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Gluten-free and casein-free diets in the management of autism spectrum disorder: A systematic literature review

| Review

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Abstract: Autism spectrum disorder (ASD) comprises a group of heterogeneous constellations characterized by deficits in cognitive, communicative, and social skills. ASD has no established etiology and the search for reliable biomarkers has proved to be difficult, giving rise to alternative theoretical accounts, including those related to nutrition. One such account posits that the proteins gluten and casein, derived from wheat and milk respectively, are causally involved in the symptomatic expression of the disorder. As a consequence, a diet devoid of such proteins has been hypothesized to ameliorate the behavioral symptoms of children with ASD. The scope of the present review is to analyze the effects of gluten-free and casein-free (GFCF) diets on children with autism. It has been shown that 8–32% of parents of affected children report the current use of a GFCF diet regimen in their children. The majority of identified dietary intervention studies failed to meet basic methodological standards of interventional science. A comparison of studies conducted with adequate scientific rigor did not show any clear-cut results. In addition to the inconsistent pattern of results, findings of challenge studies largely failed to find behavioral effects after applying gluten/casein challenges to children with ASD. Studies of potential side effects suggest that it is important to monitor both aspects of nutritional adequacy and healthy physical development in children with ASD on a GFCF dietary regimen. In conclusion, evidence for the effectiveness of the GFCF diet in the treatment of autism is sparse. Rigorous scientific evaluations found no convincing evidence of therapeutic effects of the GFCF diet. Nevertheless, more sophisticated investigations should be conducted in order to identify possible benefits and harms of such a dietary approach, particularly in subgroups of individuals with ASD yet to be identified.

Keywords: Autism spectrum disorder; complementary and alternative medicine; nutrition; gluten-free and casein-free diets.

1. Introduction

Autism or autistic disorder represents a group of heterogeneous constellations characterized by deficits in cognitive, communicative, and social skills as well as repetitive sensory-motor behaviors [1,2]. The disorder emerges during childhood and is thought to be a lifelong condition. The fifth revision of the Diagnostic and Statistical Manual of Mental Disorders introduced the umbrella diagnosis of autism spectrum disorder (ASD), which also includes the former diagnostic classes of Asperger syndrome and pervasive developmental disorder-not otherwise specified [2]. Autism has been shown to occur in approximately 0.2% of child and adolescent populations, while prevalence estimates for the whole spectrum average around 0.6% [3]. Prevalence estimates have been shown to have risen over the last two decades. The prevalence rates of autistic disorder before 1987 did not exceed 0.07%, whereas all studies published

since 2000 have consistently shown higher rates (range 0.07–0.4%). While this rise in prevalence might be attributed to a concomitant rise in the incidence of the disorder, other factors, such as changes in the concepts and diagnostic criteria as well as a growing awareness in Western societies, have been put forward as alternative explanations [3]. In regard to the etiology of ASD, more biologically oriented accounts have suggested the involvement of exposure to certain risk-inducing environmental agents and have also discussed the potential role of nutrition within a "gene x environment" framework (see reference 4). From this point of view, an unbalanced diet could potentially induce biological vulnerability, or an otherwise balanced diet might disturb the organism's homeostasis in the case of metabolic insufficiency [4]. Whether such an explanatory model is valid is a matter of ongoing debate [5], which has continued, in recent decades, against the background of world-wide changes in dietary habits, including higher intake of unhealthy fats and lower intake of fiber [6]. Suggestive evidence such as this should be treated with caution, however, and should be integrated with current knowledge regarding the etiology of autism.

Etiological accounts of autism place heavy emphasis on biological factors in regard to both the organism (genes) and the environment (e.g. exposure to neurotoxic agents) and seek to link the influence of these factors to confirmed biomarkers of brain and organismic function [1,7,8]. Potential biomarkers of autism may include structural brain abnormalities (e.g. increase in brain volume, especially in frontal cortex, cerebellum and amygdala), functional brain abnormalities (e.g. dis-connectivity of cortical structures with more asynchrony in activity; abnormal levels of neurotransmitters and neuropeptides) as well as more systemic indicators related to metabolism (e.g. indicators of mitochondrial dysfunction, abnormal urinary excretion of organic acids) and indicators of an increased dysregulation of immune functions [7]. These observations are complemented by findings demonstrating an association between the occurrence of the disorder and polymorphisms of genes related to cell structure and function, neuronal development and synaptic formation as well as with genes involved in neurotransmission [7]. There are also indications of an increase in prevalence of autism following exposure to certain environmental agents such as pesticides and solvents, which could potentially affect brain development [7]. The search for biomarkers has led to some progress in the field. However, there are as many problems as there are answers (see reference 8): none of the biomarkers identified to date have proven either sensitive for the identification of autism (presence of biomarker reliably predicts occurrence of disorder) or specific for autism (presence readily distinguishes autism from other disorders or healthy groups). This hinders the development of causal therapeutic approaches as well as the development of routinely administered biological diagnostic procedures [8]. It is likely that autism is a highly complex disorder with multiple causative pathways involved in its etiology. If this is the case, the identification of reliable biomarkers for routine use in diagnosis and intervention may be impossible. Therefore, current treatment approaches to autism should be regarded as symptomatic, as they aim to improve the core deficits associated with the disorder in order to optimize the outcome of affected children (see reference 1).

