 1989; Barthélémy et al., 1997).
It contains 19 items listed in a table, grouping the principal signs of autism defined with the DSM III (Annexe I).

This scale has been adapted to use in very young children (0-3 years): BSE-N (N = Infants).

Use of a quantitative scale makes it possible to evaluate the severity of the syndrome globally and in particular to define the severity of each behavioural item. The course and outcome of scoring of each item under the influence of given intervention makes it possible to measure the impact of the latter on the child's behavioural profile. Currently, the BSE is considered as the instrument of choice in therapeutic trials.
The *Behavioral Summarized Evaluation* (BSE; Barthelemy et al., 1992) is the English version of the BSE. The revised version of the BSE (BSE-R) and the *Infant Behavioral Summarized Evaluation* (IBSE) have also been validated as diagnostic instruments for autism.

1.2.2.2 ABC scale (*Aberrant Behavior Checklist*, Aman et al., 1985)

This tool, which is not specific for autism, evaluates behavioural disorders. It is more often administered to the parents. Its advantage is based on the limited number of items whose grouping into 5 factors has been validated. It is adapted to a population with medium to severe mental retardation (such retardation exists in 75% of autistic subjects).
The scale is based on 58 items whose scoring ranges from 0 to 3: 0 (no problem) > 3 (very serious problem) (Annex I).

The examiner also takes into account the frequency with which the behaviour occurs. This scale is also a reference scale in the measurement of behavioural disorders, in particular in the conduct of therapeutic trials.

1.3.3 Conclusions

No consensus exists on the optimum combination of such scales to evaluate the response to an intervention. The wide variety of symptoms in the autistic spectrum can make it necessary to use several tools. Conversely, it may be wise to choose a target behaviour whose quantitative and qualitative measurement would allow assessment of efficacy of the therapeutic trial.

In all cases, the evaluation of response to a given treatment strategy involves the use of standardised measurement tools and ideally it should be carried out by health care professionals who are specially trained and who are not involved in the child's management.

1.4 Conventional and alternative management of autism and view point of the families of patients

1.4.1 Management of autism

Education or rehabilitation of autistic children - using methods specifically adapted to them - and the training of instructors and parents are very generally considered as the principal if not the only effective means of intervention (Lord & McGee, 2001; Francis, 2005; Myers et al., 2007). A large number of data indicate that appreciable benefits can be obtained by using techniques appropriate to the child's needs, ideally according to an individualised programme (Lord & McGee, 2001).
In spite of appreciable differences in their concepts, their strategy and their implementation, the majority of programs of management (behavioural intervention, re-education in communication, etc.) recognize some basic principles, such as the need for intervention as early as possible and also as intensive as possible, the importance and the involvement of the child's parents and the need to adjust management depending on the precise and regular evaluation of its results. However, the outcome remains highly variable from one subject to another and there is no obvious relationship between the type of progress achieved and the use of a given technique (Lord & McGee, 2001; Levy & Hyman, 2005; Myers, 2007).

An additional psychopharmacological intervention may be necessary in light of the severity of some behavioural disorders (self-mutilation, aggressiveness…).

1.4.2 Alternative management

In spite of these efforts, childhood autism remains a major challenge for the families concerned (CCNE, 2007). The available interventions do not comprise a curative treatment. Although major advances are possible, a complete disappearance of symptoms is rare. Other disorders can arise in association with autism and worsen the child's distress and that of his parents.
Faced with the current limits of management and the frustrations that they can generate, (Liptak et al., 2006), many parents turn to methods of alternative management (AM), all the more so in that they generally consider them as not hazardous.

The use of AM in autism is a modality which has been little studied (Levy et al., 2003; Hanson et al., 2007), but which seems to affect an appreciable number of children: from 30 to 95% based on the series (Levy et al., 2003; Harrington et al., 2006; Hanson et al., 2007). Among the MA used, vitamin supplements, administration of secretin, antibiotics, food avoidance diets, prohibition of vaccinations and even administration of chelators are regularly mentioned.

AM should be subjected to as strict an examination (Charman et al., 2003; Francis, 2005) as other procedures of management, to offer parents, teachers and doctors an informed choice of the most appropriate options for such children.

1.4.3 Viewpoint of representatives of patients and their families

The working group wished to hear from autistic persons, parents and associations representing the patient and his parents to better understand the experience of persons with autism and their families, as well as the practical aspects of use of gluten-free and casein-

free diets in this setting. These hearings have provided the working group with precious information, affecting the conduct of diets and modalities of perception in autism.

1.3.3.1 On diet

Although no thorough investigation of this item exists, associations consulted estimate that use of a gluten-free and casein-free diet is frequent in France. This impression confirms that of doctors, paediatricians and child psychiatrists, in particular. It is useful to specify in this regard that a gluten-free and casein-free diet is the MA most widely used in autism in the U.S. (Levy & Hyman, 2005).

Associations have underlined that such diets represent a major constraint for parents and children and that they appreciably complicate the social life of the latter.

In addition to these difficulties, budgetary considerations associated with the cost of the diet must be added, often increased by the introduction of food supplements, or chelating agents.

The associations further report the existence of channels which, in exchange for compensation, offer a variety of services covering the different aspects of this type of intervention: laboratory tests (in particular chromatography of urinary peptides), advice for avoidance diets, or even replacement products and food supplements. In this regard, results obtained with gluten-free and casein-free diets appear highly variable from one child to another and all the more difficult to evaluate since most often they are based only on individual testimony.

Consequently, associations admit that they are embarrassed in light of the frequent invitations which they receive and they regret the absence of objective markers to guide the response to their questions.

It clearly appears that the terms of the request correspond to a real need and the project of carrying out a critical analysis of scientific data in the prospective of identifying the benefits or risks of this treatment is welcomed with interest.

1.3.3.2 On the perception of disorders in autism

The testimony of persons with autism has attracted the attention of the working group on two items:

- the very particular acuity of perception in autistic subjects can, in some cases of a relatively ordinary appearance, induce intense sensations of unease which can trigger reactions of incomprehensible amplitude for a candid observer;
- in a mirror image, the changes in behaviour observed in autistic subjects more likely are attributed to the disorder, thus discouraging the search for a somatic trigger factor.

2 EVALUATION OF THE EFFICACY OF FOOD AVOIDANCE REGIMENS IN AUTISM

2.1 Origin of avoidance regimens in autism

Dohan (1966) appears to have been the first to establish a relationship between the frequency and severity of a mental disorder (schizophrenia) and the gluten or milk content of the diet. The exclusion of such proteins seemed to improve symptoms and the re-introduction of gluten without the knowledge of patients on the contrary appeared to produce a worsening of their condition (Dohan & Graberger, 1973). Gluten has even been considered a primary factor in the occurrence of schizophrenia (Singh & Kay, 1976), a hypothesis which has not been confirmed (Anonymous, 1983).

Almost simultaneously, attention was focused on the possible role of gluten in autism by its coexistence with coeliac disease in a six year old child. The apparent improvement in behaviour during treatment, including a gluten-free diet (Goodwin & Goodwin, 1969; Goodwin et al., 1971) opened the door to a possible direct relationship between autistic symptoms and coeliac disease, all the more so since coeliac disease itself can be accompanied by neurological and psychiatric disorders (Asperger, 1961; Bushara, 2005). However, since then, this eventuality has been ruled out (see chapter 4).

Then, the origin of autism was attributed to abnormal functioning of the opioidergic system. This concept appears to have been developed from speculation on possible similarities between autism and opioid addiction (Kalat, 1978). Somewhat later, behavioural disorders induced by administration of low doses of morphine (1 mg/kg) in rats (Panksepp et al., 1979a) were compared to some characteristics of autism in children, leading Panksepp (1979b) to postulate that behavioural disturbances in autism can result from abnormal activation of the opioid system, due to an excess of agonists in the brain.

Gluten in cereals and casein in milk then were incriminated as the source of peptides with opioid activity (exorphins) (Reichelt et al., 1981; Knivsberg et al., 1990; Reichelt et Knivsberg, 2003). According to this hypothesis, some of the disorders in autism can be related to excessive amounts of dietary exorphins, a theory which still has not been confirmed (see 4) but which still comprises the main rationale for avoidance diets in this disease.

A gluten-free and casein-free diet therefore has become one of the most widely used methods of alternative management in autism (see 1.3.2), to an extent of having been the subject of an article in the American scientific lay press, published under the title “the Autism Diet” (Alpert, 2007). The use of a search engine confirms this circulation, revealing several dozen web sites in several different languages dedicated to this topic on the internet.

2.2 Scientific publications dedicated to the effects of a gluten-free and casein-free diet in autism

Beyond the popularity which gluten and casein-free diets seem to enjoy, it is essential to look for and analyse scientific data in evaluating their efficacy.

2.2.1 Bibliographical search

A search of the bibliographical references has been conducted with the aim of identifying publications providing original data on the following:

- the effect of a gluten-free and/or casein-free diet;

- the effect of a gluten-free and/or casein load;

on expression and verbal function and/or cognitive function and/or motor function and/or behaviour,

and concerning:

- children and/or adolescents;
- with autism or pervasive developmental disorders.

2.2.2 Identification of communications

The search has been conducted by searching three international reference databases (MEDLINE, EMBASE and Web of Science). Results of this search have been cross-checked with references measured in the articles, general reviews and meta-analysis treating the subject.

The interviews were conducted by using the key words most appropriate for each database, among which:

- autism, Asperger syndrome, autism spectrum disorders, pervasive development disorders, child development disorders;
- gluten, casein, free, diet, dietary intervention.

2.2.3 Pre-selection of communications

Only original communications (articles presenting data unpublished up until then in opposition to compilations of general reviews) published in journals with a review board have been chosen. The information contained in dissertations or theses as well as those contained in book chapters were not taken into consideration.

2.2.4 Evaluation of communications

Pre-selected articles have been analysed to evaluate the level of evidence they provide, in particular taking into account the following:

- criteria of definition and homogeneity of the group of study subjects;
- the existence of a control group and relevance of its choice;
- method of allocation of an investigational diet (randomization or not);
- knowledge that subjects have of treatment, their family and different observers (open label, single blind or double blind method);
- the more or less strict feature of the diet;
- assessment of compliance;
- choice of evaluation criteria, in particular tools used to assess the course of treatment;
- the existence of confounding factors such as concomitant medicinal therapy or simultaneous use of one or more techniques which can affect the evaluation criteria chosen.

2.2.5 Selection of communications

Fourteen articles studying the effects of a gluten-free or casein-free diet or of a gluten and/or casein load have been identified (Table 1):

- 3 report the case of a single child (Bird et al., 1977; O'Bannion et al., 1978; Knivsberg et al., 1999) and led to contradictory conclusions;
- 2 involved the effects of a load of gluten;
    - the first trial administered it in a single dose after randomization (15 subjects; 1 g of gluten, then 1 g of saccharose or in reverse, at a 1 week interval; Goodwin et al., 1971); the control group received the same treatment (gluten, then saccharose or in reverse) was comprised of brothers and sisters (14

subjects) of the siblings; the authors estimated that evaluation of behaviour was too imprecise, and did not conclude on this item;
    - the second study (McCarthy & Coleman, 1979) included 8 autistic subjects in which a gluten supplement was administered at a dose of 20 g/d in addition to a normal diet for 4 weeks; this study did not contain a specific behavioural evaluation, because its aim was to look for an alteration in the intestinal mucosa in response to gluten;
- 9 articles had the aim of evaluating the effect of a gluten-free and/or casein-free diet on behaviour of children, adolescents, or even adults; the authors of 4 of these articles came from the same group (Reichelt et al., 1990; Knivsberg et al., 1990, 1995, 2002).

2.2.6 Description of trials on food avoidance diets (see synthetic table in Annexe 1)

2.2.6.1 Reichelt et al., 1990 (a)

This study involved 15 subjects, 3 to 17 years of age, in whom the diagnosis of autism was based on criteria in the *Diagnostic and Statistical Manual on mental disorders III* (DSM-III). The subjects were classified into 3 groups according to peptiduria[1] (study endpoint) and consequently treated with a gluten-free diet and a reduction in milk intake (exclusion of "liquid milk"; n = 8) or a milk free diet with a reduction of gluten intake (exclusion of milk and use of bread without gluten; n = 3) or a gluten and milk free diet (n = 4). The diet was continued for a year. The outcome of behaviour with treatment was assessed by parents and teachers who took care of the children using a clinical questionnaire before the start and after one year of the diet. A deterioration in behaviour which occurred in 9 subjects after week 5 of the diet led to prescription of thioridazine (50 mg/d) for 2 to 3 months. Furthermore, 4 children were following an anti-epileptic treatment. Results of the year of treatment with this diet were compared to the spontaneous outcome of the previous year, based on data obtained retrospectively. Results of the clinical questionnaire suggest that at least half of the children improved with the diet, an effect that the authors consider very positive compared to retrospective data.

Observations

The group of subjects included was very heterogeneous (age, use of DSM-III). The trial did not contain a control group to differentiate the effect of diet from outcome without treatment. The only term of comparison was comprised of data collected retrospectively. Parents and teachers were informed of treatment or were directly involved in its implementation and, at the same time, played the part of evaluator. Actually, the authors recognise that only a double-blind study would authorise a reliable conclusion. The procedure for evaluation of the results is not detailed and the "clinical questionnaire" was not subjected to prior evaluation. Analysis of the different behavioural characteristics is not available in all subjects (4, 10, 11, 13 or 15 subjects, according to items). The effects of these 3 diets were not differentiated and no statistical analysis apparently was performed. Lastly, the effect of medication (anti-epileptic [n = 4] and thioridazine [n = 9]) was not evaluated even though it may comprise an important confounding factor.

