Evidence of the gluten free and casein free diet in autism spectrum disorders (ASDs): a systematic review

Salvador Marí-Bauset, DPhar[1], Itziar Zazpe, MD, PhD[2,3], Amelia Mari-Sanchis, MD[4], Agustín Llopis-González, PhD[1,5] and María Morales-Suárez-Varela, MD, PhD[1,5]

1 Unit of Public Health and Environmental Care, Department of Preventive Medicine, University of Valencia, Valencia, Spain

2 Department of Nutrition and Food Sciences and Physiology, University of Navarra, Navarra, Spain

3 Biomedical Research Centre Network on Obesity and Nutrition (CIBERobn), Physiopathology of Obesity and Nutrition, Institute of Health Carlos III, Madrid, Spain

4 Clinical Nutrition and Dietetics Unit, Navarra Hospital Complex, Navarra, Spain

5 CIBER Epidemiologia y Salud Pública (CIBERESP), Institute of Health Carlos III, Madrid, Spain

Corresponding to:

Maria M. Morales Suarez Varela MD, PdD.

Unit of Public Health and Environmental Care.

Department of Preventive Medicine. University of Valencia.

Avd. Vicente Andres Estellés s/n, 46100 Burjassot, Valencia (Spain) Tel.: +34 963544951

e-mail: maria.m.morales@uv.es

There are 2422 words in this manuscript

Abstract

In autism spectrum disorders, many parents resort to alternative treatments and these are generally perceived as risk free. Among these, the most commonly used is the gluten-free casein-free diet. The objective of this work was to conduct a systematic review of studies published from 1970 to date related to the gluten-free casein-free diet in autism spectrum disorders patients. Few studies can be regarded as providing sound scientific evidence since they were blinded randomised controlled trials, and even these were based on small sample sizes, reducing their validity. We observed that the evidence on this topic is currently limited and weak. We recommend that it should be only used after the diagnosis of an intolerance or allergy to foods containing the allergens excluded in gluten-free casein-free diets. Future research should be based on this type of design, but with larger sample sizes.

Keywords:

Gluten-free. Casein-free diet. Autism. Autism Spectrum Disorders (ASDs). Review.

Introduction

Autism spectrum disorders are a clinically characterised by difficulties with reciprocal social interactions, verbal and non-verbal communication deficiencies and restricted, repetitive and stereotyped behaviors and interests[1]. According to a recent publication analysing 2008 data[2], the prevalence has increased to 11.3 per 1000 people, and it is notably more common in men (ratio 4:1). On the other hand, no significant differences have been reported as a function of socioeconomic level or cultures[3].

Gluten-free casein-free diet: background

So far, there are no curative treatments for this disorder, and though there is some hope of advances[4]. In this context, many parents have turned to alternative treatments[5,6,7] driven by the frustration and concern caused by the diagnosis rather than with sound justification, and this issue is compounded by the fact that the treatments are generally thought to be risk-free. These alternative treatments include the adoption of elimination diets, in particular the gluten-free casein-free diet[8], the focus of this paper.

In relation to this, the elimination of gluten implies the exclusion of all food items containing wheat, oats, barley or rye, that is, all flours, bread, rusks, pasta, pastries and other bakery products made with these cereals, while the elimination of casein means no intake of dairy products: milk, including breast milk, yogurt, cheese, butter, cream or ice cream, among others.

On the other hand, in relation to autism spectrum disorders children, these diets involve significant changes to their routine and such changes may, in themselves, affect their eating behaviors[9,10,11]. Additionally, the adoption of elimination diets works against efforts to improve the social integration of such children, in that a personal diet is an isolating factor[12].

Opioid theory for autism spectrum disorders

The most commonly cited theory to justify adoption of a gluten-free casein-free diet is related to neurotransmitters[13] and concerns the release of peptides with an opioid activity in the intestines. After digestion, certain types of proteins could cross the intestinal mucosa intact[14], if this were more permeable than normal, this

being the case when it is impaired by immunological factors or by lesions in the case of celiac disease. If these peptides, transported by the blood stream, were to cross the blood-brain barrier and reach the central nervous system in large quantities it would affect brain functioning[15]. The hydrolysis of proteins from cereals and milk would generate exogenous neuropeptides (exorphines) such as gluteomorphins from gluten and beta-casomorphins from casein.

It should, however, be highlighted that exorphins have a low affinity for opioid receptors and that in dietary proteins there are also amino acid sequences with antagonist activity on opioid receptors which, despite having been known of for many years, tend not to be considered in this context[16].

What is more, experiments have failed to find abnormally high concentrations of opioid peptides in either plasma or the nervous system of patients with autism spectrum disorders [17]. As for urinary excretion, urinary opioid peptides have not been detected in people with autism spectrum disorders using modern methods with great sensitivity and specificity (namely, mass spectrometry coupled with high-performance liquid chromatography)[18,19,20].

Prevalence

The adoption of gluten-free casein-free diet, as an alternative treatment, is a poorly studied phenomenon. In the literature, figures are highly variable, indicating that this approach is tried in 20 to 70% of cases. For instance, Harrington et al[6] reported that 66% , Wong et al[21] found that 30%, Herndon et al[22] reported 31.1%, Bandini et al[23] indicated that 20.7%, Hall et al[24] and Sharp et al[25] reported rates of 30%.