No biomarkers of the disorder have as yet been identified [8]. Several different etiological accounts therefore coexist. The "opioid excess theory", proposed by Panksepp [9], draws parallels between the disorder's symptoms and the acute behavioral effects of opiates, i.e. the disorder is linked to increased activity in the endogenous opioid system. On the basis of this suggestion, Reichelt et al. [10,11] were able to demonstrate a possible nutritional link to autism, theorizing that certain food proteins, such as gluten and casein, can be transformed to opioid peptides during digestion. These peptides, hypothesized to be metabolized insufficiently, were suggested to accumulate and hence to be able to enter the blood stream through a "leaky gut", i.e. an increased permeability of the intestinal membrane [12]. Through systemic circulation, these peptides might cross the blood-brain barrier and act directly upon the central nervous system [13]. As such an account conceptualizes autism as a disorder of the "gut-brain-axis" [14], the account predicts heightened urinary peptide levels of these opioid peptides as a biomarker of the disorder. As a therapeutic consequence, a diet low in such proteins was hypothesized to normalize the urinary peptide levels and hence to ameliorate the behavioral symptoms of affected children [10,13]. Another prediction pertains to the presumed increased permeability of the intestinal membrane, allowing for systemic entry of food-derived opioid-like peptides.

Preclinical evaluations of central nervous system effects of these food-derived peptides (e.g. β-casomorphine) using rodent models were able to show behavioral effects such as a reduction in pain sensitivity, motor activity, and (social) orientation [15,16]. Other studies failed to show acute behavioral effects of gluten-

or casein-derived opioid peptides [17–20], while others found evidence for lasting behavioral changes following chronic administration during development [17,21].

Studies investigating the urinary profiles of individuals with autism showed increased levels of certain peptides [10,11,22–26]. Additionally, several reports [22,24,25] demonstrated reductions both in these peptide levels and in autistic symptomatology in individuals adhering to a diet free of gluten and/or casein (GFCF diet). This has lent some scientific credibility to the etiological account underlying the GFCF diet and advanced the diet's popularity, e.g. in media reports and cookbooks [27,28].

Several interventional studies have investigated the effects of a GFCF diet on autistic symptoms. Some of this literature has been summarized in a Cochrane review by Millward et al. [29], which included only two small randomized controlled trials and found mixed results regarding dietary effects. Mulloy et al. [30,31] conducted a more comprehensive review of evidence and included 14 studies for their systematic evaluation of dietary effects. Building on the work of Mulloy et al., we conducted an updated review of the empirical literature concerning diet effects as well as diet prevalence [32, 33]. Since our previous review [33], several dietary intervention trials, food challenge studies and prevalence surveys have been published [34–41], which allow for an updated review of empirical knowledge. The present review provides an update of empirical evidence regarding the effects of gluten-free and casein-free (GFCF) diets in children with autism. Additional aspects related to the GFCF diet (possible harms, prevalence of its use) will also be reviewed and updated.

2. Methods

2.1. Literature search procedure

A literature search, including publications up until October 2016, was conducted using PubMed, Medline, ERIC and Google Scholar. Search results were screened for relevant articles involving human subjects and any of the following: evaluations of GFCF diet effects (intervention studies), evaluations of gluten/casein challenges in children adhering to GFCF diets (challenge studies), evaluations of nutrition status and health in children adhering to a GFCF diet (side effects studies) as well as surveys dealing with GFCF as a treatment for autism (survey studies). The reference lists of identified studies were screened for additional trials. Identified studies were grouped into clusters according to their design and the outcome measures used. A total of 16 dietary intervention studies (5 case studies, 11 group studies), nine gluten/casein challenge studies (3 case studies, 6 group studies), 19 survey studies and another six studies concerning potential side effects of GFCF diets were identified and included. One dietary study [42] included in the review by Mulloy et al. [30] was not considered, as it involved only comorbid cases of ASD and attention deficit/hyperactivity disorder and a multimodal treatment involving a minimum of 8 interventions received simultaneously by participants, thereby complicating any straightforward interpretation of study results.

2.2. Selection and grouping of studies

The identified studies were grouped into clusters according to their outcome measures and their design (intervention studies, challenge studies, survey studies, studies related to side effects of a GFCF diet). Studies solely related to the construct validity of the "opioid excess theory" were excluded from systematic analysis. These include studies investigating the presence of urinary peptide levels in ASD populations as well as those dealing with other relevant predictions such as the presence of a "leaky gut". The analysis of dietary effects on urinary peptide levels, which was sometimes included in published dietary studies [22,25,43], was also excluded from this systematic review. While this kind of analysis may be important in establishing the validity of the etiological account underlying the use of GFCF diets in autism, it was not considered essential for the purpose of this review. It might be argued, on theoretical grounds, that an urinary peptide level analysis, performed before and after diet implementation, is indispensible in establishing a causal link between gluten/casein and autistic symptomatology. However, from a methodological point of view, it would seem at least as important to place the focus of analysis on the link between diet implementation and changes in autistic symptomatology. As the underlying theoretical account claims to be of therapeutic relevance, the demonstration of positive effects of the GFCF diet on aspects of autistic symptomatology, cognitive and motor skills and other relevant domains would seem to be among the most important yardsticks. This type of evaluation of diet effects will therefore be the main focus of this review.

2.3. Evaluation of studies according to the guidelines of Reichow et al. (2008) [44]

The current review of dietary intervention studies adopts a standardized set of evaluative guidelines in order to judge the scientific value of identified studies. Reichow et al. [44] recently offered such a set of methodological guidelines for the evaluation of therapeutic practices in autism. Their evaluative method seeks to establish a general framework which allows for the integration of results from different types of studies, i.e. single case studies and group research designs. This is achieved by explicating separate quality indicators for each type of study. In determining the report strength of a single study according to the level of its employed methodological rigor, the strength of the respective study can be judged from a predefined set of requirements as either "strong", "adequate" or "weak".

Table 1 gives on overview of the methodological quality indicators, on the basis of which a study's report strength is judged. As can be seen, there are so-called primary and secondary quality indicators for each type of interventional study (single vs. group).