2.2.6.2 Knivsberg et al., 1990, 1995 (b and c)

These two articles involved a cohort of 15 children (6 to 22 years of age) in whom the diagnosis of autism was based on DSM-III criteria and who were followed for 4 years. Subjects were classified into 3 groups (sample size not specified) according to peptiduria[2] and treated with a gluten-free diet and a reduction in intake of milk or a milk free diet and reduced gluten content or a milk-free gluten-free diet. The reduction in milk intake included the elimination of cheese but not milk; that of gluten intake was obtained by use of bread and cookies made without gluten. The avoidance diet was not uniformly followed, one subject

1. Absorbance of the eluate at 280nm on a Sephadex G25 ® column; see. 4.1.5.
2. Idem note 1

stopped following it after 9 months and another did so 1 week before evaluation at one year. Two other subjects completely abandoned it at the end of year one. Another subject occasionally consumed gluten and one stopped following this diet just before the end of year 4 of treatment. The first of two articles specified that 4 children were receiving treatment with anti-epileptic agents.
Evaluation of the effect of diet was based on parents' observations, those of teachers, and use of standardised evaluation grids: *Diagnosis of psychotic behaviour in children* adapted to Norway (DIPAB); *observation of skills needed for play and activity* (Tajford); Illinois test of psycholinguistic abilities (ITPA; between 4 and 10 years of age); a short version of Raven matrices (C-Raven test). The DIPAB was used for maintenance therapy conducted with parents from 0 to 1 year; teachers used the Tajford from 0 to 1 year; the IPTA was administered to 10 children at 0, 1 year and 4 years of age; the C-Raven was used in 15 children at 0, 6 months, 1 year and 4 years of age. The authors concluded that an improvement occurred relatively early in the first 6 months of the diet and that the effect was no longer as clear (non C-Raven test) after one year.
Observations
The authors specified that subjects included in this study comprised a very heterogeneous group, and that only spontaneous outcome could provide appreciable progress in the majority of children in the field evaluated by the study apart from diet. This comment emphasizes the importance of a control group to differentiate the effects of treatment from course and outcome over time. As in the previous report, 3 different diets were used and their results combined. There was an appreciable number of abandonment of diets (6 out of 15) with no evaluation of the effects on the final result. The method of verification of compliance with the diet moreover was not indicated. The use of questionnaires and standardised evaluation grids is an advance compared to the previous publication. However, children and teachers were informed of treatment and contributed to its evaluation. Because of the absence of a control group, the authors concluded that the results have to be considered as preliminary.

2.2.6.3 Sponheim, 1991 (d)

This study was conducted on 4 subjects (17 to 33 years of age) subjected to a gluten-free diet for 6 months and on 3 children (8 and 12 years of age) who received a gluten or placebo load administered with double-blind method after following a gluten-free diet for one year. The outcome of behaviour was estimated on a visual analogue scale using a *real life rating scale.* The authors concluded in the absence of a relationship between diet and behaviour and suggest that avoidance diets can complicate more socialisation of such children.
Observations
The sample size of this study does not offer the power necessary to conclude with certainty in the absence of the effect of an avoidance diet.

2.2.6.4 Lucarelli et al., 1995 (e)

Thirty-six children (8 and 13 years of age) were included in this trial. The diagnosis of autism was established based on DSM-III-R criteria. These children were subjected to a diet excluding milk proteins for 8 weeks. Furthermore, susceptibility to other dietary allergens was sought by intradermal reaction, leading to their elimination from the diet in case of a positive response (13 subjects). At the end of 8 weeks of the avoidance diet, children who responded favourably to the diet were again exposed to the excluded allergens (in particular egg, rice soy), in a loading test conducted with double blind method versus a placebo. Their behaviour was evaluated with a *behaviour summarized evaluation* (BSE) before and 8 weeks after the avoidance diet, and then within 2 weeks following the loading test. The exclusion diet resulted in a significant improvement in results of the BSE (in 5 out of the 7 categories evaluated). On the contrary, the loading dose test resulted only in a modest deterioration in the BSE (3 out of 7 categories). The authors concluded in a possible relationship between food allergies and childhood autism.

Observation

Recruitment criteria chosen (DSM-III) did not ensure better homogeneity of the study group than in the previous studies. Here too, the absence of a control group does not allow the results obtained to be interpreted, all the more so in that the observers were informed of the diet. Simultaneous elimination of other dietary components in a third of the subjects complicates the analysis even more. The authors insisted on the difficulty of evaluating the clinical outcome in relation to diet and asked the reader to interpret their results with caution.

2.2.6.5 Whiteley et al., 1999 (f)

This study evaluated the effects of a gluten-free diet followed for 5 months by 22 of 31 children included. The diagnoses included were: autism (n=9), Asperger syndrome (n=4), autism syndrome (n=5), "semantic pragmatic disorders" (n=2) and dyspraxia (n=2). These diagnoses meet DSM-IV criteria and/or those of the International Classification of Diseases 10th edition (ICD-10). In addition, 5 children (4 autistic and 1 autistic syndrome; receiving a gluten-free diet for over 6 months) received a gluten load whose quantity was not specified. Six other autistic children comprised a group exempt from any dietary intervention (control group). The outcome of children was assessed with the BSE scale, in 6 of the 16 Kaufman sub-evaluation groups (K-ABC) and parental satisfaction scale (PASS) administered at the end of the intervention.

Parents' observations suggest that a certain number of children showed appreciable improvements with diet. The latter were manifest especially after month 3. On the contrary, the behaviour of children subjected to a gluten load was altered only modestly (change of 5 to 7.5%); improvements were even observed, for example with the PASS questionnaire. The authors indicate that the majority of parents (16 out of 22) observed an initial regression of behaviour (for 1 to 3 weeks) after set up of the exclusion diet.

The observations of teachers did not show any significant difference during the gluten-free diet and could not be utilised during the loading test, as a result of a too large number of missing data. Four children subjected to the gluten-free diet and 4 subject to the loading dose test were evaluated with the K-ABC before and after changes in diet. They were compared to 4 children who did not receive any dietary intervention. The 4 children subjected to the gluten-free diet presented with a significant improvement in 3 of the 6 fields evaluated between the two tests. There was no significant difference between children subjected to the loading test and those without treatment, in the two evaluations carried out.When asked to provide an overall assessment of the study, only 67% of parents reported an improvement in their child's behaviour, and yet 94% of them reported their intent to continue the avoidance diet at the end of the study.

Observation

The subjects studied comprise a very heterogeneous clinical set and the various diagnoses proposed were not verified by the investigators. The reference group was a very small sample and was not comprised by randomization at time of treatment allocation. The dietary intervention was conducted without blinding of the parents and of teachers who contribute directly to the evaluation of results. There were many missing data, in particular in observations made by teachers. The statistical analysis procedures were not stable (for example uni- or bi-directional according to the need compared). The use of parametric tests is debatable with small and heterogeneous sample size. Lastly, the consistency of results obtained from the different methods of evaluation appears low. For example, the observations of parents led to believe that the effects of the loading dose test tended to be negative while it tended to be positive with the PASS questionnaire.

2.2.6.6 Cade et al., 2000 (g)

This study included 149 children and adolescents with autism or Asperger syndrome, 3.5 to 16 years of age. The diagnosis was based on DSM-III criteria. Follow-up involved 70 children maintained on a gluten-free or casein-free diet for one year or more. The evaluation involved the existence and the intensity of 9 autistic characteristics (scored 0 to 4), assessed by

parents, some teachers and independently by doctors. Then a mean was calculated based on parents' and doctors' scores. These evaluations were carried out after one month of diet and then every 3 months for one year. The treatment resulted in an appreciable improvement starting with month 3 in 57 children, while no change was detected in the other 13. The authors indicate that initiation of a diet induced disorders in many children.

Observation

The subjects studied comprised a heterogeneous clinical set in terms of diagnosis and age. Their outcome over time was not compared to that of a similar group without dietary intervention. The treatment was not conducted with blinding of observers, and evaluation was carried out with a grid whose validity is not established. Furthermore, the authors indicate that it was not always possible for them to assess compliance with the diet, in particular in children who remained at home.

2.2.6.7 Knivsberg et al., 2002 (h)

This article seems to use data previously presented in a book chapter (Knivsberg et al., 1998). Twenty autistic children (59 to 127 months of age) and with abnormal peptiduria[3] were recruited. Each was the subject of an evaluation of autistic symptoms (DIPAB), from level of intellectual development with Leiter's international performance scale, logistic aptitude with the IPTA, or with *Reynells språktest* according to age, and motor capacities with a revised version of the *test of motor impairment* (TOMI). Diagnostic criteria were not specified. Children then were matched for severity of autistic syndrome, age and level of mental development, and then members of each pair were randomly assigned to groups with dietary intervention (gluten-free and casein-free diet; n = 10) or without dietary intervention (control group; n = 10). Another evaluation was carried out after one year of diet. The group which received a diet showed an appreciable improvement in autistic syndrome but no difference in linguistic aptitude or motor capacity. The authors indicate a significant difference in mental development in support of the treated group.

Observations

Since inclusion criteria were not detailed, it is difficult to judge the clinical homogeneity of the two groups of children. The reality of the difference observed in the level of mental development was not established insofar as the values at one year were very dispersed (86 $\pm$ 38 and 74 $\pm$ 31) and the confidence interval of the difference was very wide (-20 to 44; z = 0.75, p = 0.45; Millward et al., 2004). Therefore, this study observed an improvement in the autistic syndrome. However, evaluations were based on an interview of the parents and on tests conducted in the presence of teachers, all of whom were informed of the type of diet and possible knowledge of the treatment affecting the results cannot be ruled out.

2.2.6.7 Elder et al., 2006 (i)

Fifteen children (2 to 16 years of age) were recruited. Inclusion was based on DSM-IV criteria and a minimum score for each group of symptoms of autism evaluated with the*autism diagnostic interview revised* (ADI-R). Children with a previous medical history, in particular coeliac disease, or sensory deficiencies were not included. A more precise description of characteristics of this cohort was obtained by combining the *childhood autism rating scale* (CARS; autistic syndrome) and the ADI-R (social interactions, communication and stereotypes). The course and outcome were followed with the CARS and the *ecological communication orientation language sampling summary* (ECO). Furthermore, video recordings were made in the children's home, and the parent - child interaction scored by evaluators who were unaware of treatment. All children received a gluten-free and casein-free diet and a normal diet successively according to a randomised order, with each period lasting 6 weeks. Meals and snacks were provided to participants, such that children, parents and the investigator's team were not aware of the type of diet. The CARS and ECO did not reveal any difference between an avoidance diet and placebo.

3. Reverse phase HPLC and absorbance at 215 and 280 nm.

Observations

In spite of the inclusion and non-inclusion criteria which were much more precise than those of previous studies, the children's characteristics remained highly heterogeneous, in particular regarding intellectual aptitude and severity of autism. The low sample size, the high inter-individual variability and the short duration of observation may explain the apparent absence of a diet-related effect. Moreover, the authors recognise that the power of the study was low, and that a type II error (not recognising a real difference) cannot be ruled out. Lastly, the statistical test used is not the most appropriate in this type of trial (Hills & Armitage, 1979).

2.2.7 Overall observations

Although the question has been raised over 30 years ago (Goodwin et al., 1971; Bird et al., 1977), the number of studies dedicated to effect of gluten-free and/or cow's milk casein-free diet in autism remain very limited. It was possible to identify 8 studies, corresponding to 9 publications. Unfortunately, the majority of them had serious methodological weaknesses, which considerably limit interpretation of their results and compromise their conclusions. (Christison & Ivany, 2006; Millward et al., 2004, 2008).

Six out of 8 were conducted without a control group (or period). Yet, the spontaneous outcome can result in an appreciable improvement in some of the characteristics measured over time, simply as a result of the child's development (Reichelt et al., 1990; Knivsberg et al., 1995). The duration of the study (several months, a year or more) manifestly increased this risk. Advances appear all the more likely, independently of any diet insofar as autistic children are also committed to programs of education or behavioural therapies (Whiteley et al., 1999) from which an undeniable benefit is expected (Lord & McGee, 2001). Therefore, constitution of a control group is essential to assess the effect of these confounding factors (time, associated measures, etc.) and to distinguish the effects of diet.

To be valid, a comparison assumes that characteristics of children placed under treatment are as similar as possible to those of the control group. This depends directly on choice of inclusion and non-inclusion criteria. In this regard, it is striking that the heterogeneity of recruitment of these studies is almost unanimously emphasised by the authors. The high inter-individual variability probably reflects an inadequate definition of phenotype and implies that the tools used for screening (DSM-III, DSM-IV; see 1.2) are not the most appropriate. The wide age disparity of subjects included probably contributed to this heterogeneity.

Almost all studies (7 out of 8) were conducted without the type of diet being masked for the children, parents or the teachers, even though these adults participated directly in evaluation of treatment. The high commitment of parents can alter their perception of their child's behaviour, which can affect evaluations based on their impressions. This risk also affects various staff (doctors, teachers, etc.) who contribute to estimation of results, if they are involved with full knowledge of cause in conduct of the study. The intervention of irrational factors in assessment of these situations is illustrated by the fact that three-fourths of parents of autistic children still believe in the usefulness of treatment with secretin, in spite of the fact that they all have been informed of its inefficacy (Sandler et al., 1999). The situation seems comparable for avoidance diets since almost all parents wanted to continue them even though they seem beneficial only in two-thirds of children (Whiteley et al., 1999).

The choice of an "open-label" protocol also ignores the important placebo effect induced by clinical trials, in particular in this context (Sandler & Bodfish, 2000). For example, intravenous administration of secretin to autistic children resulted in a very appreciable qualitative and quantitative improvement in some characteristic symptoms of the disease (eye contact, stereotypical behaviours, communication, etc.) and regression of concomitant disorders (diarrhoea, sleep disorders) in 30 % of children in the control group and in the treated group

(Sandler et al., 1999). The importance of this placebo effect was confirmed by at least 10 control studies, including over 500 children. Its amplitude is probably even higher than in non controlled studies (Sandler, 2005).
The *placebo* effect is not specific of PDD. The simple inclusion of a subject in a clinical research study “improves” the results of treatment. This remains true even if they are assessed with quantitative items (Horwitz & Horwitz, 1993). The placebo effect not only can change the subjective perception of the illness, but also affects its course. This is also possible in management of autism, in particular through behaviour of the patient’s family. The high motivation which underlies the set-up of difficult non-conventional treatment, such as a gluten-free and casein-free diet, can be accompanied by changes in parental attitude and interactions which may affect the child’s development by themselves (Sandler, 2005).