Behavior and Physiological Perspective

The first author to establish an association between the frequency and severity of schizophrenia and the intake of foods containing gluten and dairy products was Dohan: their withdrawal improved symptoms and their reintroduction worsened them[26,27]. Subsequently, Panksepp[28] suggested that the behavioral changes associated with autism were the result of an abnormal activation of the opioid system due to an excess of agonists in the

brain. It has been considered that gluten from cereal and casein from dairy products could be responsible, as they are a source of "exorphins", peptides with opioid activity[29-32].

Considering publications since 1970, excluding theses or book chapters, we found relatively few original studies on elimination diets that analyse their impact on behavior in autism spectrum disorders. Among these, demonstrated significant improvements in intervention vs. control groups, and Whiteley et al[33] reported the appearance of a possible diet-related autism phenotype that seems to be emerging supportive of a positive dietary effect with slight improvement in certain groups with autism spectrum disorders. On the other hand, Sponheim[12] did not observe any improvement after introduction of the elimination diet, but rather behavioral regression due to stigmatisation. Elder et al[34] and Seung et al[35], did not find any improvement in the behavior of participants in the intervention group.

Having discussed the questionable effectiveness of this nutritional intervention on cognitive-behavioral function, we will now assess its safety. Cornish[11] did not find any significant nutritional differences between autism spectrum disorders children as a function of whether they were on the gluten-free casein-free diet, similar to the findings of Johnson et al[36]. On the other hand, Arnold et al[37] observed a significantly lower concentration of amino acids, including tryptophan in children with autism spectrum disorders on gluten-free casein-free diets. Higher homocysteine levels have been observed in patients on a gluten-free diet long-term compared to typical development children, and this implies deficiencies in folates and vitamin B_6, increasing cardiovascular risk in the medium and long term[38-40]. Mariani et al[41] reported that patients on a gluten-free diet had high intakes of proteins and lipids but low intakes of carbohydrates, fibre, calcium and iron. In line with this, Marcason[42,43] warns about the risk of gluten-free diets resulting in deficient intake of both macro and micronutrients, the associated restrictions making it much more difficult to achieve a balanced diet than when a broader variety of foods are consumed.

Similarly, a casein-free diet could result in calcium deficiency[44-47]. Aldamiz-Echevarria et al[48] indicated that 76% of patients on a casein free diet had a total lipid intake within the recommended range, but 85% had high ratios of ω6/ω3 and low plasma levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), a similar pattern being described by Schuchardt et al[49]. Further, slower bone development was observed in autism spectrum disorders children on a casein free diet than among those without dietary restrictions[50], while Neumayer et al[51] demonstrated that children with autism spectrum disorders had a lower bone density than

controls. In this latter study, the total energy and macronutrient intakes did not differ significantly between groups, but the intakes of vitamin D and calcium were lower in children with autism spectrum disorders and this may be attributable to less consumption or even the elimination of milk and other dairy products.

All the above justifies this systematic review of the studies published since 1970 concerning dietary restriction and its impact on autism spectrum disorders. Specifically, the objectives of this study were to determine, on the basis of the available scientific data: a) their apparent efficacy, and b) any possible associated metabolic risks.

Methods

We conducted a systematic review of the medical literature related to gluten-free casein-free type diets. The date of the last search was 30 September 2013. We based our search on the Medline database, in accordance with the proposals of the Spanish National Health System In addition, we also consulted other databases (Cochrane Library, Scielo, ScienceDirect and Embase). For the searches, we used the following keywords*: gluten-free, casein-free diet, autism, Autism Spectrum Disorders (ASDs)* and *review*, with the corresponding Boolean operators. This paper complies with the methodological norms established for the publication of systematic reviews[52,53] and the PRISMA recommendations[54].

We first retrieved systematic reviews and full original articles published from 1970 to 2013. These publications were then included in the analysis provided that: the participants, of any age, met the DSM-IV-TR criteria for autism spectrum disorders, that they were put on a diet excluding gluten, casein or both; and that the outcome variables were related to the potential biomedical or behavioral symptoms of autism spectrum disorders. We did not restrict the searches by language. On the other hand, studies in which the diet was not under supervision of the researchers and any which did not report on health outcomes were excluded.

To guide the evaluation of the data in the papers retrieved, we defined levels of evidence, on the basis of their methodological quality in terms of the study design. We then established grades of recommendations for the planning of dietary guidelines for patients with autism spectrum disorders. For this classification of the evidence and recommendations, we employed an instrument proposed by the Scottish Intercollegiate Guidelines Network[55]. The scale proposes that two characteristics of the source be used for assessing the quality of the scientific evidence provided (level of evidence): the study design and the risk of bias. Numbers from 1 to 4 are used to rate the study design, while signs (++, + and -) indicate the assessed risk of bias, according to the degree of fulfillment of key criteria related to this potential risk (Table 1). Based on this assessment of the quality of the scientific evidence in the source, grades are used (Table 2) to classify the strength of associated recommendations (A,B,C,D).