The primary quality indicators are a set of essential requirements which a respective study type must fulfill in order to provide meaningful and valid results [44]. For example, a single case study must provide some proof for a valid and stable measurement baseline in the measures of interest, which are then shown to be reliably and repeatedly influenced by the introduction of the independent variable, i.e. the treatment. A group study, on the other hand, must involve a comparison condition which allows the determination of effect specificity of the independent variable. Furthermore, a group study has to fulfill criteria related to statistical testing of observed effects against chance (use of proper levels of analysis and statistical procedures), while a single case study must provide all relevant data and allow for detailed visual analysis of treatment effects. Other primary quality indicators are shared by both types of study design and deal with the maximization of a study's replicability. This, of course, allows the presentation of precise and detailed information regarding the participants involved, the precise treatment procedure implemented and the dependent measures taken.

The secondary quality indicators form a set of requirements, which might not be essential elements of a study's design in the production of valid results. Nevertheless, these design features improve the significance of a study's results by establishing agreement across objective information sources (interobserver agreement, kappa values, blindness of raters), by demonstrating real-life changes and endurance of effects (generalization/maintenance, social validity), by ensuring the quality of treatment throughout the study period (fidelity) and by controlling for/reporting on participant-dependent effects in group research (random assignment, analysis of attrition). These secondary indicators are also important for the establishment of a study's scientific strength.

These evaluation guidelines were adopted in the evaluation of both GFCF dietary intervention and gluten/casein challenge studies.

Table 1. Quality indicators of single case and group research studies (adapted from reference 44)

Single case studies	Group research studies
Primary quality indicators	
Participant characteristics	
Independent variable	
Dependent variable	
Baseline condition	Comparison condition
Visual analysis	Link between research question and data analysis
Experimental control	Use of statistical tests
Secondary quality indicators	
Fidelity of treatment	
Blindness of raters	
Generalization/maintenance	
Social validity	
Interobserver agreement	
Kappa	Randomization
	Attrition
	Effect size

3. Results

3.1. Survey studies

3.1.1. Prevalence of GFCF diet use

We identified a total of 19 survey studies, 18 of which sought to determine the prevalence of the GFCF diet in ASD populations (see Table 2). The GFCF diet was one of several treatment options in these studies, the aim of which was to assess the popularity of so-called complementary and alternative medicine (CAM) treatments among parents of children with ASD. While a number of the surveys were conducted in postal/analog form, the majority of the more recent studies took the form of an online survey (see Table 2). Two studies [45,46] were medical chart reviews or registry studies, which involved comparatively large populations. In terms of

CAM use prevalence, 54–81% of families reported the use of one or more of the various CAM treatment options available at some time in their lives, while 28–62% of parents reported current use of at least one CAM treatment option in their children with ASD. In reference to specific data on the use of the GFCF diet, the studies found somewhat diverse rates for the use of GFCF diets, with results indicating current use in approximately 8–32% of families and a previous use in around 20–55% of families (see Table 2). From these studies, it is evident that parents frequently report the use of multiple CAM treatments, particularly dietary treatment forms. These involve dietary supplementation with vitamins or minerals as well as specific forms of diet (Feingold diet, sugar free, GFCF etc.), of which the GFCF diet appears to be the most common [35,47,48]. Green et al. [47] showed that parents report the current use of an average of seven different treatment modalities (including CAM) for their children. This high number of different treatment options used is substantiated by some [49,50] but not all [51] studies and should be assessed more thoroughly on different national as well as socio-demographic levels. There are indications that higher levels of parental education, more severe symptoms, comorbid disorders and younger age of children with ASD are associated with CAM treatment use (see Table 2). It therefore seems obvious that intervention studies should assess and control for these alternative treatments and their potential effects regarding ASD symptomatology more thoroughly. As these alternative (background) treatments may also mitigate treatment effects when study groups are not controlled for them, this point should not be overlooked (see below).

3.1.2. Parental perception of GFCF diet effects

With respect to effects on ASD symptoms, only a subset of six survey studies assessed for parental perception of GFCF dietary effects [34,48,50,52–54]. These studies found up to 41–69% of parents reporting positive dietary effects, when collapsing across symptom domains. A recent UK survey study [50] questioned parent and expert groups about their experiences and perceptions regarding the use of a variety of treatment options. The parents reported current use of an average of four treatment modalities, and more than 80% reported the current use of a form of dietary intervention (with 29% reporting the use of a GFCF diet). When asked about perceived effects of the GFCF diet on various symptom domains, only 20–29% of the parents reported significant improvement (on a 5-point Likert-type scale, ranging from "significant decline" to "significant improvement") on the ASD core dimensions (communication, social interaction, repetitive behaviors/restricted interests). However, 54% of parents reported significant improvements regarding GI symptoms, and 42% reported significant improvements in concentration and attention in their child [50]. This finding of a greater dietary effect on comorbid problems is supported by the result of a survey conducted by Pennesi and Klein [55], which found that parents reported more positive effects of a GFCF diet when their children showed gastrointestinal symptoms or signs of allergy. This finding lends support to the possibility that there may be a subset of children with ASD who could benefit from such a GFCF diet. However, this possibility needs further exploration and should be validated by clinical observations in addition to those of parents.

3.1.3. Methodological problems of survey studies

Whether the representativeness of the survey studies may be generalized to the prevalence of CAM among all cases of ASD is unclear. The online studies cannot offer any information in this regard and the postal surveys produced a fairly low response rate (26–42%, see Table 2). Medical chart reviews or case registry studies could yield more information as they involve all documented cases within a defined time frame. However, this kind of study may also not be representative of the ASD population as a whole. For example, it might over-represent parents who rely mainly on conventional or evidence-based treatment options, as offered within medical and academically oriented settings. In this case, one might expect to find lower rates of reported CAM use. This is, in fact, what the results suggest. Since both registry studies show comparatively low rates of CAM use (28–32% CAM use, 8–15% GFCF diet use), these figures could be seen as an underestimation of the true prevalence of CAM use in children with ASD. At the same time, however, postal and internet surveys might attract highly committed parents and those actively looking for alternative treatment modalities in ASD, thus leading to an overestimation of CAM use prevalence. The true prevalence could be somewhere between the prevalence rates as estimated by these different kinds of studies. This question should be addressed more thoroughly in studies of representative samples of children with ASD.