2.2.8 Synthesis and conclusion

Six studies (a to g) did not comply with minimum universally-recognised conditions, as essential to objective evaluation of an intervention (in particular: control group and allocation of treatment by randomization). Therefore it is impossible to take their results into account (Christison & Ivany, 2006; Millward et al., 2004, 2008).

One study (h); contained a control group and randomization, but parents and teachers were aware of the diet. If an improvement in some autistic characteristics existed, the importance of variance makes the other differences doubtful (Millward et al., 2004). In addition, bias related to information of parents and teachers cannot be ruled out (Sandler, 2005).

Only one study (i) associated the principal characteristics required of a controlled clinical trial, i.e. control group, allocation of treatment by randomization and double blind method. Its result did not show any improvement following diet, but the sample size was small and the variance large. A type II error cannot be excluded.

Therefore current scientific data do not make it possible to conclude in a beneficial effect of a gluten-free and casein-free diet on the outcome of autism.

3 EVALUATION OF SAFETY OF FOOD AVOIDANCE DIETS IN AUTISM

To affirm the safety of a gluten-free and casein-free diet in autism implies demonstrating the absence of any adverse event of this intervention in terms of nutrition. In theory, the spectrum of effects to be sought is vast, ranging from the most marked (altered growth) to the slightest (reduction of stores of a micronutrient).

The demonstration of possible harmful effects involves comparing growth and nutritional status of autistic children subjected to an avoidance diet to those of children with normal development and eating a varied diet.

This step is necessary but insufficient. In fact, the existence of an impact of autism on spontaneous diet and nutritional status of children should be excluded in order to be able to incriminate the avoidance diet in the occurrence of such disorders.

3.1 Spontaneous food intake and nutritional status of autistic children

3.1.1 Spontaneous food intake of autistic children

There are few studies dedicated to eating patterns in autistic children. Yet, Leo Kanner (1943) had reported eating disorders at the outset, at least during the first 8 months of life. Anecdotal relations regularly report the existence of difficulties in this field and various sources of information for the public, including dedicated sites on the internet, also mention their existence.

The first attempt at systematic analysis of spontaneous eating patterns in autistic children showed that over half of children included are selective eaters, accepting or rejecting food in particular based on their texture or type (Ahearn et al., 2001). Unfortunately, the extent of this study is very limited because the sample size was small, and most importantly, it did not comprise a control group. A more recent study confirmed this selectivity, but it is exposed to the same criticism (Field et al., 2003)
The absence of a control group is a serious deficiency when we know that children with normal psychomotor development can also select their food and have food manias without having any apparent effect on their nutritional status (Davis, 1928).

The comparison between autistic children and a reference population (control group) confirms that food choices in the former are more restricted than those in the latter, which may lead to concern that autistic children are more exposed to a risk of nutritional disorders (Schreck et al., 2004; Twachtman-Reilly et al., 2008).

However, evaluation of quantities of nutrients consumed by autistic children based on weekly diaries suggest that they lie at least on the same level as those of the control group (Raiten & Massaro, 1986). Intake of energy, carbohydrates and fat assessed over 3 days did not seem to deviate from nutritional recommendations and the consumption of proteins was higher than the safety requirement just as in children with normal development (Levy et al., 2007). This limited information suggests that selectivity of eating patterns does not overtly affect the coverage of macronutrient requirements.

3.1.2 Nutritional status of autistic children

Growth is highly sensitive to the adequate response between nutritional requirements and intake, all the more so when the child is younger. Therefore, height and weight growth curves are precious methods of evaluation even though indirect.

The growth in height of a cohort of 420 subjects (from age 2 years to adulthood) with pervasive developmental disorders (208 autistics) appears all the more superimposable to that of a reference population of healthy subjects (*Centres for Disease Control)* (Lainhart et al., 2006). Growth in height may be slightly faster during the first year of life than in a reference population (van Daalen et al., 2007).
In the UK, a shift in growth curves was observed to high values compared to the reference population. For example, the majority of a group of 50 boys with PDD was above the 50th percentile for height (70%), weight (74%) body mass index (BMI=W/H^2; 80%) (Whiteley et al., 2004). In the US, the prevalence of overweight in autistic children is the same as in the general population (Curtin et al., 2005).
Conversely, in other countries, distribution of BMI in autistic boys appears shifted to lower than normal values (Mouridsen et al., 2002), a difference which may be related more to hyperactivity than eating pattern disorders (Bölte et al., 2002).

One has to admit, with follow-up of nearly 65 years, that a major alteration in the curve of height and weight appears very rare (Keen, 2007), in spite of the original worrisome description (Kanner, 1973) and the recurrent mention of disorders of eating behaviour.

More selective nutritional depletion or deficiencies have been reported but they only involve a small series for iron (Latif et al., 2002), methylmalonic acid excretion (Wakefield et al., 1998), or isolated subjects for other nutrients (Clark et al., 1993; Uyanik et al., 2006). Analysis of hair content of some minerals and metals, moreover, has not demonstrated a difference with control subjects (Shearer et al., 1982). The possibility of amino acid deficiencies also has been suggested based on their plasma concentrations (Arnold et al., 2003), a measurement inappropriate to this type of judgement. The only objective anomaly described, based on a consequent population (75 children) concerned a deficiency in bone development which was manifest by reduction in thickness of cortical bone (Hediger et al., 2007). This observation suggests that intakes of calcium, vitamin D or both are insufficient in autistic spectrum disorders (ASD).

There are few studies dedicated to evaluation of growth and nutritional status in autistic children, and their results appear to agree and do not demonstrate an appreciable difference with populations taken as a reference.

3.2 Eating patterns and nutritional status of autistic children receiving a gluten-free and casein-free diet

Exclusion of products containing gluten and milk caseins must necessarily be compensated for by the introduction of replacement foods to ensure coverage of nutritional requirements.

The exclusion of products containing gluten involves the elimination of all wheat-based foods (wheat, grain and “spelt bread”), rye and barley. These foods are flour, bread, biscuits, cookies, pasta, pastry and Danish pastries. Exclusion of caseins results in that of milk (whole or skimmed), infant formula, fermented dairy products and yoghurts, cheeses, dessert creams or ice cream.
Many industrial products which contain gluten or milk proteins as ingredients or technical additives must also be avoided (this dual exclusion prohibits the intake of almost all commercially prepared baked goods).

Therefore, the initiation of a strict diet is possible only by educating the child’s parents, on one hand, to avoid foods which may contain gluten or casein, and also to buy replacement products or to make their own.
For the family this is a bothersome and costly diet (Annex II), because it calls for wide use of specific dietetic preparations.

It also involves major changes for the child in terms of his habits, which can affect his eating patterns (Sponheim, 1991; Whiteley et al., 1999; Cade et al., 2000; Cornish, 2002).

3.2.1 Eating patterns with an avoidance diet

A single study sought to analyse eating patterns of autistic children subjected to a gluten-free and/or milk casein-free diet or not (Cornish, 2002). This investigation was conducted with a dietary questionnaire extending over 3 days, to which a minority of invited parents (26%) replied. Twelve families had previously used a gluten-free and/or casein-free diet at one time or another[4] and 25 had never used it. At time of the investigation, 8 out of 29 children were following a gluten-free and/or casein-free diet. The degree of compliance with exclusion diets was not specified. The investigation did not reveal any difference between children who were on this diet and others, in terms of energy and protein intake. However, a certain number of children may have ingested an insufficient quantity of several micronutrients in the 2 groups.

3.2.2 Nutritional status with an avoidance diet

Only one study (Knivsberg et al., 1995; see. 2.2.6) followed autistic children receiving a gluten-free and casein-free diet for a sufficient duration (4 years) to evaluate with definite reliability the nutritional tolerance to this type of diet. Unfortunately, the authors did not provide any indication on this subject. The information was also absent from studies involving exclusion regimens of shorter duration (several months to one year; see. 2.2.6).
The cortical bone development anomaly which affects autistic children (see. 3.1.2) was more pronounced in those who followed a casein-free diet (without dairy products): the difference compared to standards in children receiving an exclusion diet was nearly two times higher than those who did not follow it, suggesting a relationship at least partial with food intake (Hediger et al., 2007).

3.2.3 Gluten-free and casein-free diet: theoretical estimation of nutritional intake

The insufficient amount of data on eating patterns and nutritional status of autistic children following a gluten-free and casein-free diet has led to simulate the effects of diet on nutritional intake by substituting for foods which contain them “homemade” preparations without gluten and industrial products without gluten such as pasta, bread and biscuits. Milk and dairy products are replaced by soy preparations (drinks, dessert creams and yoghurts enriched in calcium or not) or with chestnut-based, almond or hazelnut-based drinks arbitrarily estimating it for the latter, an intake of about 400 mL/d.

The calculation was used as the basis for mean food intake (energy and macronutrients) in a 6 year-old child, based on the INCA 2 survey (Afssa, 2007). However, the lack of information on the nutrient content of food without gluten (see. 3.2.5) limits this extrapolation to proteins, calcium and some B vitamins.

Protein intake of a gluten-free, casein-free diet remains higher than safety intake for age (ANC, 2001), even if we use chestnut or almond-based drinks, which are erroneously referred to as “milk”. On the contrary, the intake of calcium which is of about 780 mg/d (ANC 700mg/d) apart from an avoidance diet, decreases to 550-650 mg/d with an avoidance diet using soy-based preparations enriched with calcium, and further to 170-200 mg/d with replacements not enriched with calcium (Annex III). Intake of thiamine and riboflavin remains close to ANC (Annex IV).

4. In one of these children, the avoidance diet produced such a reduction in food intake that it had to be abandoned.

A gluten-free and casein-free diet does not appear to compromise intake of proteins, vitamin B1 and B2 and calcium, provided it contains milk substitutes enriched with calcium.

It should be emphasized that this conclusion is very tenuous since it is based solely on a theoretical calculation. A reduction in food intake in children in response to introduction of diet (Ahearn et al., 2001; Cornish, 2002; Francis, 2005) would make it completely null and void.

It is impossible to take a stand on other nutrients.

3.2.4 Other possible effects of an avoidance diet

A gluten-free and casein-free diet may have two other effects on which studies published to date (see. 2.2.6) have given little interest.

The first possible effect results from the apparent contradiction which exists between the effort used to promote better social insertion of autistic children and the set-up of a restrictive diet, which can on the contrary reinforce their isolation (Sponheim, 1991). Moreover, some studies report worsening of behavioural disorders during the weeks following initiation of the diet (Reichelt et al., 1990; Cade et al., 2000).

The second effect is associated with spontaneous eating patterns in autistic children who are very affected by the colour, smell or texture of foods (see. 3.1.1). The simultaneous exclusion of gluten and of milk casein represents an upset of habits with the risk of exacerbating eating difficulties (Ahearn et al., 2001; Francis, 2005). Although this risk is perfectly identified (Cornish, 2002), the frequency and severity of disorders induced have not been evaluated.

3.2.5 Long term nutritional effects of a gluten-free diet in non autistic children: experience with coeliac disease

In coeliac disease, some wheat gluten peptides, but also barley and rye prolamines, produce inappropriate activation of intestinal immunity in genetically predisposed subjects. The effect is the occurrence of intestinal lesions - a diagnostic criterion of the disease - diarrhoea and malnutrition evidenced by growth retardation.

The elimination of gluten from the diet quenches the abnormal immune reaction and within a few weeks or months clinical signs improve. This improvement, which is often spectacular, corresponds to restoration of the normal structure of the intestinal mucosa.
Therefore, benefits of diet are major (correction of deficiencies, resumption of growth) and manifest in the short and mid-term, such that few doctors have wondered if it could be harmful in the long term, in particular by eliminating nutrients provided by cereals, such as vitamins, trace elements and fibres.

And yet the question is relevant, since the vitamin B content of food without gluten (thiamine, riboflavin and niacin), iron, and fibre in particular can differ from that of natural foods that they are supposed to replace.

There are very few longitudinal prospective follow-up studies evaluating the long term effects of a gluten-free diet. In adolescents, a gluten-free diet tends to enhance the nutritional imbalances usual at this age (too many proteins or fats, not enough carbohydrates, and fibre), but does not seem to appreciably alter the quantities of energy, macronutrients, iron, calcium and fibre ingested compared to the general population (Hopman et al., 2006).

In adults who have been on a diet for over 20 years, mean plasma concentrations of homocysteine are higher than those in the general population, which reflects folate and

vitamin B6 deficiency, which exists in 37 and 20% of patients respectively who are on a gluten-free diet (Hallert et al., 2002). It also seems that body composition seems to differ from that of healthy subjects (reduced fat-free and fat masses; Bardella et al., 2000).

This limited information indicates the existence of a nutritional risk, but does not allow its importance to be evaluated.
This conclusion should encourage caution in autistic children, all the more so since it calls for the avoidance of all foods containing casein in addition to a gluten-free diet.

3.2.6 Nutritional effects of a milk protein-free diet in non autistic children: experience with milk protein intolerance

Acquired experience with diet which excludes milk, dairy products, cheeses, butter, fresh cream and all industrial products containing milk differs appreciably from that of the gluten-free diet insofar as the symptoms of allergy to milk proteins often disappear before the age of 3 years, while coeliac disease continues throughout life.

However, diet can prove insufficient in terms of some nutrients, in particular calcium (Monti et al., 2007; Konstantynowicz et al., 2007; Aldámiz-Echevarria et al., 2008) and can even result in severe malnutrition if it is not given under medical supervision (Noimark et al., 2008).