In addition to the aforementioned system of levels, we considered the following features, as applicable, to assess the level of evidence provided by the selected articles: (A) Degree of homogeneity of the group studied (as determined by definitions and criteria applied); (B) use of a control group and the appropriateness of the selection; (C) type of experimental design (randomized or not); (D) knowledge of the intervention by patients,

relatives and other observers (open, simple or double blind trial); (E) nature of the dietary regimen (level of strictness) and degree of adherence; (F) selection of assessment criteria, including the instruments used (questionnaires, scales, etc.) for assessing changes in patient status under the treatment; and (G) the presence of confounding factors including any types of pharmacological treatments provided, or the use of one or more intervention procedures that could affect the assessment criteria selected.

For evaluating and synthesizing the scientific evidence, we also considered the internal validity of the studies, whether there was statistical significance and the accuracy of the results, as well as their clinical relevance. We then characterized the recommendations on the basis of the quantity, generality, and clinical relevance of the results as well as the quality of the scientific evidence.

Results

The studies retrieved were analysed in terms of the following characteristics, as applicable: sample size, study design, assessment and intervention criteria, and the results, as well as the level of evidence and the grade of recommendation. Tables 3 and 4 summarise the characteristics of the studies the results of which have been referred to above.

Effectiveness

Scientific literature on this topic is relatively scarce. Among the studies that refer to the effectiveness, only four[12,34,35,56] may be considered to provide high scientific evidence. The studies of Harland[57] and Hyman[58] not yet completed. Millward[59] and Mulloy[60,61] in their papers present systematic reviews, evaluated with the highest level of evidence and grade of recommendation. Notably, in our analysis, the studies that reported positive results were classified with the lowest levels of evidence, while the rest reported negative results with regards this type of dietary intervention. None of the studies identified provided conclusive evidence because they had poor validity (Table 3).

Safety

There are similarly few publications addressing the safety of the gluten-free casein-free diet. Among those identified, the studies of Konstantynowicz et al[46] and Hediger et al[50] provide the highest level of evidence. Nevertheless, in the results found there was a certain degree of consensus on the risks that could be associated with following this type of restriction diet (Table 4).

Discussion

Data in the literature in this field is very limited both in quantity and quality. To assess the effectiveness and safety of the gluten-free casein-free elimination diet, we considered both behavioral (verbal and non-verbal communication, stereotypy and disruptive behavior) and biomedical variables (e.g. urinary peptides, gliadin and endomysial antibodies, as well as other laboratory data and nutrient intake). Methodological limitations identified were associated with a range of factors: the lack of a control group and/or clear definitions of inclusion criteria, very small sample sizes and analysis being based on single individuals or anecdotal information, groups being heterogeneous in terms of age, failure to control for phenotypic variability between individuals, and interventions being of variable duration and generally short, as well as lack of pre-/post-intervention comparisons. There was also a risk of bias in data on the behavioral variables attributable to memories of parents and other caregivers being distorted over time and that their perception of changes in the behavior of participants may be subjectively influenced by the fact of being included in non-blinded trials. Similarly, a placebo effect may have had an impact on results. Lastly, alternative explanations were not always considered, such as the of risk of confounding bias, in particular, it being possible that behavioral improvements were due to ongoing development and behavioral therapy given, rather than to gluten-free casein-free diets *per se*. Finally, it should be noted that the literature search may not have identified all the relevant publications, and the review itself may be sensitive to information bias.

Recommendations

On the basis of this review, we conclude that the evidence to support gluten-free casein-free diets is limited and weak, such dietary restrictions being associated with social rejection, stigmatisation, deficits in socialisation and integration, and a misuse of resources, as well as potential adverse biomedical effects. Hence, we advise against resorting to elimination diets in an attempt to treat autism spectrum disorders. Specifically, until there is conclusive evidence of the benefits of gluten-free casein-free diets in autism spectrum disorders, they should only be introduced after the diagnosis of an intolerance or allergy to allergens in the foods that would be eliminated in such a diet. Similarly, the results retrieved do not support the opioid theory.

Implications for the practice

As a final recommendation, we underline that, when used, elimination diets must be at least as closely monitored as other types of intervention, to allow doctors, parents, and other caregivers to optimise treatments and hence health outcomes for these children. On the other hand, a diet-related specific end phenotype may be a target for future research and even a marker for the gluten-free casein-free dietary intervention.

Based on the results of this review, future research should be focussed on blinded randomised controlled trials, and include larger samples sizes.

Author Contributions

All five coauthors of this paper have contributed significantly to the design and implementation of the study, as well as the analysis and interpretation of the results. Further, all have participated in the preparation of this manuscript and have approved the final version submitted for publication.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship and/or publication of this article.

Ethical Approval

The study protocol was approved by the Ethic Committee of University Hospital “Dr. Peset” (Valencia, Spain) (code 46/10). The school accepted the study and parents of children participating in the study gave written informed consent.

References

1. Filipek PA, Accardo PJ, Baranek GT, et al. The screening and diagnosis of autism spectrum disorders. *J Autism Dev Disord.* 1999;29(6): 439-484. *Review. Erratum in: J Autism Dev Disord.* 2000; 30(1) : 81.

2. CDC. Centers for Disease Control and Prevention. Prevalence of autism spectrum disorders. *MMWR Surveillance Summaries.* 2012; 61(3) 1-18. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/ss6103a1.htm . Accessed on 11 September 2013.