Future survey studies of treatment options used should also employ a questionnaire involving an agreed-upon set of relevant treatment options. For example, Green et al. [47] based their list of 111 available treatment options on an earlier and comprehensive scientific review of available options [56]. By agreeing upon such a list,

researchers keep their study results comparable across national borders, allowing the analysis of trends in the use of different treatment options. To date, every survey has created its own list of treatment options (see Table 2). Furthermore, published studies should specify whether they asked respondents about current or former use of different treatment options. As some of the published reports failed to do this [45,52,57] (see Table 2), the prevalence rates need to be treated with caution when comparing them with other studies.

3.1.4. Summary of survey findings

In summary, the results presented above show that the GFCF diet is a CAM treatment option used by approximately 25 % of families with a child diagnosed with ASD. Furthermore, the diet is perceived by the majority of parents to have positive effects on various aspects of the child's functioning. There are indications that the core dimensions of autistic symptoms may not be those that are influenced most effectively by the diet. This point certainly deserves further consideration in dietary intervention studies, designed specifically for the identification of possible effect moderating variables. Future prevalence surveys should be conducted with a stronger focus on comparability of results across studies in order to allow for the analysis of trends in the use of treatment options.

Table 2. Summary of survey studies related to the prevalence and perceived effects of GFCF diet use

Authors	Type of study	Groups	Treatment-related measures	Main results
Smith & Antolovich (2000) [48]	Postal survey regarding prevalence of different treatment options (USA; response rate 42%)	121 completed questionnaires in children with autism (no further demographic information provided)	Questionnaire on the previous/current use of different treatment options; questions concerning perceived effectiveness	50% previous/current used elimination diets (mainly GFCF diet); 66% rated diets as helpful
Cornish (2002) [58]	Postal survey regarding nutritional status of autistic children on GFCF diet (U.K.; response rate: 26%)	37 completed questionnaires	(Among others) questions related to GFCF status	21% of sample using GFCF diet
Levy et al. (2003) [45]	Medical chart review of CAM use among children with autism (USA)	284 medical charts of children with autism	Unstructured interview questions concerning CAM use	32% reported CAM use; 15% reported GFCF use
Green et al. (2006) [47]	Internet survey regarding prevalence of different treatment options (mainly North American respondents)	552 completed questionnaires in children with autism	Questionnaire on use of different treatments (111 options listed); questions concerning current and previous use	25% current GFCF use; 20% former GFCF use; current use of an average of 7 treatments
Hanson et al. (2007) [52]	Postal survey regarding prevalence of different treatment options (USA; response rate 35%)	112 completed questionnaires in children with autism	Questionnaire on (current/previous?) use of different treatment options (15 categories); questions regarding perceived helpfulness of interventions	74% reported CAM use; 38% reported modified diet (incl. GFCF); 41% of users found modified diet to be helpful; more CAM use among more severe cases
Goin-Kochel et al. (2007, 2009) [49, 59]	Internet survey regarding prevalence of different treatment options (mainly North America)	479 completed questionnaires children with autism	Questionnaire on current/previous use of different treatment options (18 options listed)	13% current GFCF use; 32% previous GFCF use; current use of an average of 5 treatments; more treatments among younger children and more severe cases
Christon et al. (2010) [53]	Internet survey regarding prevalence of different treatment options (U.S. respondents)	248 completed parental questionnaires	Questionnaire on use of different treatment options (11 options listed; previous/current and current use, questions regarding perceived helpfulness of interventions	71% previous/current use, 51% current CAM use (more CAM among severe cases); 29% previous/current, 14% current GFCF use; 55% at least some symptomatic improvement after GFCF

Continued

Table 2 continued. Summary of survey studies related to the prevalence and perceived effects of GFCF diet use