The analysis of eating patterns of older children, following an avoidance diet to prevent development of food allergy, provides important practical precision: complete elimination of food allergens from the diet is difficult, often incomplete, or even simply impossible to do in many cases (Vlieg-Boerstra et al., 2006).

3.2.7 Conclusion

In spite of the selective nature of spontaneous eating patterns, a few studies evaluating growth in autistic children do not show manifest difference with populations of children taken as a reference.

On the contrary, no data exists on growth or nutritional status of autistic children subjected to a gluten-free and casein-free diet. Therefore, it is not possible to confirm that such a diet has no harmful effects in the short, medium or long term, all the more so since there may be an unfavourable effect on eating patterns.

The implementation of an exclusion diet (for example, without gluten or without cow's milk proteins) always carries a risk for nutritional status and growth of a child. Simultaneous exclusion of two important food groups can only appreciably increase the probability of this hazard. Therefore, the conduct of such a diet should be carried out only under strict monitoring provided by qualified nutritionists, who alone can assess its tolerability. Lastly, it should not be overlooked that practice of this diet, which is far removed from usual eating patterns of other children and other adults, can reinforce their isolation.

4 INDIRECT EVIDENCE PROPOSED IN SUPPORT OF A GLUTEN-FREE AND CASEIN-FREE DIET

It may appear surprising that the use of a very restrictive avoidance diet, which has not provided evidence of its efficacy (see 2), nor demonstrated its safety (see 3), is as widely used. This perhaps is related to much evidence proposed in support of it[5], which can pass for true justification of use of this type of diet with a person who is not informed.

The most commonly mentioned theory in support of a gluten-free and casein-free diet involves the action of neuromediators (see 2.1.). It postulates that peptides with opioid activity are released in the intestine during the digestion of certain proteins, cross the intestinal mucosa intact, are transported by the blood, cross the blood-brain barrier and arrive in the central nervous system where their presence in high quantities alters brain functioning.

The "opioid theory" is based on complementary hypotheses, for example:

- Abnormally high urinary elimination of dietary opioid agonists may be both evidence of their presence in the circulation, and a characteristic of the disorder: the existence of these peptides in the urine could even be a diagnostic component;
- Autism might be associated with gastrointestinal disorders and/or intestinal permeability promoting the passage of exogenous opioid agonists across the intestinal mucosa.

The implication of each allegation contained in the opioid theory justifies examination of the elements on which they are based.

4.1 Opioid peptides: origins and biological effects

The central nervous system physiologically produces opioid agonists (endogenous opioids). They consist of about twenty neuropeptides distributed into three classes (endorphins, enkephalins and dynorphins), each one derived from partial proteolysis of a distinct precursor. Endogenous opioids can interact with three families of receptors (δ, κ and μ). All have a central analgesic effect in common, and depending on type of endogenous opioid receptor implicated, euphoria-inducing effects or action on cognitive function, eating pattern or locomotor activity, olfaction and respiration, neuroendocrine secretions, intestinal motility or diuresis can also be observed. Endogenous opioids include 4 or more amino acids (review in Gutstein & Akil, 2005; Bodnar, 2007).

Opioid agonists can also be of exogenous origin (exorphins). Therefore, it is these exorphins, and thus their dietary sources, that the opioid theory has implicated for over 20 years in autism (Reichelt et al., 1981; Reichelt et al., 1991; Reichelt et Knivsberg, 2003). The exclusion of gluten and casein from the diet is the direct result of the role that the opioid theory attributes to exorphins in the manifestations of the disorder.

4.1.1 Sources of opioid peptides in food

Milk and cereal proteins, in particular, have been implicated due to the presence of peptide sequences with potential opioid activity, encoded in their primary structure, and which may be released during their digestion.

5. A search using the words *gluten and casein-free diet* identified over 130,000 items on the web. The same search conducted in French identified over 15,000.

4.1.1.1 Milk casein

One of the best known examples is comprised of the β-casomorphin group, a derivative of enzymatic hydrolysis of bovine casein. These peptides are derived from residues 60 to 70 (tyr-pro-phe-pro-gly-pro-ile-pro-asn-ser-leu) in the β-casein sequence. β-casomorphin 7, covering residues 60 to 66 (tyr-pro-phe-pro-gly-pro-ile), is the one which has been the most studied.
However, peptide sequences with potential opioid activity also exist in other cow's milk proteins, such as β-lactoglobulin or α-lactalbumin. This involves tyr-gly-leu-phe sequences or α-lactorphin and tyr-leu-leu-phe or β-lactorphin.
Identical sequences or homologues are also present in milk proteins from other domestic mammals (ovine, caprine, etc.), just as in that from humans.
Indeed, human breast milk, in particular colostrum, contains relatively high levels of free 5 to 7 β-casomorphins (Jarmolowska et al., 2007).

4.1.1.2 Cereal gluten

In cereals (wheat, barley, rye, oats, etc.), gluten is a mixture of very abundant proteins which can, during digestion, generate gluteomorphins (Fukudome & Yoshikawa, 1993) which can bind to μ receptors.

4.1.1.3 Other plant and animal proteins

In reality, sources of opioid peptides are not limited to gluten in cereals nor to milk proteins. Many other proteins also contain them (Table 2):

- the β, γ, δ and ε chains of haemoglobin can be broken down into various haemorphins resembling casomorphins (for example haemorphin 4: tyr-pro-trp-thr) (Nyberg et al., 1997);
- bovine albumin also contains a peptide with opioid activities (serorphin);
- cytochrome b, which exists in mitochondria of all nucleated cells, has a tyr-pro-phe-thr sequence (cytochrophine; present in meat, fish and vegetables);
- rice and maize, very used in gluten-free diets, also contain opioid sequences (Zioudrou et al., 1979);
- similarly, soy which is widely used in diets excluding cow's milk proteins, has a ß-conglycinin whose hydrolysis can produce soy morphins, with the shortest sequences being identical to casomorphin-4 (Ohinata et al., 2007);
- lastly, rubisco, the foliar enzyme and one of the most abundant compounds in the biosphere, also contains peptide sequences (rubiscolin 5 and 6) with opioid activity.

Therefore, dietary sources of agonist peptides are very diverse since proteins in many plant and animal foods contain them. All available foods were not evidently the subject of such extensive analyses. A search of databases (for example, http://expasy.org/) allows them to be scrutinized by looking for the existence of peptides with potential opioid activity in the primary structure of proteins sequenced to date. This investigation suggests that their distribution may be more extensive than is currently thought.

4.1.1.4 Characterisation of pharmacological properties of these peptides

The agonist activity of these peptides toward opioid receptors has been established by analysis of the pharmacological properties of hydrolysates and/or peptides resulting from enzymatic digestion of their protein precursor (Teschemacher, 2003). It should be underlined that the exorphins have low affinity for opioid receptors (Table 2)

Sequences with opioid receptor antagonistic activities also exist in dietary proteins such as in bovine kappa caseins (tyr-ile-pro-ile-gln-tyr-val-leu-ser-arg) in human caseins. Activities of the same type are found in lactoferrin in residues 339-344, 544-548 and 681-687. Paradoxically, the opioid theory does not take into account these antagonistic activities, even though their existence has been known for a long time (Zioudrou et al., 1979).

4.1.1.5 Conclusion

Contrary to its alleged properties, the gluten and casein-free diet does not allow elimination of all potential sources of opioid peptides from the diet.

In light of current knowledge, actual exclusion of proteins containing peptide sequences with opioid activity would lead to the exclusion of almost all foods, including human breast milk.

Tableau 1: Origin and sequence of ligands and principal dietary peptides with opioid activity. Opioid activity is measured by IC 50 value in test on target organs rich in the indicated receptor. (Paroli, 1988)

Source	Type of opioid	Sequence	IC 50 (µM) GPI rich in R mu	IC 50 (µM) MVD rich in R delta
Agonists				
Endomorphin1		tyr-pro-trp-phe	0.015	0.029
Endomorphin2		tyr-pro-phe-phe	0.011	0.022
Medicines				
Morphine			0.1	0.9
Opioids of other origins				
β casein	β casomorphin 4	tyr-pro-phe-pro	21.9 (27.6)	84.3
β casein	β casomorphin 5	tyr-pro-phe-pro-gly	5.0 6.5 (13.5)	35 42.1
β casein	β casomorphin 7	tyr-pro-phe-pro-gly-pro-ile	55 6.5 (29.0)	> 500 42.1
β casein	β casomorphin 8	tyr-pro-phe-pro-gly-pro-ile-pro		
Serum albumin	serorphin	tyr-gly-phe-gln-asn-ala	230	8.5
α casein	α casomorphin	arg-tyr-leu-gly-tyr-leu-glu		30
α lactalbumin	α human lactorphine	tyr-gly-leu-phe	300	>1000
β lactoglobulin	β bovine lactorphine	tyr-leu-leu-phe-	160	>1000
Kappa chain immunoglobulin	kappa aporphin	tyr-ser-phe-gly-gly		
Gluten	Gluten-exorphins	gly-tyr-tyr-pro-thr	1000	60
		gly-tyr-tyr-pro	>1000	70
		tyr-gly-gly-trp-leu	0.05	0.017
		Tyr-gly-gly-trp	1.5	3.4
Haemoglobin	Haemorphin 4	tyr-pro-trp-thr	45.2	
Cytochrome b	Cytochrophine 4	tyr-pro-phe-thr		
Antagonists				
κ casein	Casoxin A Casoxin C	tyr-pro-ser-tyr-gly-leu-asn-tyr tyr-ile-pro-ile-gln-tyr-val-leu-ser-arg		

GPI: myenteric plexus rich in µ receptor
MVD: mouse *vas deferens* rich in delta receptors.
*concentration which inhibits 50% binding of a radio-labelled ligand (^{3}H)
Without parenthesis bovine origin, with parenthesis human origin.

4.1.2 Intestinal production and absorption, urinary excretion of opioid peptides in humans

4.1.2.1 Production of dietary opioid peptides in the intestinal lumen in animals and in humans

The existence of a sequence with potential opioid activity in the primary structure of an ingested protein does not implicate that it is released in its totality during digestion, that is, in the form of a biologically active peptide (Schmelzer et al., 2007), nor that it is present in sufficient quantity to produce a physiological effect.

Several *in vivo* studies have demonstrated the existence of β-casomorphins in the intestinal lumen after ingestion of milk or dairy products, both in animals (mini-pig) (Meisel, 1986) and in humans (Svedberg et al., 1985).
Opioid peptides have also been revealed by immunochemistry in samples of intestinal content collected from healthy volunteers after ingestion of a litre of milk (Svedberg et al., 1985).

Therefore, the release of opioid peptides in the intestinal lumen occurs during digestion of their protein precursors.

4.1.2.2 Passage of opioid peptides across the intestinal mucosa

The normal intestinal mucosa behaves as a barrier which opposes entry into the body of molecules contained in the lumen of the digestive tract, in particular, proteins and peptides.
Cells in the mucosa are closely joined together and allow only small molecules to pass between them. Therefore, paracellular diffusion of a tetrapeptide is much lower than that of a dipeptide (Adson et al., 1994). The administration of increasing length polyethylene-glycol molecules, in humans as well as in animals, demonstrates that their absorption is considerably reduced as molecular weight increases (Amidon & Lee, 1994). Therefore, molecules with a molecular weight greater than 200 may be weakly absorbed by paracellular pathway (Lennernäs, 2007).

In the apical membrane of the enterocyte, opioid peptides (4 or more amino acids) are not uptaken by carriers which ensure the rapid intestinal transport of di and tripeptides across the mucosa (Daniel, 2004; Ganapathy & Miyauchi, 2005). Generally, the efficacy of intestinal peptide transport is inversely proportional to the size of these molecules (Roberts et al., 1999).

Many peptidase-like activities exist in the intestinal epithelium, which can break down peptides with opioid activity, for example β-casomorphins (Kreil et al. 1983). Experiments conducted *in vitro* with oligopeptides (> 4 amino acids) in an intestinal epithelium model (Caco-2 cells in culture) or in a Ussing chamber have confirmed that the sensitivity of peptides to peptidases in the mucosa has a decisive effect on the speed of their transport (Tomé et al., 1987; Shimizu et al., 1997; Iwan et al., 2008).

Passage of small quantities of peptides, or even of entire proteins, can however occur by endocytosis of antigen-presenting cells (or M cells in the Peyer's patches) or by transcytosis, through the enterocytes. A study conducted in humans confirms that absorption of large peptides (24 amino acids) is possible (Chabance et al., 1998). However, incubation of bioactive peptides containing 7 amino acids in the presence of Caco-2 cells in culture suggest that passage of small peptides across the epithelial cell layer may occur in a quantity insufficient to trigger a physiological effect (Vermeirssen et al., 2002).

4.1.2.3 Bioavailability and biological effects *in vivo* of dietary bioactive peptides

As instructive as they may be, *in vitro* studies do not allow the actual bioavailability of dietary bioactive peptides to be evaluated, nor their biological activity *in vivo* in humans.

Angiotensin converting enzyme (ACE) inhibitors have been chosen as a model because they have been studied much more than exorphins as a result of their potential usefulness for millions of persons with arterial hypertension worldwide. They are small peptides (lacto-di and -tripeptides) which can be produced during digestion of protein precursors, in particular resulting from cow's milk. The size of these peptides allows them to be transported quickly by active systems present on the surface of the intestine unlike opioid peptides (Daniel, 2004). A recent double blind, placebo-controlled, cross-over study conducted on healthy volunteers demonstrated that the ingestion of food enriched with lactotripeptides results in a significant change in their plasma concentrations (Foltz et al., 2007). However, the increase in plasma concentrations of bioactive tripeptide in response to the consumption of enriched product remained less than several orders of magnitude than that required to inhibit ACE by 50% (Foltz et al., 2007). Bioavailability of lactotripeptides and their stability in the plasma compartment appears inadequate to obtain an appreciable pharmacological affect *in vivo* (Vermeirssen et al., 2004; Engberink et al., 2008).