3. Baghdadli A. *Recommandations pour la pratique professionnelle du diagnostic de l'autisme*. Fédération française de psychiatrie et haute autorité de santé. 2005. Available at: http://has-sante.fr/portail/display.jsp=c 468812. Accessed 30 of September 2013.

4. Francis K. Autism interventions: a critical update. *Dev Med Child Neurol.* 2005; 47: 493-499.

5. Levy SE, Hyman SL. Novel Treatments for autistic spectrum disorders. *Ment Retard Dev Disabil Res Rev.* 2005; 11: 131-142.

6. Harrington JW, Rosen L, Garnecho A, Patrick PA. Parental perceptions and use of complementary and alternative medicine practices for children with autistic spectrum disorders in private practice. *J Dev Behav Pediatr*. 2006; 27: 156-161.

7. Hanson E, Kalish LA, Bunce E, et al. Use of complementary and alternative medicine among children with autism spectrum disorder. *J Autism Dev Disord.* 2007; 37(4):628-636.

8. Alpert M. The autism diet. *Sci Am.* 2007; 296: 19-20.

9. Whiteley P, Rogers J, Savery D, Shattock P. A gluten-free diet as an intervention for autism and associated disorders: preliminary findings. *Autism.*1999; 3: 45-65.

10. Cade R, Privette M, Fregly M, et al. Autism and schizophrenia: intestinal disorders. *Nutr Neurosci.* 2000; 3: 57-72.

11. Cornish E. Gluten and casein free diets in autism: a study of the effects on food choice and nutrition. *J Hum Nutr Diet.* 2002; 15: 261-269.

12. Sponheim E. Gluten-free diet in infantile autism. A therapeutic trial. *Tidsskr Nor Laegeforen*. 1991; 111: 704-707.

13. Amidon GL, Lee HJ. Absorption of peptide and peptidomimetic drugs. *Annu Rev Pharmacol Toxicol.* 1994; 34: 321-341.

14. Daniel H. Molecular and integrative physiology of intestinal peptide transport. *Annu Rev Physiol.* 2004; 66: 361-384.

15. Janecka A, Staniszewska R, Gach K, Fichna J. Enzymatic degradation of endomorphins. *Peptides.* 2008; 29: 2066-2073.

16. Zioudrou C, Streaty RA, Klee WA. Opioid peptides derived from food proteins. The exorphins. *J Biol Chem.* 1979; 254: 2446-2449.

17. Lennernäs H. Intestinal permeability and its relevance for absorption and elimination. *Xenobiotica.* 2007; 37: 1015-1051.

18. Sponheim E, Myrhe AM, Reichelt KL, Aalen OO. Urine peptide patterns in children with milder types of autism. *Tidsskr Nor Laegeforen.* 2006; 25: 1475-1477.

19. Dettmer K, Hanna D, Whestone P, et al. Autism and urinary exogenous neuropeptides: development of an on-line SPE-HPLC-tandem mass spectrometry method to test the opioid excess theory. *Anal Bioanal Chem.* 2007; 388: 1643-1651.

20. Cass H, Gringras P, March J, et al. Absence of urinary opioid peptides in children with autism. *Arch Dis Child.* 2008; 93: 745-750.

21. Wong HH, Smith RG. Patterns of complementary and alternative medical therapy use in children diagnosed with autism spectrum disorders. *J Autism Dev Disord.* 2006; 36(7): 901-910.

22. Herndon AC, DiGuiseppi C, Johnson SL, et al. Does nutritional intake differ between children with autism spectrum disorders and children with typical development? *J Autism Dev Disord.* 2009; 39: 212-222.

23. Bandini LG, Anderson SE, Curtin C, et al. Food selectivity in children with autism spectrum disorders and typically developing children**.** *J Pediatr.* 2010; 157(2): 259-264.

24. Hall SE, Riccio CA. Complementary and alternative treatment use for autism spectrum disorders. *Complementar Ther Clin Pract.* 2012; 18(3): 159–163.

25. Sharp WG, Jaquess DL, Lukens CT. Multi-method assessment of feeding problems among children with autism spectrum disorders. *Res Autism Spectr Disord*. 2013; 7(1): 56–65.

26. Dohan FC. Cereals and schizophrenia. Data and hypothesis. *Acta Psychiatr Scand.* 1966; 42: 125-152.

27. Dohan FC, Graberger JC. Relapsed schizophrenics: earlier discharge from the hospital after cereal-free, milk-free diet. *Am J Psychiatry.* 1973; 130: 685-686.

28. Panksepp JA. A neurochemical theory of autism. *Trends Neurosci.*1979; 2: 174-177.

29. Knivsberg AM, Wiig K, Lind G, et al. Dietary intervention in autistic syndromes. *Brain Dysfunction.* 1990; 3: 315-327.

30. Reichelt KL, Knivsberg AM, Lind G, Nødland M. Probable etiology and possible treatment of childhood autism. *Brain Dysfunction.* 1991; 4: 308-319.

31. Knivsberg AM, Reichelt KL, Høien T, Nødland M. Effect of dietary intervention on autistic behavior. *Focus Autism Other Dev Disabl.* 2003; 18(4): 247–256.

32. Reichelt KL, Knivsberg AM. Can the physiopathology of autism be explained by the nature of the discovered urine peptides? *Nutr Neurosci*. 2003; 6: 19-28.