Authors	Type of study	Groups	Treatment-related measures	Main results
Carter et al. (2011) [54]	Interview concerning prevalence of different treatments (Australia/ Sydney)	84 completed interviews in children with autism	Interview concerning CAM and conventional treatment use; questions related to current use	62% current CAM use; 32% currently GFCF use; 51% reported autistic symptom improvements
Bowker et al. (2011) [51]	Internet survey regarding prevalence of different treatment options (mainly North America)	970 completed question-naires	Open questions concerning current and previous treatments of children	14% (19%) current (previous) use of modified diet; current use of an average of 2 treatments
Frye et al. (2011) [60]	Internet survey regarding prevalence of treatments in children with ASD and comorbid seizures (USA)	290 completed parental questionnaires	19 different, non-traditional seizure treatments (incl. GFCF)	41% previous/current GFCF diet use in control ASD children
Pennesi & Klein (2012) [55]	Internet survey concerning factors moderating the effects of GFCF diet implementation (mainly North America)	387 completed question-naires: 223 strict GFCF followers, 70 incomplete GFCF followers, 94 non-users	Measures of diet imple-mentation (duration, strictness etc.), symptom ratings, gastrointestinal and allergy symptoms	More positive effects after stricter, longer diet (> 6 months); more positive effects in children with gastrointestinal symptoms and/or allergy
Perrin et al. (2012) [46]	Medical registry review of CAM use in children with autism (USA)	3173 completed data sets	Questionnaire on current use of different CAM treatments (23 categories)	28% CAM use (positively related to core/comorbid symptom severity; negatively related to use of prescribed drugs); 17% special diets (about 9% GFCF)
Huang et al (2013) [57]	Postal survey regarding prevalence of different treatment options (USA, response rate 36%)	22 completed parental questionnaires	10 CAM treatments (incl. GFCF diet)	9% (n=2) ASD children on GFCF diet; 82% (n=18) previous/current CAM use
Winburn et al. (2014) [50]	Internet survey regarding prevalence of different treatment options (U.K. respondents)	258 completed parental questionnaires	Questionnaire concerning use of different treat-ments (21 options), current use and perceived helpfulness of interven-tions	29% current GFCF use; current use of an average of 4 treatments; 20–29% significant improvements in autistic symptoms after GFCF diet; 42–54% significant improvements in gastrointestinal symptoms and attention
Akins et al. (2014) [61]	Data from population-based catchment area study of ASD children (CHARGE study, USA)	453 completed parental interviews	8 categories of different CAM treatments (incl. GFCF diet, current/past use)	39% (18%) previous/ current CAM (GFCF diet) use; 38% GFCF diet use in ASD children with gastro-intestinal symptoms
Granich et al. (2014) [62]	Data from self-selected study sample (postal survey in Australia)	169 completed parental questionnaires	7 categories of CAM treatment use (including special forms of diet)	54% previous/current CAM use (related to comorbid symptoms: gastrointest., ADHD); 8% previous/current diet use
Valicenti-McDermott et al. (2014) [63]	Interview/questionnaire study conducted in clinical settings (USA)	50 completed parental interviews	13 different CAM treat-ment options (including GFCF diet)	58% previous/current CAM use, 26% previous/ current GFCF diet use; CAM use associated with higher levels of parental education and comorbid problems in the child
Salomone et al. (2015) [35]	Internet survey concerning prevalence of different treatment options in ASD children (Europe)	1389 completed parental questionnaires	27 different CAM treatment categories (incl. GFCF diet)	47% (14%) CAM (GFCF diet) use during previous 6 months; CAM diet use associated with higher education and lower child verbal ability
Hopf et al. (2016) [34]	Internet survey concerning prevalence of different treatments (U.S. respondents)	194 completed parental questionnaires	120 different CAM treatments (incl. GFCF); perceived helpfulness of interventions	81% (55%) previous/ current CAM (GFCF diet) use; GFCF diet perceived to “make things better”

3.2. GFCF dietary intervention studies

3.2.1. Case studies

The five identified case studies form a group of highly diverse publications in terms of scientific quality, ranging from purely anecdotal case reports [64,65] to more scientific trials seeking to establish a causal role of the GFCF diet in autistic symptom relief [66–68]. The upper part of Table 3 gives an overview of these studies and provides some basic information regarding the measures used, the duration of dietary manipulation, their main results and their scientific strength in terms of the guidelines by Reichow et al. [44]. All of these case studies found evidence for positive dietary effects at least for some of the measures employed (e.g. physical development, autistic symptomatology, and cognitive skills). As an example, Knivsberg and colleagues [66] were able to follow-up a seven-year-old girl for a period of two years after the introduction of a GFCF diet by her parents. The authors provided anecdotal evidence from behavioral reports that the girls' communicative patterns normalized and she responded when addressed by others. The girl's social interest grew stronger, i.e. she participated in gaming activities and had a close friend by the time of follow-up. These reports were substantiated by parental and teacher observation ratings as well as by formal tests of cognitive abilities, which documented an ongoing development of cognitive skill at the follow-ups after one and two years (in linguistic abilities, and nonverbal reasoning skills). The authors attribute this (positive) development to the introduction of the GFCF diet [66].

As can be seen from Table 3, no single case study has been conducted with adequate scientific rigor and thus the results from these studies have to be considered as weak evidence at best. None of these studies implemented an experimental protocol involving repeated introduction and discontinuation of the GFCF diet with an accompanying assessment of effects. Only one study [68] ensured a measurement baseline at least for some of the employed measures. Another problematic aspect of the two studies using standardized testing procedures [66,68] relates to their inadequate use of test/measurement data, i.e. the calculation of mental age scores from raw data [68] or the use of raw data itself [66] in order to determine treatment progress. This procedure might seem feasible in short-term evaluations of treatment effects or in adult populations, where follow-up assessments are not as strongly affected by normative developmental spurts. However, in long-term evaluations and in the age ranges covered by the published case studies of GFCF dietary effects (3–12 years of age), every attempt should be made to control for time or maturational effects. This could be achieved by using standardized and normed assessment instruments, which allow for the calculation of age-sensitive standard scores (e.g. percentile ranks). By doing so, the question of whether the introduction of a GFCF diet leads to improvements in the child's standard scores could be analyzed, and more insight could be gained into positive dietary effects in the course of a child's development. A mere statement that a child progressed in mental age, as made by Hsu et al. [68], would not seem sufficient to link this progress to the GFCF diet rather than simple developmental progress or maturation. Another problematic aspect is the use of parents as an information source: each of the five studies gained information concerning the child's autistic behavior symptoms from parents, who, as unblinded providers of treatment, may be biased in their perception of diet effects. This point deserves further consideration, and future case studies should implement observational measures and clinician-administered rating procedures to arrive at more objective ratings across observers.

3.2.2. Group studies

Table 3 provides an overview of the 11 identified group studies concerned with the evaluation of GFCF dietary intervention effects. As can be seen, a subset of four studies did not strictly eliminate both foods from a child's diet, but employed either a gluten-free [37,69–71] or a casein-free diet [72]. Another study [73] can be dismissed as unscientific due to a complete lack of formal definitions of improvement, procedural information on diet implementation or descriptions of information sources. The authors present results of GFCF dietary effects in a subset of 61 children diagnosed with ASD, who were switched to some form of elimination diet (including gluten-free, casein-free, and/or soy-free diets or combinations thereof). The displayed results imply that the dietary interventions led to clinical improvements in 56 of 61 children (91.8%) [73]. No indication is given of what improvement means, in which symptom domain it occurred, and how exactly it was established. These results are therefore valueless.