Data concerning exorphins are much more limited. Material with an antigenic structure related to β-casomorphins can be detected in the blood of young animals, calves (Umbach et al. 1985) and puppies (Singh et al. 1989), after milk intake. However, these compounds were not found in the plasma of adult animals before, nor even after milk intake.

In humans, Teschemacher et al. (1986) were not able to demonstrate any material antigenically related to β-casomorphins in the plasma of normal subjects, after milk intake. Similarly, Koch et al. (1986, 1988) did not detect any epitope of β-casomorphin in adults, men or women, outside of pregnancy.

Measurement of plasma concentrations of β-endorphins conducted in autistic patients has not resulted in conclusive results: it was either decreased (Weizman et al., 1988), or increased (Sandman, 1988; Leboyer et al., 1994). Results of measurements made in the cerebrospinal fluid were not in agreement either: one study demonstrated a decrease in concentrations of β-endorphins (Gillberg et al., 1990), while another did not demonstrate any abnormality (Nagamitsu et al., 1997). Thus, experimental data do not confirme the existence of abnormal concentrations of opioid peptides in the plasma or the nervous system of autistic patients.

In the neonate, crying induced by blood sample collection with a lancet can be attenuated by intake of milk (Blass, 1997), but the role of opioid peptides has never been confirmed by use of an antagonist.

In adult subjects, no data exist demonstrating any systemic opioid effect related to intake of milk, casein or its hydrolysate (Teschemacher, 2003). Moreover, the use of opioid peptides in clinical pharmacology has resulted in a failure, in particular as a result of the absence of their activity after oral dosing (*in* Janecka et al., 2008). Therefore, it remains to be demonstrated that intake of exorphins or their dietary precursors can have a systemic effect in humans (Teschemacher, 2003).
In spite of the fact that they are widely present in food, no evidence exists that exorphins or their precursors administered orally can produce a systemic biological effect in humans.

4.1.2.4 Urinary excretion of opioid peptides

It has been said that autistic subjects could be differentiated from normal development subjects by the presence of abnormal quantities of urinary peptides (abnormal peptiduria). Such peptiduria would make it possible to establish the diagnosis of the disease (Reichelt et al., 1986), or even differentiate progressive subgroups of it. Presented as both the result and evidence of excessive intestinal absorption of peptides released into the intestinal lumen by

digestion of dietary proteins, such peptiduria may also be used to assess the efficacy of a gluten-free and casein-free diet (Reichelt et al., 1986, 1990),
Abnormal in quantitative terms, peptiduria would also be so qualitatively: it could consist of peptides with opioid activity which would reflect their accumulation in the circulating compartment, and therefore probably in the central nervous system (Reichelt et al., 1986, 2003).

The existence of abnormal peptiduria initially was demonstrated by exclusion chromatography (Sephadex G25, separation of molecules by size) (Tristan et al., 1980) and absorbance at 280 nm (detection of elution products). The absorption at this wavelength is essentially the result of aromatic amino acids but does not prejudge the structure in which they are incorporated (unbound or bound to macromolecules, peptides or proteins). Moreover, the existence of abnormal peptiduria in schizophrenia has been suggested by the same team (Reichelt et al., 1981). Subsequently, this technique has lead to differentiate different profiles of urinary peptides in autism (Reichelt et al., 1986) and to suggest that such peptiduria may disappear with a gluten-free and milk-free diet (Reichelt et al., 1990).
This analytical study was further carried out using high performance liquid chromatography (HPLC) with measurement of absorbance at 215 nm (corresponding to peptide bonds) and has led to identify a characteristic urinary profile in autism (Reichelt et al., 1997), whose sensitivity and diagnostic specificity appear exceptional (100 % in both cases) (Reichelt et al., 2003).
Unfortunately, the exact type of urinary peaks has not been characterised and although Reichelt et al. (1997) in fact indicate that they identified casomorphin 1-7, the article mentioned in the reference does not contain this information.

Another team, using similar methodology, has also reported abnormalities in urinary peptide excretion in autistic subjects vs. control subjects, as well as the probable existence of methionine-enkephalin in one of the abnormal peaks (Israngkun et al., 1986). Combining exclusion chromatography and HPLC with absorbance at 215 nm, Cade et al. (2000) also observed abnormal urinary peaks in autistic and schizophrenic patients.
Shattock's team (Alcorn et al., 2004) published similar results as those of Reichelt, but sensitivity and specificity reached only 53 and 75 %, respectively (calculated according to Alcorn et al., 2004). Subsequently, a tryptophan derivative with no opioid effect, indolyl-3-acryloylglycine, seemed characteristic of the urine of autistic subjects (Anderson et al., 2002; Bull et al., 2003). However, this marker has been abandoned because it has no specificity (Whiteley et al., 2003; Wright et al., 2005).

After publishing results similar to those of Reichelt's team, Gillberg et al. (1982) revised their first conclusion and indicated that the new procedures of analysis used did not allow to reproduce the initial results, nor to identify any specific peptide profile in the urines s of autistic subjects (Le Couteur et al., 1988). This result agrees with those obtained by Gilroy et al. (1991), as well as with those of more recent studies (Hunter et al., 2003), which did not find a significant difference between the HPLC profile of urine from autistic patients and of control subjects, a conclusion which KL Reichelt appears to have accepted, at least for certain forms of autism (Sponheim et al., 2006).

In reality, the mixture of low molecular weight peptides in the urine is too complex to be characterised by single-dimension chromatographic techniques. Therefore, using much more sensitive and specific methods than those used to date (tandem mass spectrometry coupled with HPLC; LC-MSMS), Hunter et al. (2003) were unable to reveal the presence of opioid peptides in the urine of autistic subjects. They also illustrated the limits of HPLC in this application: the chromatographic profile remained unchanged in spite of the deliberate addition of opioid peptides (spiking) to the analysed urine samples. Conversely, this spiking was perfectly detected by LC-MSMS.

Dettmer et al. (2007) also looked in the urine of 69 autistic patients and controls for gliadinomorphine, β-casomorphin and deltorphin, using LC-MSMS. This sensitive method (detection limit values: 0.25 ng/mL) did not detect opioid peptides in autistic subjects nor in controls. Recently, a study compared urinary samples of 65 boys with autism to 158 matched controls and could not demonstrate any difference between the HPLC profiles of the two groups (Cass et al., 2008). Furthermore, urinary peaks were analysed by matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF) and no opioid peptide was identified.

Methods of reference currently used for analysis of peptides and proteins have not demonstrated any significant difference between autistic subjects and control subjects in terms of peptiduria. Consequently, the analysis of urinary peptides cannot be considered as a component of the diagnosis of autism as a test useful to its monitoring, nor to the assessment of dietary management.

Furthermore, characterisation of urinary peptides by these methods has not made it possible to identify opioid peptides in the urines of autistic subjects.

4.1.3 Effects of opiate antagonists in autism

The "opioidergic" hypothesis (see 2.1.1 and 4) has led to use opiate antagonists in the treatment of children with autism, and in particular, use of naloxone and naltrexone, which can bind to δ and κ receptors, and mainly to μ.

A recent review of the literature (Elchaar et al., 2006) mentioned relevant data concerning use of naltrexone in children with autism, collected in 8 series of cases and 14 clinical studies.

4.1.3.1 Open-label series

The series of cases consists of a total of 33 subjects, 2 years and 20 months to 17 years of age. Naltrexone was administered at doses ranging from 0.4 to 2 mg/kg, in a single dose or several doses, administered sporadically or daily, for a duration which could reach several weeks.
The most frequently observed benefit was a reduction in self-mutilation behaviour, which moreover was the effect sought. Also mentioned, but less consistently, was a decrease in hyperactivity, stereotypical behaviour, panic attacks and aggressiveness, improvement of social behaviour, an increase in social initiatives and in verbal production.

4.1.3.2 Controlled clinical trials

Fourteen clinical trials are available and results are summarised in table 3. It appears that naltrexone was used in some children with autism as soon as the 3 years of age. Dosages vary greatly, from 0.5 mg/kg once a week, to 2.35 mg/kg in a single dose. The majority of studies used doses of between 0.5 and 2 mg/kg.d in one daily dose. Some results suggest the existence of a dose-effect relationship while the efficacy of treatment seems to reach a plateau at around 1.5 mg/kg.d in other studies. Overall, clinical data suggest that naltrexone decreases self-mutilation in children with autism. Other improvements were reported but inconsistently, concerning hyperactivity, agitation, aggressiveness, irritability, access of rage, social withdrawal, attention, visual contact and stereotypical behaviours (Malone et al., 2005).
It is possible that the benefit of treatment with naltrexone is limited to sub groups of autistic patients, in particular those with self-aggressive behaviour (Parikh et al., 2008). Unfortunately, currently no factor allows therapeutic response to be predicted and no measurable biological correlate of it exists.

Table 2: Clinical trials of naltrexone in autism

Reference	Type of study	Age (years)	Diagnosis, symptoms	Dose of naltrexone	Result	Comments
Campbell et al., 1990	DB, VP, N=18	3-8	Autism, MR	0.5-1mg/kg.d for 21 d	CGCR: improvement CPRS: ↓ hyperactivity CGI: no change	No improvement in learning
Leboyer et al., 1992	R, DB, VP, CO N=4	4,12, 12, 19	Autism, SAB, MR	0.5, 1 or 2 mg/kg/d in 2 divided doses for 7 days	Improved socialisation, ↓ SAB and agitation.	Dose-dependent response
Campbell et al., 1993	R, VP, N=41	2.9 – 7.8	Autism	0.5 to 1 mg/kg.d after 7 d for 21 d	↓ hyperactivity and ↓ SAB (rebound effect on discontinuation)	No improvement in central symptoms of autism
Ernst et al., 1993	N=5 See Campbell et al., 1993				Positive correlation between BEP, hetero-aggressiveness and stereotypical behaviours	
Gonzalez et al., 1994	See Campbell et al., 1993				No correlation between serum concentrations of naltrexone and response	
Willemsen et al., 1995	R, DB, VP, CO, N=20	2.8-7.4	Autism	40 mg (1.48-2.35 mg/kg) single dose	No improvement in stereotypical behaviours nor social behaviour; ↓ hyperactivity, access of rage, improvement in attention, activity, irritability	
Bouvard et al., 1995	R, VP, DB, CO N=10	5 - 14	Autism (6 SAB)	0.5 mg/kg.d, 30 d	CPRS : improvement in hyperactivity, hostility. BSE: improvement in sociability, communication, attention;	
Kolmen et al., 1995	DA, VP, CO N=13	3.4 – 8.3	Autism	1 mg/kg.d, 14 d	CGI (parents and teachers) improvement, hyperactivity, improvement	Variability of response
Willemsen et al., 1996	R, VP, DB, CO N=20	2.8- 7.4	Autism	20 mg, 40 mg (0.74-1.18 mg/kg.d) for 28 d	Improvement in behaviour:↓ Hyperactivity, irritability	
Scifo et al., 1996	R, VP, CO, N=12	7-15	Autism	0.5-1.5 mg/kg.48 h for 15 weeks	↓ stereotypical behaviours, social withdrawal, improvement in attention and eye contact.	Dose-dependent effects
Kolmen et al, 1997	see Kolmen et al., 1995				Hyperactivity, attention: improvement	No improvement in learning
Cazzullo et al., 1999	Open-label N=11	3.2-8.11	Autism	2 mg/kg.d for 3 months	↓ SAB associated with improvement of social functioning and behavioural problems; ↓ BSE/IBSE	
Feldman et al., 1999	See Kolmen et al, 1997 N=24				No improvement in communication between parent and child	
Willemsen et al., 1999	See Willemsen et al., 1996 N=6 (study respondents)			20 mg for 6 months	No maintenance of improvement in hyperactivity	

BEP: β-endorphin; DB: Double Blind; VP: Versus Placebo; MR: Mental retardation; R: controlled randomization, CO: cross-over, SAB: Self-aggressive behaviour

The fragility of these results should be emphasised, in particular as a result of the absence of homogeneous methods of measurement of efficacy of naltrexone, and the high risk of carry over in crossover studies which comprised the essential part of data. In addition, these treatments were evaluated over short periods, such that their long term effects are unknown.

4.1.3.3 Conclusion

The majority of studies conducted to date have not demonstrated any change in symptoms in the autism triad in response to opiate antagonists. It is possible that the benefit of treatment with naltrexone is limited to subgroups of autistic patients, in particular those with self-aggressive behaviours.

The hypothesis of a primordial role of opioid peptides in the occurrence and/or course of autistic disorders appears barely compatible with the absence of an effect of opiate antagonists in the clinical triad characteristic of autism.

4.2 Autism, gastrointestinal disorders and change in intestinal permeability

The association between autism and gastrointestinal disorders was suggested very early, in particular in relation to coeliac disease (see. 2.1.1). The existence of abnormalities of the digestive tract remains an add-on hypothesis to the opioid theory. It postulates that digestive tract lesions, or more simply an increase in intestinal permeability, contribute to passage of dietary exorphins in excessive quantities into the blood and into the central nervous system (Reichelt et al., 1986).
Therefore, the question raised is to determine whether autism is associated with specific gastrointestinal disorders (coeliac disease, chronic inflammatory bowel disease, etc) or if autistic children have gastrointestinal disorders with a specific incidence.

4.2.1 Autism and specific gastrointestinal disorders

4.2.1.1 Autism and coeliac disease

Coeliac disease is a chronic enteropathy, the result of digestive hypersensitivity to gluten, occurring in genetically predisposed subjects. It is characterised by a malabsorption syndrome, secondary to atrophy of the duodeno-jejunal mucosa. The main symptoms of it are diarrhoea, weight loss and growth retardation. Its diagnosis is based on the existence of histological lesions of the mucosa and their disappearance in response to a gluten-free diet.

The chance discovery of coeliac disease in a 6-year-old boy with autism (Goodwin & Goodwin, 1969) and the apparent improvement in his behavioural disorders after initiation of a gluten-free diet raised the possibility of a relationship between the two diseases (Goodwin et al., 1971) (cf. 2.1.1).
This eventuality may seem all the more plausible, since coeliac disease itself can induce neurological and psychiatric disorders (Asperger, 1961; Bushara, 2005).