33. Whiteley P, Shattock P, Knivsberg AM, et al. Gluten and casein-free dietary intervention for autism spectrum conditions. *Front Hum Neurosci.* 2013; 6: 344.

34. Elder JH, Shankar M, Shuster J, et al. The gluten-free, casein-free diet in autism: results of a preliminary double blind clinical trial. *J Autism Dev Disord.* 2006; 36(3):413-420.

35. Seung HK, Rogalski Y, Shankar M, Elder J. The Gluten-and Casein-Free Diet and Autism: Communication Outcomes From a Preliminary Double-Blind Clinical Trial. *J Med Speech Lang Pathol.* 2007;15 (4): 337- 345.

36. Johnson CR, Handen BL, Zimmer M, et al. Effects of Gluten Free - Casein Free Diet in Young Children with Autism: A Pilot Study. *J Dev Phys Disabil.* 2011; 23(3): 213-325.

37. Arnold GL, Hyman SL, Mooney RA, Kirby RS. Plasma amino acids profiles in children with autism: potential risk of nutritional deficiencies. *J Autism Dev Disord.* 2003; 33(4):449-454.

38. Thompson T. Folate, iron, and dietary fiber contents of the gluten-free diet. *J Am Diet Assoc.* 2000; 100:1389-1396.

39. Hallert C, Grant C, Grehn S, et al. Evidence of poor vitamin status in celiac patients on a gluten-free diet for 10 years. *Aliment Pharmacol Ther.* 2002; 16: 1333-1339.

40. Ali A, Waly MI, Al-Farsi YM, et al. Hyperhomocysteinemia among Omani autistic children: a case-control study. *Acta Biochim Pol.* 2011; 58(4):547-551.

41. Mariani P, Viti MG, Montuori M, et al. The gluten-free diet: a nutritional risk factor for adolescents with celiac disease?. *J Pediatr Gastroenterol Nutr.* 1998; 27(5):519-523.

42. Marcason W. What is the current status of research concerning use of a gluten-free, casein-free diet for children diagnosed with autism? *J Am Diet Assoc.* 2009; 109 (3): 572.

43. Marcason W. Is there evidence to support the claim that a gluten-free diet should be used for weight loss?.*J Am Diet Assoc.* 2011; 111(11): 1786.

44. Black C, Kaye JA, Jick H. Relation of childhood gastrointestinal disorders to autism: nested case¬control study using data from the UK General Practice Research Database. *BMJ.* 2002; 325:419–421.

45. Monti G et al. Multiple bone fracture in an 8 years old child with cow's milk allergy and inappropriate calcium supplementation. *Ann Nutr Metab.* 2007; 51: 228-231.

46 Konstantynowicz J, Nguyen TV, Kaczmarski M, et al. Fractures during growth: potential role of a milk-free diet. *Osteoporos Int.* 2007; 18: 1601-1607.

47. Agostoni C, Turck D. Is cow's milk harmful to a child's health? . *J Pediatr Gastroenterol Nutr.* 2011; 53(6): 594-600.

48. Aldámiz-Echevarria L, Bilbao A, Andrade F, et al. Fatty acide deficiency profile in children with food allergy managed by elimination diet. *Acta Paediatr.* 2008; 97(11):1572-1576.

49. Schuchardt JP, Huss M, Stauss-Grabo M, Hahn A. Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children. *Eur J Pediatr.* 2010; 169(2): 149-164.

50. Hediger ML, England LJ, Molloy CA, et al. Reduced bone cortical thickness in boys with autism or autism spectrum disorder. *J Autism Dev Disord.* 2008; 38: 848-856.

51. Neumeyer AM, Gates A, Ferrone C, et al. Bone Density in Peripubertal Boys with Autism Spectrum Disorders. *J Autism Dev Disord.* 2013; 43(7):1623-1629.

52. Friedenreich CM. Methods for pooled analyses of epidemiologic studies. *Epidemiology.* 1993; 4(4):295-302.

53. Froom P, Froom J. Deficiencies in structured medical abstracts. *J Clin Epidemiol.* 1993; Jul*;46(7):591-594.*

54. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses ofstudies that evaluate healthcare interventions: Explanation and elaboration. *BMJ.* 2009; 339:b2700.

55. Scottish Intercollegiate Guidelines Network. A guideline developers' handbook (Publication no. 50). Edinburgh: SIGN: 2001 (updated in January 2008). Available at: http://www.sign.ac.uk/guidelines/fulltext/50/index.html. (Accessed on 30 September 2013).

56. Hyman S, Stewart P, Smith T, et al. The gluten free and casein free (GFCF) diet: A double blind, placebo controlled challenge study. Paper presented at the International Meeting for Autism Research, Philadelphia, PA 2010.

57.Harland SW. A study to assess the role of a gluten free-dairy free (GFCG) diet in the dietary management of autism associated gastrointestinal disorders. Clinical Trials.gov. Available at: http://clinicaltrials.gov/ct2/show/NCT01116388?term=autism+diet&rank=2. (NCT01116388). 2010. Accessed on 11 September 2013.

58. Hyman SL .Diet and behavior in young children with Autism. Clinical Trials.gov. Available at: http://clinicaltrials.gov/ct2/show/NCT00090428?term=autism+diet&rank=2. (NCT00090428). 2004. Accessed on 05 September 2013.