Of the six uncontrolled group studies [22,24,25,69–73], all but one trial [70,71] were able to show positive dietary effects on autistic core symptoms, cognitive deficits, comorbid symptoms or gastrointestinal problems. All of these studies were rated to provide only weak scientific evidence, however, as they were lacking control procedures (see Table 3). However, this was not

the only major methodological problem inherent in these trials. None of them reported on the use of blinding procedures in the assessment of dependent measures. Additionally, all but one study [24,25] failed to report on measures taken to ensure the fidelity of treatment (i.e. the degree of adherence to a GFCF diet regimen). Half of the studies employed no or improper statistical tests [22, 73] or were underpowered to do so [70,71]. Therefore, the results from this group of studies should be treated with caution.

Of the five controlled group studies [37,43,74–79], all but one trial [77,78] were conducted with adequate scientific rigor. Although the randomized and controlled trial reported by Knivsberg and colleagues [77,78] did meet the requirements of all primary quality indicators with at least acceptable quality (the study failed to provide gender information of their sample and it did not state and control for additional treatments beyond the GFCF diet), it was able to provide only weak scientific evidence. This was due to a complete lack of additional quality indicators, including the following: no blinding procedures were employed, treatment fidelity was not controlled for, and attrition was neither analyzed nor reported. Nonetheless, this study provided consistent evidence for positive dietary effects on autistic core symptoms, cognitive performance and motor problems over a follow-up period of one year [77,78]. These findings are supported by recently published results of a randomized trial of a gluten-free diet [37]. This study employed a partly standardized supply of gluten-free foods and sought to establish and control the fidelity of treatments by means of parent manuals and advisory phone calls throughout the study period of 6 weeks. While not controlling for additional treatments received in both groups, study and control groups were matched for age and sex and were comparable in comorbid attention/hyperactivity problems. As this study was conducted with adequate scientific rigor, it is interesting to note that positive dietary effects of a gluten-free diet occurred after only six weeks of diet adherence. These effects were rated solely by (unblinded) parents and encompassed both autistic core symptoms and gastrointestinal symptoms. These two favorable evaluations of dietary effects are contrasted with the results of two dietary trials involving six [43, 74] to twelve weeks [79] of diet adherence. Both of these studies were conducted with adequate scientific rigor, but differed in their respective study design. Elder and colleagues [43,74] employed a double-blind, randomized (counterbalanced) crossover design and studied the effects of GFCF diet introduction against a within-subjects control condition involving a regular diet. Blindness was achieved by the implementation of a study kitchen providing parents with ready-cooked study foods either devoid of or including gluten- and casein-containing ingredients. As well as parental ratings of autistic symptoms, the study also involved at-home observations and ratings of parent-child interaction by blinded coders. This study failed to show any significant and positive effects of a GFCF diet. These negative findings are supported by those reported by Johnson and colleagues [79], who conducted a small, randomized controlled study of the GFCF diet involving young children with ASD (mean age 3.3 years). Parental ratings of autistic symptoms three months after diet implementation did not indicate any positive gains for children put on the restriction diet, which was corroborated by (blinded) observation measures and developmental testing procedures. Another GFCF dietary trial conducted with adequate scientific rigor [75,76] failed to show consistent positive dietary effects on autistic symptoms, attention/hyperactivity symptoms or neurodevelopmental ratings obtained from (unblinded) parents. Across the multiple contrasts conducted throughout the groups and follow-up period of up to two years, only a subset (not corrected for multiple testing) showed significant positive effects of the GFCF diet, while the majority of contrasts failed to do so.

3.2.3. Summary of dietary intervention studies

Taken together, the studies reviewed above show a highly divergent picture of results, not allowing for any clear-cut conclusions regarding GFCF diet effects in children with ASD. While the majority of studies conducted without adequate scientific rigor provided evidence for positive effects of GFCF diet adherence, more rigorous scientific evaluations failed to provide a consistent pattern of results. Many studies are hampered by methodological flaws, such as a strong reliance on (unblinded) parental reports as the sole information source regarding ASD symptoms, a frequent lack of control procedures (control groups, measurement baselines, control for additional treatments) or attempts to monitor and assess treatment fidelity. This clearly needs to be considered in interpreting the overall pattern of results.

Future studies in this field should seek to assess and control for additional treatments received by children and should include ratings of autistic symptoms performed by uninvolved (and blinded) clinicians. As shown by Ghalichi and colleagues [37], treatment effects may occur as early as six weeks after diet introduction. Nonetheless, longer

follow-up periods involving multiple assessments would seem advisable, given the many positive results reported by case/group studies with substantially longer follow-up periods (see Table 3).