To demonstrate such an association, it is necessary to demonstrate the coexistence of signs of the two diseases in a given subject. In this regard, it should be kept in mind that growth retardation in terms of height and weight, which accompany symptomatic coeliac disease, in particular during the first years of life, appears rare in autism (see. 3.1.2).
McCarthy & Coleman (1979) studied 8 autistic children whose parents had observed an improvement in their behaviour during treatment with a gluten-free diet. Each child received a gluten load (20 g/day) for 4 weeks. This load did not produce any appreciable change in body weight nor intestinal disorders. In particular, no histological abnormality was detected in a biopsy of the jejunum, which led to rule out the diagnosis of coeliac disease. Furthermore, no major change in behaviour was observed during this loading dose test. Similarly, a cohort

study of 148 children with PDD did not reveal any serological and histological signs of coeliac disease (Wakefield et al., 2005).
Conversely, Pavone et al. (1997) sought autistic symptoms in 120 children with coeliac disease (62 boys and 58 girls, 2.6 to 16 years of age, mean age 9.6 years). Three groups were comprised: patients before a dietary regimen, patients with a strict gluten-free diet and patients with a diet which was little followed. None of the 120 children had symptoms of autism.
The authors simultaneously sought markers of coeliac disease (anti-gliadin and anti-endomysium antibodies) in 11 autistic children (9 boys, 2 girls, 3 to 12 years of age, mean age 7 years). No case of coeliac disease was demonstrated. Biopsies of the jejunum were normal.

The existence of neurological disorders in children or young adults with coeliac disease also was sought by Zelnik et al. (2004). This study involved 111 patients (mean age: 20.1 years) and 211 control subjects matched for age and sex. Although neurological disorders (hypotonia, cerebellar ataxia, epilepsy or headache) were more common in patients with coeliac disease (51.4 %) than in control subjects (19.9 %), this study did not demonstrate an association between coeliac disease and autism.

Analysis of clinical descriptions also allowed an approach to this question. In 2000, Gillberg and Billstedt examined different co-morbidities reported in autistic subjects. It emerged from this analysis that coeliac disease did not occur among the disorders associated with autism or Asperger's syndrome in all of the disorders reported. Lastly, a recent review of neurological and psychiatric syndromes associated with coeliac disease did not reveal a link between this disorder and autism (Bushara, 2005)**.**

Clinical studies, like the analysis of symptomatic presentations of autism and of coeliac disease, indicate that there is no link between autism and coeliac disease and that their co-existence is random.

4.2.1.2 Autism and inflammatory digestive tract diseases

CIBD
The possible association between autism and chronic inflammatory bowel disease (CIBD) was suggested by Wakefield et al. (1998). A survey, conducted in 3 French departments to evaluate the nature of mental and medical disabilities in a population of school children 6 to 16 years of age identified one case of Crohn's disease and one of ulcerative colitis, but none of them involved autistic children (Fombonne et al., 1997). A similar study, conducted on a larger sample size in the London metropolitan area, confirmed that cases of CIBD identified did not affect subjects with autism (Fombonne, 1998). These epidemiological surveys did not show the existence of an association between autism and CIBD.

Enterocolitis of autism
Wakefield et al. (1998) reported the clinical characteristics of 12 children with PDD and referred to a department of paediatric gastroenterology for management of gastrointestinal disorders (pain, diarrhoea, etc.). In reality, the principal disorder present in almost all these children was severe constipation with major dilation of the recto-sigmoid colon (megarectum) (Murch et al., 1998). Ileocolonic endoscopy was normal in 3 of them and showed nodular lymphoid hyperplasia (NLH) in the ileum and/or colon in 9 others. Histology confirmed the existence of ileal lymphoid hyperplasia in 7 children and revealed a more or less intense infiltration of the colonic mucosa with monocytes and lymphocytes.
This first report was supplemented by a new article (Wakefield et al., 2000) on 60 children (3 to 16 years of age) including those who appeared in the initial description. Endoscopy of the ileum and colon was carried out in each of them. Contrary to the first study which did not have a control group, endoscopic data were compared to those in 37 children with normal development and those suspected of having CIBD. Histological results were compared to

those of 22 children (out of the aforementioned 37) without CIBD and to those of 20 children with ulcerative colitis. Endoscopy revealed a significant increase in nodular lymphoid hyperplasia in the ileum (93% of children with PDD *vs*. 14% for controls) and the colon (30% *vs*. 5% of control subjects without CIBD). Microscopically, follicular hyperplasia existed in nearly 90% of biopsies of the ileum performed in children with PDD and in almost 30 % of biopsies of children with ulcerative colitis. No follicular hyperplasia was observed in control subjects without ulcerative colitis. An inflammatory infiltrate was also observed in 88% of the 60 children with PDD, in less than 5% of children without CIBD (1/22) and in all children with ulcerative colitis. As in the first article, the authors concluded in the existence of a chronic inflammatory enterocolitis associated with autism. In addition, they suggested that it may involve a specific group of children by their symptoms and the origin of the disorders. In fact, one of the hypotheses considered questioned the mumps, measles and rubella vaccination. This suggestion has been the subject of a retraction (Murch et al., 2004).

These two published reports received a follow-up on 148 children with PDD (including children included in the first two studies) and 30 control subjects (Wakefield et al., 2005). In children with PDD, the most frequent symptoms were constipation (51%), diarrhoea and alternating diarrhoea and constipation. In case of diarrhoea, x-rays of the abdomen revealed major stercoral stasis and symptoms improved with use of enemas and laxatives. This team had previously reported that the majority of parents observed a spectacular improvement in their child's behaviour following intestinal preparation for colonoscopy, a sort of "honeymoon" which seemed to continue as long as return to constipation was avoided (Murch et al., 1998). Endoscopy and histology confirmed the existence of NLH of the ileum and/or colon. The inflammatory infiltrate affected 22% of children with PDD in the ileum but was much more common in the colon.
Nearly half of the children included (69) followed a gluten-free and/or casein-free diet, with compliance with the regimen being very good. In these children, NLH of the ileum was as common as in those who followed a normal diet. NLH of the colon was even more frequent with an avoidance diet than with a normal diet, but with no difference depending on type of avoidance.
All these studies resulted in the conclusion that autistic children with gastrointestinal disorders (mainly severe constipation) present with nodular lymphoid hyperplasia of the ileum and/or colon. It is not possible to extrapolate these results to all subjects with autism, because inclusion of these children corresponded to their transfer to a department of paediatric gastroenterology for management of gastrointestinal disorders, which is a major selection bias. In addition, these lesions were not present in all autistic children with GI disorders. For example, comparison of endoscopic and histological data in another cohort (6 children with PDD) to those of 9 control subjects matched for age did not reveal any histological abnormality of the colon (DeFelice et al., 2003). Moreover, the extent of disorders was qualified in another endoscopic and histological analysis conducted on 21 children with PDD (Furlano et al., 2001). The authors recognised that endoscopic lesions of the mucosa and histological alterations of the colon are subtle, such that this raises a real difficulty in establishing its diagnosis.
Lastly, it should be noted that NLH is routinely observed, in particular in young children, and that it is not always associated with clinical symptoms (Riddlesgberger & Lebenthal, 1980). Walker-Smith et al. (1983) moreover indicated that this symptom can be considered as non serious, in light of its frequency in asymptomatic children. Its association with gastrointestinal disorders such as abdominal pain or diarrhoea has often seemed to be a coincidence, and some authors consider that NLH is not a disorder (Williams & Nicholls, 1994). Long term follow-up of children with NLH, moreover, has not revealed any sequelae (Colón et al., 1991).
The existence of NLH in children, apart from any disorder, suggests that this manifestation is not specific to autism. A retrospective analysis of endoscopic and histological data accumulated in a continuous series of 140 children with normal development showed that 67% of them did not present with any sign of colonic inflammation (Kokkonen & Karttunen,

2002). However, among these, nearly 40 % had NLH of the colon, and 85% of those with involvement of the ileum presented with NLH in the ileum. Some of these children also underwent oesogastroduodenoscopy. Twelve of 22 subjects presenting with NLH of the colon also had NLH of the duodenum. This retrospective study confirmed that NLH is a frequent endoscopic and histological manifestation in childhood, in particular in the ileum.

Teams working on chronic inflammatory enteropathy in autism have also sought to characterise the type of immune cells present in the different levels of the digestive tract. The baseline histological aspects are relatively contradictory; sometimes normal in the duodenum (Torrente et al., 2002), on the contrary showing gastritis (Torrente et al., 2004) or an inflammatory infiltrate in the oesophagus, stomach, duodenum, ileum and colon (Ashwood et al., 2003). Inflammation of the mucosa presented as spreading to the entire GI tract was again qualified as subtle (Torrente et al., 2004). A published report from the same group reporting the results of oesogastroduodenal endoscopy conducted on 74 children with PDD also qualified the previous results: endoscopic and histological anomalies appeared as moderate and their frequency was similar in children with PDD and control subjects (Wakefield et al., 2005).
Lastly, Horvath et al. (1999) reported the coexistence in 36 children with PDD of an increased response to secretin (27 times), reflux oesophagitis (25 times), duodenitis (25 times), reduction in activity of intestinal disaccharidases (21 times) and chronic gastritis (15 times). However, it is difficult to judge the clinical significance of an inflammatory infiltrate observed in these children due to the absence of a control group. In addition, disaccharidase activity was considered abnormal when it was less than the standard error of the mean of normal values, a level which does not have functional relevance.
All these studies draw attention to the existence of GI disorders in children with PDD. Unfortunately a recruitment bias does not allow the type or prevalence to this total population to be extrapolated. However, the majority of articles put severe constipation in the front line of symptoms and several report the benefit which apparently can be obtained from its treatment in terms of behaviour.

Observations
The reality of chronic inflammatory enterocolitis, which would be a new intestinal inflammatory disease, affecting a subpopulation of autistic children (with regression) (Ashwood et al., 2003), was not demonstrated by endoscopic and histological analysis. Analysis of published data on this topic between 1965 and 2005 is not in support of the existence of a chronic inflammatory bowel disease specific of autism (MacDonald & Domizio, 2007). Lastly, the occurrence of regression does not appear associated with a specific medical disorder (Ming et al., 2008).

4.2.2 Autism and food allergies

Bidet et al. (1993) apparently were the first to report the results of basophil degranulation tests *in vitro* in autistic children (DMS III). Five environmental or dietary antigens were evaluated in 10 autistic patients and 10 control subjects, 6 of whom with psychiatric disorders. Placed in the presence of these antigens, the basophils from 7 autistic patients out of 10 reacted, and for 5 of them, against at least one dietary antigen (milk and/or egg). On the contrary, only one control subject presented a positive reaction against an environmental antigen. These data suggest that autistic subjects may be sensitised to food antigens. However, these results have been obtained *in vitro* on a very small number of subjects whose screening criteria are unknown.

In the setting of a study of exclusion of milk proteins (see. 2.2.6), Lucarelli et al. (1995) studied susceptibility to dietary allergens (casein, lactalbumin, β-lactoglobulin, egg, rice, soy) by intradermal injections in 36 autistic children (8 to 13 years of age) and 20 control children (5 to 14 years of age). Thirteen autistic children out of 36 developed a positive skin response,

vs. one child out of 20 in the control group. Plasma concentrations of specific IgE of the tested antigens were similar in the two groups but total IgE were higher in the autistic children. Skin tests suggest that a small group of autistic children may present with hypersensitivity to dietary proteins. However, no information exists on the existence of allergic symptoms, nor even of gastrointestinal disorders in the studied children in support of this hypothesis.

Thirty autistic children (DSM-IV; 2 to 4 years of age) were compared to a control group of 39 children matched for age and sex and recruited in the department of neurology of the same Turkish hospital for problems of seizures, epilepsy or delayed development (Bakkaloglu et al., 2008).
A validated questionnaire was used to detect immediate hypersensitivity reactions in autistic subjects and in control subjects and intradermal injections were given with 12 dietary and environmental antigens solely in the autistic children. Thirsty percent of the autistic children and 2.5% of the controlled subjects had a family history of atopy. The incidence of a previous history of allergy in autistic children seems to correspond to that observed in the Turkish population. Skin tests were positive (at least one reaction) in 48% of autistic children. These reactions were triggered by environmental antigens and not by dietary antigens. The frequency of responses and antigens involved were comparable to what was reported in Turkish children 2 to 3 years of age. Plasma concentration of IgA, IgG and IgM were within normal limits, while concentrations of IgE were elevated in 13.3% of children (4/30 children). However, these 4 children did not have IgE specific of antigens which produce a cutaneous response. This study indicates that immediate hypersensitivity reactions may be present in a certain number of autistic children but that their frequency is no higher than that in the general population. On the contrary, the frequency of allergic reactions in this population of autistic children was lower than that expected according to their familial history of atopy. Lastly, only environmental antigens and not dietary antigens induced a hypersensitivity skin reaction.