59. Millward C, Ferriter M, Calver S, Connell-Jones G. Gluten- and casein-free diet for autistic spectrum disorder. Cochrane Database Systematic Review 2008; 16 (2):CD003498. Available at: http://www.update-software.com. Accessed on 16 September 2013.

60. Mulloy A, Lang R, O'Reilly M, et al. Gluten-free and casein-free diets in the treatment of autism spectrum disorders: A systematic review. *Res Autism Spectr Disord.* 2010; 4: 328–339.

61. Mulloy A, Lang R, O'Reilly M, et al. Addendum to "gluten-free and casein-free diets in treatment of autism spectrum disorders: A systematic review". *Res Autism Spectr Disord.* 2011; 5: 86–88.

62. Goodwin MS, Cowen MA, Goodwin TC. Malabsorption and cerebral dysfunction: a multivariate and comparative study of autistic children. *J Autism Child Schizophr.* 1971; 1: 48-62.

63. Bird BL, Russo DC, Cataldo MF. Considerations in the analysis and treatment of dietary effects on behaviour: a case study. *J Autism Child Schizophr.* 1977; 7: 373-381.

64. O'Bannion D, Armstrong B, Cummings RA, Stange J. Disruptive behaviour: a dietary approach. *J Autism Child Schizophr.* 1978; 8: 325-337.

65. McCarthy DM, Coleman M. Response of intestinal mucosa to gluten challenge in autistic subjects. *Lancet.* 1979; 2:877-878.

66. Reichelt KL, Ekrem J, Scott H. Gluten, milk proteins and autism: dietary intervention effects on behaviour and peptide secretion. *J Appl Nutr.* 1990; 42: 1-11.

67. Lucarelli S, Frediani T, Zingoni AM, et al. Food allergy and infantile autism. *Panminerva Med.* 1995; 37: 137-141.

68. Knivsberg AM, Reichelt KL, Nødland M, Høien T. Autistic syndromes and diet: A follow-up study. *Escanear Educ Res.* 1995; 39: 223–236.

69. Adams L, Conn S. Nutrition and its relationship to autism. *Focus Autism Other Dev Disabl.* 1997; 12(1): 53-58.

70. Knivsberg AM, Reichelt KL, Nødland M. Dietary intervention for a seven year old girl with autistic behaviour. *Nutr Neurosci.* 1999; 2: 435–439.

71. Knivsberg AM, Reichelt KL, Høien T, Nødland M. A randomized, controlled study of dietary interventions in autistic syndromes. *Nutr Neurosci.* 2002; 5: 251-61.

72. Irvin D. Using analog assessment procedures for determining the effects of a gluten-free and casein-free diet on rate of problem behaviors for an adolescent with autism. *Behav Interv*. 2006; 21: 281–286.

73. Patel K, Curtis LT. A comprehensive approach to treating autism and attention-deficit hyperactivity disorder: a prepilot study. *J Altern Complement Med.* 2007; 13(10):1091-1097.

74. Whiteley P, Haracopos D, Knivsberg AM, et al. The ScanBrit randomised, controlled, single-blind study of a gluten- and casein-free dietary intervention for children with autism spectrum disorders**.** (NCT00614198). *Nutr Neurosci.* 2010;13(2): 87-100.

Table 1. Levels of evidence

	LE	Characteristics
1	1++	high-quality meta-analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias
	1+	well-conducted meta-analyses, systematic reviews of RCTs, or RCTs with a low risk of bias.
	1-	well-conducted meta-analyses, systematic reviews of RCTs, or RCTs with a high risk of bias.
2	2++	high-quality systematic reviews of case-control or cohort or studies.
	2+	high-quality case-control or cohort studies with a very low risk of confounding, bias, or chance and a high probability that the relationship is casual.
	2-	well-conducted case control or cohort studies with a low risk of confounding, bias, or chance and a moderate probability that the relationship is casual.
3		non-analytic studies, e.g. case reports, case series.
4		expert opinion.

Abbreviations: SIGN, *Scottish Intercollegiate Guidelines Network* (2008); LE, levels of evidence; RCT: randomised and controlled trials.

Table 2. Grades of recommendation

GR	Definition
A	at least one meta-analysis, systematic review, or RCT rated as 1++, and directly applicable to the target population; or a systematic review of RCTs or a body of evidence consisting principally of studies rated as 1+, directly applicable to the target population, and demonstrating overall consistency of results
B	a body of evidence including studies rated as 2++, directly applicable to the target population, and demonstrating overall consistency of results; orextrapolated evidence from studies rated as 1++, or 1+
C	a body of evidence including studies rated as 2+,directly applicable to the target population, and demonstrating overall consistency of results; or Extrapolated evidence from studies rated as 2++
D	evidence level 3 or 4; or extrapolated evidence from studies rated as 2+

Abbreviations: SIGN, *Scottish Intercollegiate Guidelines Network* (2008). GR, Grade of Recommendation; RCT: randomised and controlled trials.

Table 3. Studies considering the effectiveness of the gluten-free casein-free diet.