Table 3. Summary of intervention studies of GFCF dietary effects on various dependent measures, grouped by quality of design (C: controlled study; UC: uncontrolled study)

Authors	Measures	Type/duration	Information sources	Results
Weak report strength				
Fields & Fields (1976) [65]	ASD symptoms	Case, several years	Parents	Positive effects of GFCF diet on several behavioral aspects
Adams & Conn (1997) [64]	ASD symptoms	Case, unknown	Parents	Positive effects of GFCF diet on several behavioral aspects
Knivsberg et al. (1999) [66]	ASD symptoms, cognitive skills	Case, 2 years	Parents, test	Positive effects of GFCF diet on several behavioral aspects and cognitive skills
Hsu et al. (2009) [68]	ASD symptoms, behavior problems, developmental level	Case, 1 year	Parents, test	Positive GFCF diet effects on several behavioral aspects, gastrointestinal symptoms, cognitive and physical development (growth)
Herbert & Buckley (2013) [67]	ASD symptoms, comorbidity	Case, several years	Parents	Amelioration of autistic symptoms while on GFCF diet (no improvement in immunological and gastrointestinal symptoms)
Knivsberg et al. (1990, 1995) [24, 25]	ASD symptoms, cognitive & psycholinguistic functioning	UC/Group, 4 years	Parents, tests	Stable improvement in symptomatic behaviors and cognitive/linguistic skills
Lucarelli et al. (1995) [72]	ASD symptoms	UC/Group, 8 weeks	Parents	Improvement in autistic behaviors following CF diet implementation
Gemmell & Chambliss (1997) [70], **Pontino et al. (1998)** [71]	Measure of treatment progress in applied behavioral analysis	UC/Group, 9 months	Treatment provider	No clear and consistent positive changes in rate of skills achievement following implementation of a GF diet
Whiteley et al. (1999) [69]	ASD symptoms, cognitive skills	UC/Group, 3 months	Parents/ teachers, test	Behavioral (motor, feeding, attention) and some cognitive improvement
Cade et al. (2000) [22]	ASD symptoms	UC/Group, 1 year	Parents/ physicians	Stable improvement in autistic behaviors throughout follow-up period
Jyonouchi et al. (2002) [73]	ASD symptoms, comorbidities	UC/Group, unknown	parents/ teachers/ physicians	Improvements in any of several problem domains in >90% of children (autistic behaviors, gastrointestinal sympt., sleep, concentration, speech)
Knivsberg et al. (2002, 2003) [77, 78]	ASD symptoms, cognitive, linguistic and motor functioning	C/Group, 1 year	Parents, tests	Improvements in almost all autistic behavior domains and in cognitive and motor performance during study period
Adequate report strength				
Elder et al. (2006) [43], **Seung et al. (2007)** [74]	ASD symptoms, language and social functioning, parent-child interactions	C/Group, 12 weeks	Parents, coders	No significant treatment effects, although indications of positive effects in subjective reports of several parents
Whiteley et al. (2010) [75], **Pedersen et al. (2013)** [76]	ASD symptoms, ADHD symptoms, and neuro-developmental ratings	C/Group, 1–2 years	Parents, children	Several improvements in autistic and related behaviors after 8 and 12 months; not consistent across study groups and throughout study period
Johnson et al. (2011) [79]	Problem behavior ratings, behavioral observations, developmental testing	C/Group, 3 months	Parents, coders, tests	No significant treatment effects in behavior or developmental domain; no differences between groups in terms of nutritional adequacy (more adherence problems in GFCF diet group)
Ghalichi et al. (2016) [37]	Gastrointestinal symptoms, ASD symptoms	C/Group, 6 weeks	Parents	Significant improvements in gastro-intestinal and ASD symptoms in children adhering to a gluten-free diet
Strong report strength				
No studies found				

Table 4. Summary of challenge studies of gluten/casein effects on various dependent measures, grouped by quality of design

Authors	Measures	Type/duration	Information sources	Results
Weak report strength				
Bird et al. (1977) [80]	Observation of situational behaviors	Case, 7–11 days	Coders	No observable dietary challenge effects on behavior
O'Banion et al. (1978) [81]	Observation of situational behaviors	Case, 4–72h observation after repeated challenges	Coders	Acute increases in behavioral reactivity to challenge with certain foods including gluten/wheat; report on long-term cycling reaction to wheat
Irvin (2006) [82]	Observation of situational behaviors	Case, 12–21 days	Coders	No acute, observable dietary challenge effects on behavior problems
Lucarelli et al. (1995) [72]	Autistic behavior	UC/Group, < 2 weeks after a single challenge	Caregivers	Acute increases in motor disturbances, inappropriate emotional responses and disturbances in concentration; no changes in social isolation, verbal communication, eating behaviors and reactions to sensory stimuli
Whiteley et al. (1999) [69]	Autistic behavior, cognition/ language	UC/Group, n.a.	Parents, tests	No further behavioral impairment; no significant decrease in cognit. functions
McCarthy & Coleman (1979) [83]	Autistic behavior	UC/Group, 4 weeks	Parents, investigators	No gross and acute changes in behavior due to challenge or after restoration of a gluten-free diet
Pusponegoro et al. (2015) [41]	Gastrointestinal symptoms, autistic behavior	C/Group, 1 week	Parents	No significant differences between challenge and control group in autistic symptoms or gastrointestinal symptoms (trend towards increased gastrointestinal symptoms in challenge group)
Adequate report strength				
Navarro et al. (2015) [40]	Inattention, hyperactivity, irritability, intestinal permeability	C/Group, 4 weeks	Parents, biomedical testing	No significant differences between challenge and control group in (comorbid) behavior problems and/or intestinal permeability
Strong report strength				
Hyman et al. (2016) [38]	Stool quality, sleep quality, ADHD symptoms, ASD symptoms,	C/Group, 24 h observation after different challenges	Parents, investigators, coders, teachers, actigraphy	No significant (within-subject) effects of food challenges (gluten-only, casein-only, gluten+casein vs. control) on any of the functional domains assessed