Some studies have characterised the response *in vitro* of innate and acquired immune cells to dietary antigens, in particular to milk proteins and gluten. A first study on 71 children with PDD, 23 brothers and sisters and 17 unmatched control subjects, demonstrated *in vitro* a higher production of pro-inflammatory cytokines by circulating mononuclear cells (CNC) in children with PDD than in control subjects, in the absence of any antigenic stimulation (Jyonouchi et al., 2001). This study was followed by a second study on 72 children with PDD, 26 brothers and sisters, 15 control subjects not matched and 24 children with dietary protein intolerance (DPI) (Jyonouchi et al., 2002). The frequency of atopy among children with PDD was 23 %, that is, the same order of magnitude as that observed in brothers and sisters and the control subjects. In the group of autistic children, cytokine production with CMC exposed to dietary proteins *in vitro* (gliadin, cow's milk protein and soy) was higher for IFNγ and TNFα (Th1 cytokines involved in the cell mediator response), which was not the case with interleukin-5 (Th2 cytokine implicated in humoral mediated response). This response was comparable to that observed in the group of children with DPI. It should be noted that parents, teachers or healthcare staff had previously reported an improvement in behaviour following adoption of a casein-free, gluten-free or soy diet in 56 children with PDD out of 60. However, this article does not provide any information on existence of GI disorders in autistic children.
Another study was conducted in 90 children with PDD, separated according to existence (n=71) or absence (n=29) of GI disorders, 13 non-matched control subjects and 14 children with DPI; all children were on a normal diet (Jyonouchi et al., 2005a). Concomitantly, 77 children with PDD with (n=68) or not (n=9) GI disorders and 16 with DPI were included; all these children were following a restricted diet. Here too, the prevalence of signs of atopy among children with PDD was similar to that of the normal population. CMC were incubated *in vitro* with bacterial lipopolysaccharide (LPS) (innate immunity) and production of diverse cytokines was measured. The authors indicate that stimulation of CMC by LPS results in

increased production of cytokines in children with PDD or DPI, a statement which is difficult to accept, at least regarding IL-10. No obvious difference in response existed between children on a normal diet and those who were following a restriction diet. Among children with PDD, the responses did not seem very different depending on whether gastrointestinal disorders existed or not. The use of dietary antigens was reported but results were not provided.
This study was followed by another published report on 109 children with PDD (75 with GI disorders and 34 without), 19 unmatched control subjects and 15 children with DPI (Jyonouchi et al., 2005b). The prevalence of atopy among children with PDD was similar to that of the general population. CMC were incubated *in vitro* in the presence of dietary protein (raw cow's milk proteins, β-lactoglobulin, α-lactalbumin, gliadin) and production of various cytokines was measured. CMC of children with PDD produced more TNF-α and IL-12 than control subjects. CMC of children with DPI produced more IFN-γ, TNF-α, IL-10 and IL-12 than control subjects. The authors suggest that GI symptoms in PDD children may be related in part, to non-allergic dietary hypersensitivity to milk proteins, and in particular β-lactoglobulin and α-lactalbumin. The production of TNFα and IL-12 under stimulation by gliadin was less frequent and less intense than with the previous antigens. Lastly, casein induced only a weak response, with no difference groups of children with PDD, DPI or control subjects.

Observations
Clinical data in these studies indicate that the prevalence of atopic disorders is no different in children with PDD than what it is in the general population. Studies conducted on CMC *in vitro* suggest that some children with PDD have non-allergic hypersensitivity to some dietary antigens. Moreover, β-lactoglobulin and α-lactalbumin may be more often implicated than casein, and in particular gluten.
However, this type of study leads to formulate certain reservations. First, stimulation of CMC *in vitro* and detection of cytokines in the culture supernatant is not considered as a reference technique for the study of dietary allergens (Murch, 2005). Second, it is difficult to extrapolate results obtained *in vitro* to clinical manifestations. The fact that a difference in secretion of certain cytokines in the presence or absence of GI disorders is low or even non existent confirms that the clinical significance of this study is not clear.
In this regard, it is necessary to keep in mind that only a clinical response to a loading dose test conducted according to a double blind design, with use of a placebo, makes it possible to characterise food intolerance or allergy (Niggeman & Beyer, 2007).

4.2.3 Prevalence of GI disorders in autistic children

Available data have been acquired either in population surveys or during a count of symptoms in gastroenterology consultations.

4.2.3.1 Population surveys

Black et al. (2002) sought to determine the prevalence of GI disorders (chronic inflammation, coeliac disease, food intolerance, but also diarrhoea, vomiting, etc.) in autistic children before the diagnosis of their disorder, to compare this frequency with that observed in the general population of children of the same age and with normal development. This study conducted based on a "UK general practice database" involved all children born after 1 January 1988 and recorded in this database within 6 months of their birth (n = 211,480). Autistic children were identified *post hoc.* Data were collected from dossiers kept by general practitioners and were completed if necessary by archives of specialist or hospital consultations. Therefore, this procedure did not require the memory of the children's parents.
Nine percent (9/96) of autistic children had presented with GI disorders before the diagnosis of autism was established. The population of children with normal development (controls

matched for age and sex) presented an identical prevalence of GI disorders (41/449). According to these results, autism does not appeare as a risk factor for GI disorders.

Fombonne and Chakrabarti (2001) sought to clarify the links existing between autism, MMR vaccination and gastrointestinal disorders. Their investigation involved a geographically defined population of 15,500 children, born between 1992 and 1995. Among these children, 96 presented with PDD (including 26 typical autistic, and 56 atypical autistic, 13 children with Asperger's syndrome and one with disintegrative syndrome). Medical data, and in particular information on GI disorders, were obtained form questionnaires sent to paediatricians as well as to parents. This study indicates that GI symptoms were present in 18.8% of children with PDD. Constipation was the most commonly identified problem since it was present in 9.4% of cases.

Taylor et al. (2002) used computerised data on children born between 1979 and 1998 and associated with 5 areas of healthcare services in northeast London. Only GI disorders of duration of more than 3 months were noted. Out of 473 children with a typical (n=278) or atypical autistic disorder (n=195), 8.8% had chronic constipation, 4% diarrhoea and 1.5% both. Some children also had food allergy (7/473), or again non-specific colitis with nodular lymphoid hyperplasia (2/473). In summary, 17% of children presented with intestinal disorders.

Lastly, a study published in 2003 by Molloy et al. took into account children 24 to 96 months of age, recruited in a US clinic specialising in autism and related disorders. Follow-up and recording of GI disorders was carried out by a medical team according to strict definition. Out of the 137 autistic children evaluated, 24% had a previous history of at least one chronic GI symptom. As in the two previous studies, the most frequently observed symptoms were diarrhoea (17%) and constipation (17%). This study did not demonstrate an association between chronic GI symptoms and a regression of development.
The last 3 studies provide estimates of the incidence of GI disorders in autistic children that were relatively similar (17, 18.8 and 24%, respectively). However, they did not include a control group and therefore do not allow conclusions on the significance of the figures.

Results by Fombonne and Chakrabarti (2001) and Taylor et al. (2002) produced an estimate of the frequency of constipation, the most common symptom in their study, which was approximately equivalent to that found in a population of children with normal development (Loening-Baucke, 1998).

In a study published in 2006, Valicenti-McDermott et al studied the frequency of GI symptoms in 50 autistic children, 50 children with normal development and 50 children with a "mental disability". The 3 groups were matched for age, sex and ethnic background. Autistic children were followed in the setting of the program of neurology and paediatric development at the Albert Einstein Medical School (New York) and were diagnosed according to DSM-IV-TR criteria. Data on GI disorders were obtained form questionnaires from patients. Results obtained showed a higher incidence of GI symptoms in autistic children (70%) than in a group of children with normal development (28%) or with a mental disability (42%). Constipation was the most frequently reported GI disorder (44% of autistic children). A high selectivity of foods was also recorded in 60% of autistic children. However, it should be noted that - unlike previous published reports - this was a cross-sectional study involving a limited pre-selected population of autistic children and that the reality of symptoms reported in the questionnaire was not verified. Therefore these results are to be taken with reservation.

Observations
Overall, population studies suggest that the frequency of GI disorders in autistic children does not appreciably depart from that of the normal population (Kuddo & Nelson,

2003).Moreover, this is the conclusion of the only controlled study available to date (Black et al., 2002).

4.2.3.2 Surveys based on gastroenterology consultations

It is not possible to incorporate in this evaluation the studies conducted in departments of paediatric gastroenterology. In fact, they resulted in very high frequencies of GI disorders [46% to 84%] (table 1) which may simply translate a major recruitment bias.

Table 3: Frequency of GI disorders in autistic children.

Authors	Source of studied population	PDD/Control subjects	Control group with comparable sex and age	Prevalence of GI symptoms PDD Controls
Population studies				
Black et al., 2002 Before diagnosis	Database of General Practice Research n=21 480	96/449	Yes	9% * 9%
Fombonne and Chakrabarti et al. 2001	Geographical zone n=15 000	96/0	No	18.8% ND
Taylor et al., 2002	5 "healthcare zones"	473/0	No	17% ND
Molloy et al. 2003	n=151 000	137 (24 to 96 months)	No	24% ND
Gastroenterology recruitment				
Horvath et Perman, 2002 (Revue)	?	112 /44	Siblings	76% 30%
Afzal et al., 2003	Department of paediatric gastroenterology	103/29	No	36% 10% Severe constipation

4.2.4 Autism and intestinal permeability

The increase in intestinal permeability alone or associated with a GI disease is one of the complementary hypotheses of the opioid theory.
Intestinal permeability translates the capacity of components of the intestinal lumen to abnormally cross the mucosa through tight junctions (see. 4.1.3). It can be evaluated with a lactulose-mannitol test. In this test, 2 sugars are administered simultaneously in an oral solution: D-mannitol (182.2 g/mole) makes it possible to investigate permeability to small molecules and lactulose (342.3 g/mole) that of larger molecules. These substances are not metabolised and therefore are excreted in the urine. Their concentrations then can be measured by gas chromatography. Results are expressed as the percentage of the dose administered and as a ratio of these 2 percentages.
There are few data on intestinal permeability in autistic subjects. The first study on this subject was published by Eufémia et al. (1996). In this article, the authors showed an increase in the lactulose/mannitol ratio after a permeability to sugar test in 9 autistic subjects out of 21, while the ratio was not increased in any of the 40 children in the control group. Autistic children who underwent this test did not have known intestinal disorders and were 4 to 16 years of age. Control subjects were of similar age and did not have a known GI disorder. A change in ratio was related to an increase in permeability to lactulose.
Horvath et al. also demonstrated a lactulose/mannitol ratio which was increased in 76 % of the 25 autistic children tested (2002). This study did not include a control group and the autistic children presented GI disorders whose type was not specified.

This study was repeated in 14 autistic children with a previous history of GI disorders. Seven brothers and sisters and 8 unmatched children, all with normal development and normal health, were enrolled as a control group. Contrary to the previous studies, the oral load of lactulose-mannitol did not demonstrate a difference between autistic children and a control

group (Robertson et al., 2008), a result confirmed by another loading test with lactulose-mannitol carried out on 23 children (mean age about 10 years) (Kemperman et al., 2008).

No concurring data exist in support of an increase in intestinal permeability in autism.

4.2.5 Conclusion

No item exists indicating that autism is associated with chronic inflammatory digestive diseases and its coexistence with coeliac disease is only a random finding (see. 4.2.1).
It is also very difficult to defend the reality of a chronic inflammatory disease which may be specifically associated with autism or even one of its sub groups (see 4.2.1.2.2).

The prevalence of allergy to dietary antigens seems similar to that of the general population (see. 4.2.2).

The most recent results did not show any manifest alteration in intestinal permeability (see 4.2.4).

Lastly, available data do not confirm that the prevalence of GI disorders in autistic children is higher than that observed in the population of children with normal development (see. 4.2.3; Kuddo & Nelson, 2003; Erickson et al., 2005).

The principle merit of all of these studies is to draw attention to simple GI disorders which children with PDD may suffer from. It is striking that many articles place severe constipation in the front line and that several report the benefit in terms of behaviour that can apparently be obtained by its treatment.

The difficulty in comprehending GI disorders in autistic patients and their possible effects on behaviour makes it worth to consider evaluation of GI function when these children present with major behavioural changes.

5 CONCLUSIONS

5.1 Efficacy and safety of a gluten-free, casein-free diet in autism

5.1.1 Efficacy of the gluten-free casein-free diet

It was possible to identify 9 articles, relating to 8 studies, whose objectives were to evaluate the effects of a gluten-free and/or casein-free diet in autistic children. Four articles came from a same group.
Six of the 8 studies presented defects in methodology such that their results cannot be taken into consideration. A seventh study applied a methodology which was missing from the previous ones: a control group (autistic children without a diet) and treatment allocation by randomization. However, the treatment was conducted with the knowledge and participation of parents and teachers who were also directly involved in evaluation of the results.
Only one study meets minimum criteria required for a quality clinical trial: a control group (autistic children without dietary intervention), allocation of treatment by randomization and double blind method. This study did not show any influence of diet on autistic symptoms.

Therefore, current scientific data do not make it possible to conclude in a beneficial effect of a gluten-free, casein-free diet in the course and outcome of autism.

5.1.2 Safety of a gluten-free, casein-free diet

No data are available on growth or nutritional status of autistic children subjected to a gluten-free, casein-free diet. Therefore, it is impossible to contend that such a diet has no harmful effect in the short, medium or long term.
The set-up of an avoidance diet (for example, exclusion of gluten in coeliac disease) always contains a risk for the nutritional status and growth of a child.

The simultaneous exclusion of two important food groups (those which contain gluten and those which contain cow's milk proteins) can only appreciably increase this hazard, insofar as this diet may have an unfavourable influence on eating patterns in autistic children.

We should insist on the fact that there is no reason to encourage this type of diet. However, if such a diet is initiated, the potential nutritional effects require attentive monitoring by qualified doctors. The occurrence of undesirable nutritional effects should lead to abandon a diet from which no benefit can be expected.

5.2 Indirect evidence proposed in support of a gluten-free, casein-free diet

5.2.1 Gluten-free, casein-free diet and dietary exorphins

Theoretically, a gluten-free, casein-free diet has the aim of eliminating from the diet the precursors (gluten and casein) of opioid peptides (exorphins), whose presence in excess in the brain might be responsible for symptoms of the disease. In reality, a gluten-free, casein-free diet hardly eliminates all sources of exorphins. In light of current knowledge, the actual exclusion of proteins containing peptide sequences with opioid activity would involve exclusion of almost all foods, including human breast milk, in which precursors and free forms co-exist.
The existence of peptides in the urine, in particular opioid peptides, was presented as evidence of their passage in abnormal quantities into the blood of autistic subjects and as a characteristic of the disorder. Reference methods currently used for analysis of peptides and proteins have not demonstrated any significant difference between autistic subjects and control subjects in terms of peptiduria. Furthermore, characterisation of urinary peptides by the same methods has not made it possible to identify opioid peptides in the urine of autistic

subjects. Consequently, analysis of urinary peptides cannot be considered as a component of the diagnosis of autism nor as a test useful for its monitoring or evaluation of its management.