Author	N	Design	Assessment criteria	Results	LE	GR
Goodwin et al, 1971[62]	Cases: 15 children with ASDs Age: 6-13 years old. Controls: 14 Siblings. 1-13 years.	Cross over intervention with gluten load test after GF diet Randomised. Open-label. Experimental.	Blood biochemistry. EEG. Behavioral.	Correlation between autism and malabsorption, and between gluten sensitivity and cognitive impairment	3	D
Bird et al, 1977[63]	9-year-old child with ASD. No controls	Case report. GFCF diet. Experimental.	Behavioral.	Diet does not affect behavior	3	D
O´Bannion et al, 1978[64]	One child with ASD. No controls	Descriptive. Case report.	Behavioral. Dietary restriction	Wheat, maize, tomatoes, sugar, mushrooms and dairy products cause behavioral problems in the child	3	D
Mc Carthy et al, 1979[65]	8 patients with ASD, unknown age. No healthy controls	Open label. Gluten load after GF diet	Clinical picture and intestinal biopsy	No abnormal histological findings in the intestine. No association between autism and celiac disease	2-	
Reichelt et al, 1990[66]	15 patients with ASDs 3-17 years old.	Open label. urinary peptide levels, GFCF/GF/CF, cohort study.	Peptiduria. Parent and caregivers questionnaire with retrospective comparison	Behavioral improvement after the treatment in more than 50% of children	3	D
Knivsberg et al, 1990[29]	15 patients with ASD, 6-14 years if age, No healthy control group.	Open label. urinary peptide levels, GF/CF, cohort study.	Parent and caregiver reports. Standardised assessment questionnaires	Early improvement (first 6 months), less evident after 1 year	3	D
Sponheim, 1991[12]	4 adults and 3 children with ASDs. 6-month GF diet	Experimental. Randomised. Double blind (gluten free or placebo 1 y). Cohorts.	Parent and caregiver reports. Standardised assessment questionnaires	No behavioral improvement	2++	B

Lucarelli et al, 1995[67]	36 patients with ASDs, 8-13 years old; Controls: 20 children without ASDs for the caseinload test; 2-month CF diet	Open label before and after dietary restriction. Casein load test: Double blind, placebo-controlled cohort study	Standardised assessment questionnaire. Measurement of Ig levels.	After the dietary restriction, decrease in the specific Ig levels. Behavioral improvement. Casein load test results not conclusive	2-

Abbreviations: ASD, autism spectrum disorder; GR, Grade of Recommendation; LE, level of evidence; GF, gluten free; CF, casein free; GFCF, gluten-free casein-free.

The studies classified as 1– or 2– were not used in the recommendation process given the high risk of bias

Table 3. Studies considering the effectiveness of the gluten-free casein-free diet (cont.)

Author	N	Design	Assessment criteria	Results	LE	GR
Knivsberg et al, 1995[68]	Continuation of the previous study cf. Knivsberg et al. (1990).	Experimental. Open label, urinary peptide levels, GF/CF, cohort study.	Peptiduria. Parent and caregiver reports, Standardised assessment questionnaires	No clear improvement after one year	3	D
Adams et al, 1997[69]	One boy and one girl with ASDs; Both 3 years old	Series of cases. 1) Very high doses of Mg+B6. 2) GFCF for 2 years.	Parents report	Anecdotal improvement of the general behavior	3	D
Knivsberg et al, 1999[70]	Girl with an ASD, 7 years old	Case report GF for 2 years, Experimental.	Parents report. Standardised assessment questionnaires	Behavioral improvement in communication and socializing	3	D
Whiteley et al, 1999[9]	Cases: 5 children with ASDs and 9 children with autism. No randomised controls: 6 children all diagnosed with autism, formed a control group of children not involved with any dietary intervention GF>6 months.	GF diet for 5 months. Experimental. Cohort study	Peptiduria. Parents report. Standardised assessment questionnaires	Behavioral improvement	2-	
Cade et al, 2000[10]	150 children with ASDs aged between 3.5-16 years old. No healthy controls.	Open label. Gluten load test after GF diet Experimental.	Behavioral. Peptiduria.	Improvement of the autistic signs and symptoms	2-	
Knivsberg et al, 2002[71]	Cases: Ten children with ASDs, GFCF diet for a year. Controls: 10 children with ASDs with normal diet. Both groups with a mean age of 7.5	Randomised. Simple blind. GFCF diet for a year Experimental.	Parents and caregiver report. Standardised assessment questionnaires	Behavioral improvement in communication and socializing	2+	C

	years.					
Elder et al, 2006[34]	15 people with ASDs. Aged 2-16 years old. GFCF diet for 12 weeks. No healthy controls	Randomised, retrospective, double blind, cross-over (RCT), 6 week under normal and GFCF diet, alternately	Parents report. Standardised assessment questionnaires. Urinary peptide levels	No statistically significant differences were observed	2++	B
Irvin 2006[72]	One boy with an ASD, 12 years old	Case report, GFCF for 4 days	Direct observation of the level of aggression and destructive behavior	No behavioral change	3	D

The studies classified as 1– or 2– were not used in the recommendation process given the high risk of bias.

Abbreviations: ASD, autism spectrum disorder; GR, Grade of Recommendation; LE, level of evidence; GF, gluten free; CF, casein free; GFCF, gluten-free casein-free.

Table 3. Studies considering the effectiveness of the gluten-free casein-free diet (cont.)

Author	N	Design	Assessment criteria	Result	LE	GR
Patel et Curtis, 2007[73]	10 children with ASDs and ADHD aged between 4-10 years old. No healthy controls	Open label, Experimental. Children received an integrated treatment based on nutritional (GFCF) and environmental changes, plus the chelating agents, for 3-6 months, as well as the usual behavioural therapy and physiotherapy.	Behavioral assessment by physicians/parents/ teachers. Urinary heavy metals.	Lower urinary concentration of heavy metals. Parents report a behavioral improvement	3	D
Seung et al, 2007[35]	13 children with ASDs; 2-16 years old. No healthy controls.	Retrospective, randomised, double blind, cross over study. Six weeks on a normal and GFCF diet, alternatively.	Video recording, Assessment of verbal and non-verbal communication	No statistically significant differences were observed	2++	B
Millward et al, 2008[59]		Systematic review		There is no empirical evidence base for recommending the GFCF diet.	1+	A
Hyman et al, 2010[56]	30 children with ASDs; 30-45 months	Experimental. Double blind. Cross over. Randomised, controlled (RCT), 18 weeks under GFCF and normal diet alternately.	Behavioral assessment	No significant differences in the preliminary results (2010). No empirical evidence to recommend the GFCF diet.	1+	A
Mulloy et al, 2010[60]		Systematic review		No empirical evidence to recommend the GFCF diet	1+	A
Whiteley et al, 2010[74]	72 children with ASDs. No healthy controls.	Single blind, randomised, clinical trial. Grouped by ages, stratified. Conducted in 2 phases.	Standardised assessment questionnaires Peptiduria.	Significant improvement in some ASD subgroups	2+	C
Mulloy et al, 2011[61]		Systematic review.		Confirmation of the conclusions reached by Whiteley P et al. (2010)	1+	A

Harland 2012[57]	In the recruitment phase, estimated number patients with ASDs 30; aged between 2-17 years old.	Experimental, double blind, cross over, randomised, controlled (RCT), 18 weeks on GFCF and normal diet alternately.	Behavioral assessment (activity, sleep, behaviors related to the ASD); Stool pattern, nutrition	Not yet complete	_	_

The studies classified as 1- and 2- were not used for establishing the recommendations given the high risk of bias.

Abbreviations: GFCF, Gluten-free casein-free diet; GF, gluten free diet; CF, casein-free diet; ASD, Autism spectrum disorder; ADHD, Attention-deficit/hyperactivity disorder; RCT, randomised controlled trial; LE, level of evidence; GR, Grade of recommendation; AA: amino acids.

Table 4. Studies considering the safety of the gluten-free casein-free diet.

Author	N	Design	Assessment criteria	Result	LE	GR
Cornish et al, 2002[11]	Cases: 8 children with ASDs, 3-16 years old; GFCF diet. Controls: 29 children with ASDs; 3-16 years old with no dietary restrictions	Retrospective, Case-control	3-day food diary	No differences observed for energy, macro and micronutrients	2-	
Black et al, 2002[44]	Cases: 50 children who refuse milk (CF), controls 200 children who drink milk. Age range in both groups: 3-10 years old	Observational Cross-sectional.	4-day food diary. Food frequency questionnaire. Measurement of bone density	Lower intake of calcium, Stature and bone density associated with CF diet	2-	
Arnold et al, 2003[37]	Cases: 36 children with ASDs, 26 with normal diet, 10 with GFCF diet. Controls: 24 without ASD Both groups under 5 years of age	Observational. Cross-sectional.	Blood analysis	Significant deficiency in AA (mainly tryptophan) associated with ASDs compared to controls, more pronounced on a GFCF diet	2+	C
Monti et al, 2007[45]	One 8-year-old child	Case report	Dairy product elimination diet.	Lower bone density	3	D
Konstantynowicz et al, 2007[46]	Cases: 91 children with fractures. Controls: 273 children without fractures. Age range: 2.5 to 20 years old.	Observational Grouped by age and sex. Randomised. Case-control.	24-hour recall. Bone density	Lower calcium intake and bone density in children with restriction of dairy products. Weak association between fractures and milk consumption	2++	B
Hediger et al, 2008[50]	75 children with ASDs on a CF diet. No controls	Observational. Cross-sectional.	Cortical bone density	Reduced	2++	B
Johnson et al, 2011[36]	22 children with ASDs aged 3-5 years old. Cases: 8 children on a GFCF diet for 3 months. Controls: 14 children with no dietary restrictions	Prospective, randomized, open-label cohort trial	24-h recall	No significant differences for proteins, lipids, carbohydrates, calcium and iron	2-	
Neumayer et al, 2012[51]	Cases: 18 children with ASD Controls: 19 typically developing children Age range in both	Observational. Case-control.	Bone mineral density. Analysis of blood and saliva; Physical exercise;	Lower bone density. Lower physical activity. Lower intake of lactose, vitamin D, increase	2+	C

	groups 8-14 years	3-day food diary.	of salivary cortisol

The studies classified as 1- and 2- were not used for establishing the recommendations given their high risk of bias.

Abbreviations: GFCF. Gluten-free casein-free diet; GF: gluten free diet; CF: casein-free diet; ASD: Autism spectrum disorder; FFQ: Food frequency questionnaires; RCT: randomised clinical trial; LE: level of evidence; GR Grade of recommendation; and AA: amino acids.