Lastly, the majority of studies conducted to date have not demonstrated any appreciable change in symptoms in the autism triad in response to opiate antagonists. The hypothesis of a central role of opioid peptides in the occurrence and/or course of autistic disorders appears difficultly compatible with the absence of an effect of opiate antagonists on the clinical triad that is characteristic of autism.

5.2.2 Autism and gastrointestinal disorders or intestinal permeability

Some published reports suggest that gastrointestinal disorders and/or intestinal permeability are especially frequent in autism. This would promote the passage of exorphins into the blood.

No data shows that autism is associated with chronic inflammatory gastrointestinal disorders and its coexistence with coeliac disease is only a chance finding. It is also very difficult to defend the reality of an inflammatory disease which may be specifically associated with autism, or even one of its sub groups. In addition, the prevalence of allergy to food antigens appears similar to that of the general population and the most recent results did not show any manifest alteration in intestinal permeability.

Lastly, available data do not make it possible to confirm that the prevalence of GI disorders in autistic children is greater than that observed in the population of children with normal development.

In conclusion, current scientific data do not permit to conclude that the gluten-free, casein-free diet has any beneficial effect on the course and outcome of autism.

It is impossible to contend that this diet has no harmful effects in the short, medium or long term.

Indirect evidence (excess levels of exorphins, abnormal peptiduria, concomitant GI disorders, in particular) proposed in support of this type of diet are not supported by validated facts.

Therefore, there is no reason to encourage the use of this type of diet.

Doctors should be better informed of the type of alternative managements used in autism to be able to freely discuss this topic with parents of autistic children. This would make it possible to respond in part to their need for information and, in case of use of alternative management, to avoid such management being carried out without any medical supervision (American Academy of Paediatrics, 2001).

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Annex 1: Selected interventional studies

	N	Age	Type	Control group	Construction			Assessment criteria
Goodwin et al., 1971	*15*	*6 - 13 years*	*Open-label*	*14 brothers and sisters*	*Crossover treatment, order randomly drawn*	*Gluten or saccharose load (1g/d)*		*electroencephalography; behaviour; histamine test; plasma cortisol*
Bird et al., 1977	*1*	*9 years*			*Alternating diet with and without gluten*			*behaviour*
O'Bannion et al., 1978	*1*	*8 years*			*4 phases*	*4 days normal diet, 4 days of fasting, 18 days of loading with various foods*		*behaviour*
McCarthy & Coleman, 1979	*8*	*Not specified*	*Open-label*	*No*	*Gluten load*	*20 g gluten/d*	*4 weeks*	*Clinical Intestinal biopsy*
Reichelt et al., 1990	*15 DMS III*	*3-17 years 4 with anti-epileptic treatment*	*Open-label*	*No*	*According to peptiduria profile without gluten/reduced casein without casein/or reduced gluten without gluten and without casein Compliance unknown*	*N 8 3 4*	*1 year of diet worsening of behavioural disorders after 5 weeks: thioridazine (50mg/d) for 2 to 3 months*	*Peptiduria Questionnaire for parents and teachers with retrospective comparison*
Knivsberg et al., 1990	*15 DSM III*	*6-22 years 4 with anti-epileptic treatment*	*Open-label*	*No*	*According to profile of peptiduria without gluten/reduced casein Without milk and/or reduced gluten Compliance unknown*	*N = ?*	*1 year of diet 2 discontinuations of diet*	*DIPAB C-Raven-illinois test - Tafjord*
Sponheim E, 1991	*4 3*	*17-33 years 8-12 years*	*4 in open-label 3 with a load (gluten or placebo with blind method)*	*No*	*Diet without gluten: 6 months conducted after 1 year of a gluten-free diet*			*Behaviours Visual Analogues Scale or RLRS*
Knivsberg et al., 1995	*15 DSM III R*	*6-22 years*	*Open-label*	*No*	*According to profile of peptiduria see. Knivsberg et al., 1990*		*4 year follow-up 6 abandonments of diet*	*Peptiduria DIPAB Tafjord illinois ITPA (10) C-Raven (15) Parents and teachers*
Lucarelli et al., 1995	*36 DSM III R*	*8-13 years*	*Open-label*	*No 20 children without ASD for loading test*	*8 weeks without casein Exclusion of other dietary allergens (?) after skin tests (n = 13)*		*A load versus placebo Loading test with allergens excluded in subjects who presented with improvement*	*BSE Antibodies*
Knivsberg et al., 1999	*1*	*7 years*			*Without gluten for 2 years*			

	N	Age	Type	Control group	Construction			Assessment criteria
Whiteley et al., 1999	DSM-IV 9 autistic children 4 Asperger's patients 5 ASD 2 semantic disorders 2 dyspraxia 22/31 included	68 mo 80 mo 40 mo 54 mo 102 mo 73 mo	Open-label	6 autistic (64 months) Recruited without randomization	Without gluten for 5 months			Questionnaire for parents Scoring by parents and teachers BSE, PASS K-ABC in 6 Peptiduria
	4 autistic subjects 1 ADHD				Gluten load after a gluten-free diet for 6 months			
Cade et al., 2000	270 DSM-III	120 non ASD 150 autistic	Open-label	No	Gluten-free, casein-free	149 autistic children included	70 followed with diet for at least 1 year Likert scale parents and teachers	Behaviour Peptiduria
Knivsberg et al., 2002	20 Inclusion criteria not specified	59-127 months	Randomization Placebo	Yes	Gluten-free, casein-free for 1 year		Parents and teachers informed	DIPAB / Behaviour Leiter IPS IPTA TOMI
Elder, 2006	15 DSM-IV ADI-R	2-16 years	Randomization Placebo Double blind method	cross-over	Gluten-free, casein-free vs. placebo 2 periods of 6 weeks	13 completed the study		CARS ADI-R ECO

Annex 2: Evaluation scales of behavioural disorders in autism

The different studies measuring the impact of a treatment strategy in pervasive developmental disorders (PDD) use different methods of evaluation: evaluation scales, behavioural inventories, physiological measurements. The vast majority of symptoms expressed in the autistic spectrum, however, make the use of several tools necessary. No consensus exists on the optimum battery to determine response to treatment in autism. This heterogeneity and the absence of specificity of evaluation tools are a significant limitation to determine the efficacy of a treatment modality in such disorders. Also, it appears essential in a first phase, in light of the heterogeneity of clinical expression of PDD to determine a target behaviour for treatment whose quantitative and/or qualitative measurement will be evaluated in a therapeutic trial.
Two types of instruments are to be differentiated: diagnostic instruments and instruments for measurement of behavioural traits (rating scales which measure specific signs and non specific signs of the autistic syndrome).

Diagnostic instruments

Autism Diagnostic Interview - R (ADI - R)
This standardised diagnostic instrument makes it possible to establish the diagnosis of childhood autism according to diagnostic criteria of the ICD 10 and the DSM-IV. This diagnostic interview is conducted with the child's parents and allows the child's capacities and behaviour to be evaluated. Quantitative investigation of the 3 main dimensions of the autistic syndrome: social disorders, communication disorders and stereotypical disorders is thus obtained. Each dimension is rated as a subscore based on items specific for the dimension investigated. The expected mean value at inclusion for each dimension in the sample of patients entering in the study is the threshold value, i.e. 10 for social dimension, 7/8 for communication dimension and 3 for repetitive behaviours.

B- ADOS (autism diagnosis observation scale) is an observation scale for diagnosis of autism under semi-structured conditions. The scale is organised into four modules administered. Each module has its own protocol with activities for children or for adults. A single module is administered for a given period and the choice is made according to chronological age and level of expressive language:

- module 1 is intended for non verbal children whose level of language does not exceed rudimentary phrases;
- module 2 applies to children who have access to a level of language ranging from small three word phrases including verbs, used regularly and spontaneously, to sentences exceeding the immediate context and containing logical connections;
- module 3 is used for children or adolescents who use fluid language; it contains an observational part during an interactive play and questions to collect information on social communication;
- module 4 applies to adolescents and adults whose language is more developed, and in particular based on questions and conversation.

Precise scoring criteria allow scores to be assigned ranging from 0 to 3 for each item.

C-Children Autistic Rating Scale (CARS)
The CARS is a hetero-evaluation scale of autistic symptoms based on direct observation of the child. It contains 15 items scored 1 to 4 with possible intermediate scoring. Depending on score obtained, it is possible to classify the child according to severity of symptoms:

- non autistic = 15 – 29.5;
- moderate autism = 30 – 36.5;
- severe autism = 37 - 60.

2- Scales measuring behavioural traits

A- BSE scale - Behavioural Summarised Evaluation scale of Autism

This scale contains 29 items listed in a table combining the principal signs of autism described with the DSM III. Notifying parties are aided by a glossary which provides the meaning of each item. Each item is scored from 0 to 4 (0: the disorder is never observed, 1: sometimes, 2: often, 3: very often, 4: always).

Items

1. Looks for isolation
2. Ignores other people
3. Insufficient social interaction
4. Inadequate eye contact
5. Does not seek to communicate by voice and speech
6. Difficulty in communicating by gesture and mimic
7. Stereotypical vocal or verbal utterances, echolalia
8. Lack of initiative. Reduced spontaneous activity
9. Behavioural disorders with regard to objects, or a doll
10. Uses objects in an irresistible or ritualised manner
11. Intolerance to change, to frustration
12. Stereotypical sensory motor activity
13. Agitation, turbulence
14. Bizarre mimic, posture or presentation
15. Self-aggressiveness
16. Hetero-aggressiveness
17. Minor signs of anxiety
18. Mood disorders
19. Eating pattern disorders
20. Does not try to be toilet trained (stool, urine). Faecal games
21. Specific bodily activities
22. Sleep disorder
23. Attention difficult to fix, distracted
24. Bizarre patterns of hearing
25. Variability
26. Does not imitate gestures or another person’s voice
27. Child is too relaxed, amorphous
28. Does not share emotions
29. Paradoxical sensitivity to touch, to body contact

IBSE (Infant Behavioural Summarised Evaluation) contains 33 items. Essential changes concerned are items defined as the most characteristic of very young children: absence of a smile, of contact by look, of imitation of gestures or the voice of another person; hypotonia, non-differentiation of persons, absence of manifestation of emotion, etc.

Scoring is carried out according to a direct observation technique but a retrospective study can also be applied using family movies. The latter method allows simultaneous analysis of documents by several clinicians (5 to 8 on average). These scorings conducted and discussed in common allow harmonisation of clinical judgement and homogeneity of results.

The Behavioural Summarized Evaluation (BSE) is a scale which measures changes in behaviour in autistic patients, children and adolescents. The scale should be filled out by a person who has daily contact with the child. In its original utilisation, passage was carried out once or twice a week in the setting of therapeutic interventional studies with children. Furthermore, the original version of the BSE, the revised version of the BSE (BSE-R) (Barthelemy et al., 1997) and the Infant Behavioural Summarized Evaluation (IBSE) (Adrien et al., 1992) also have been analysed as diagnostic instruments for autism.

B- The ABC scale (Aberrant Behaviour Checklist)

This tool has the advantage of presenting a limited number of items and a clearly established and validated factorial structure. It is also adapted to a population with mental retardation ranging from average to severe (with cognitive retardation present in 75% of autistic subjects).
The scale is built based on 58 items whose scoring ranges from 0 to 3: 0 (no problem) -> 3 (severe problem). It is comprised of 5 factors:

Factor 1 (F1): irritability, agitation, crying
Factor 2 (F2): lethargy, social withdrawal
Factor 3 (F3): stereotypical behaviours
Factor 4 (F4): hyperactivity, non compliance
Factor 5 (F5): inappropriate language
The examiner also takes into account the frequency with which the behaviour occurs.
This scale is a reference scale in measurement of behavioural disorders, in particular for conduct of therapeutic trials.

Annex 3: Comparative intake of protein of 3 theoretical rations: normal, gluten-free casein-free with milk substitutes consisting of soy, gluten-free casein-free with milk substitutes other than soy

Theoretical dietary protein content (g/d)	**Safety intake[1] (g/d)**	**No diet[2]**	**Gluten-free casein-free with soy**	**Gluten-free casein-free with other soy substitutes[3]**
6 years	18	58	40-45	30-35

[1] Recommended Daily Allowances for the French population CNERNA CNRS AFSSA 2001

[2] Results of the INCA 2 study (Afssa, 2007)

[3] A ration with 400 ml of vegetable “formula” containing 1 % proteins has been counted

ANNEX 4: Comparative intake of calcium in 3 theoretical rations: normal, gluten-free casein-free with soy milk substitutes, enriched with calcium, gluten-free casein-free with milk substitutes other than soy, enriched with calcium

Theoretical dietary calcium content (mg/d)	**RDA[1] (mg/d)**	**No diet[2]**	**Gluten-free casein-free with soy + calcium enrichment**	**Gluten-free casein-free with substitutes other than soy, enriched with calcium**
6 years	700	780	550-650	170-200

[1] Recommended Daily Allowances for the French population CNERNA CNRS AFSSA 2001
[2] Results of the INCA 2 study (Afssa, 2007)

Annex 5: Estimated intake of vitamins B1 and B2 in a diet with exclusion of casein and gluten with soy-based products (without taking into account products without gluten)

	Thiamine (mg/d)		**Riboflavin (mg/d)**	
	RDA[1] (mg/d)	**Estimated consumption (mg/d)**	**RDA[1] (mg/d)**	**Estimated consumption(mg/d)**
6 years	0.6	0.8-1	1	0.5-0.7

[1] Recommended Daily Allowances for the French population CNERNA CNRS AFSSA 2001