3.3. Gluten/casein challenge studies

We were able to identify a total of 9 studies (see Table 4) involving gluten/casein challenges with a concomitant observation of (negative) behavioral effects, equally distributed across different study designs, i.e. three case studies [80–82], three uncontrolled group studies [69,72,83] and three controlled group studies [38,40,41]. With the exception of one study [41], these studies assessed the effects of gluten/casein challenges in children adhering to some form of GFCF diet or after a washout period (e.g. after fasting periods of several days; see reference 81). These studies rarely justified their precise rationale for choosing a certain duration for both the challenge period and/or the ensuing observation period. From those that did [38,72,80,81], it is evident that design considerations were based on studies of food allergies. In a case study of an eight-year-old boy with autism, O'Banion and colleagues [81] demonstrated acute negative effects of gluten-/casein-containing foods. The consumption of such products, particularly wheat products, caused an acute increase in the rate of occurrence of several problem behaviors (general motor activity, laughing, screaming, biting, scratching, and throwing objects). Similar (although less extreme) increases in problem behaviors were also noted after ingestion of sugar, tomatoes and mushrooms [81]. This finding is supported by the results of another uncontrolled group study conducted by Lucarelli and colleagues [72]. These researchers were able to show that a single challenge with food allergens, including casein, in children with ASD on a casein-free diet led to some increases in behavioral symptoms during a variable follow-up period

of up to two weeks. These included significant increases in three of seven behavioral domains assessed: motor disturbances, inappropriate emotional responses, disturbances in concentration/perception.

Aside from these two studies showing acute reactivity of autistic symptomatology in single occasion challenges with gluten and/or casein, all studies failed to show any clear-cut results following such food challenges. Among these studies providing null results are two conducted with at least adequate scientific rigor [38,40], suggesting the absence of dietary challenge effects. Given that some of the studies reporting null results reintroduced Western standard diets or daily gluten-/casein challenges for periods of one to four weeks [40,80,82,83], there also seem to be no consistent effects of longer term ingestion of gluten/casein in children adhering to a GFCF diet. The most conclusive evidence in this respect stems from a small randomized double-blind placebo-controlled challenge study conducted by Navarro and colleagues [40], in which a group of 12 children with ASD underwent a GFCF diet regimen for two weeks before half were randomly assigned to experimental gluten/casein challenges or a placebo challenge group. Challenges were provided in standardized form by parents who were blinded to group status. This was achieved by supplying them with supplements equivalent in appearance and taste that either did or did not contain gluten and casein. Parents were asked to administer to their child a daily defined dose of the provided supplement for a period of four weeks, while continuing the GFCF diet regimen. Half of the children were continued on a GFCF diet for a total time period of six weeks, while the other half underwent gluten/casein challenges for a total duration of four weeks. Study results showed no consistent effects of the gluten/casein challenges on behavioral problems (hyperactivity, inattention, irritability) or the levels of gastrointestinal symptoms; the sample sizes of these studies were rather small. Taking these complex findings together, it seems that the majority of the identified challenge studies were unable to show clear-cut effects of gluten/casein on symptomatic expression of autistic symptoms, comorbid behavior problems, cognitive functioning or gastrointestinal symptoms. As only two of nine identified studies were conducted with at least adequate scientific rigor, however, this evidence must be treated with some caution and warrants further replication in more sophisticated studies in the future. It is, at present, unclear what time scale can reasonably be expected to allow an observation of behavioral effects of gluten/casein challenges in children with autism. At the present time, the form that a dietary challenge study should take remains unclear. Having based their design and study rationale on studies of food allergies, Hyman and colleagues, in a highly sophisticated study [38], excluded ASD children with putative or established allergies (such as celiac disease) to ingredients used in their gluten/casein food challenges. Future studies could potentially include children at risk for gluten/casein allergy or those with established disturbances in the metabolic breakdown of food proteins such as gluten/casein. As discussed by Whiteley [84], there may be a diet-related phenotype of autism, which could be linked to some biological aberrations related linked to abnormal functioning of the gut-brain axis. Future challenge studies could include some of the discussed biomarkers of this rather loose and yet to be established diet-related phenotype, as its presence might modulate the effects of gluten/casein challenges in children with ASD. This could also be of relevance for dietary intervention studies, as the identification of such a diet-related phenotype may help in identifying those children who could benefit from a GFCF diet regimen.

3.4. Studies of potential harms of the GFCF diet

The six identified studies of potential harms of a GFCF diet in children with autism were mainly related to one of two aspects: nutritional adequacy [39,58,85,86] or physical development [87,88].

The four studies related to nutritional adequacy investigated possible deficiencies of children on restriction diets as compared to healthy control children or children with autism on unrestricted diets. Three of these studies sought to estimate adequacy from dietary records maintained by parents; none of them found evidence of more nutritional deficiencies than in the respective comparison groups [39, 58, 86]. The fourth of these studies estimated deficiencies from plasma-derived levels of essential amino acids and found evidence for deficiencies in several of these, including important neurotransmitter precursors such as tyrosine and tryptophan [85].

The two studies of physical development compared bone development of children with autism on a casein-free diet (with a low intake of calcium) with those on unrestricted diets as well as control values [87, 88]. These studies found that children with autism generally displayed reduced bone density, with the reduction being significantly greater in the group on a casein-free diet [87, 88]. These aspects should be considered more thoroughly

and on a routine basis in future studies of GFCF dietary effects.

4. Discussion

4.1. Issues in determining strength of evidence according to Reichow et al. (2008) [44]

The judgment of quality indicators and the classification of report strength performed according to Reichow et al. [44] have proved to be efficient. The evaluation results obtained by applying the present method are similar to those presented by Mulloy et al. [30]. The only major difference in evaluation results relates to the case studies of Bird et al. [80] and Irvin [82]: While Mulloy et al. [30] judged them to provide significant amounts of evidence, they were judged to provide only weak evidence in this review and were thus rated more negatively. This was due to the inability of either study to provide an adequate measurement baseline from which to judge the effects of diet implementation. Nevertheless, both studies fulfilled the largest number of quality indicators among the single case studies. Thus, these differences in categorical judgement do not seem to be related to fundamental differences in the evaluation of research quality, but rather slightly different thresholds of categorization.

The evaluation method, as proposed by Reichow et al. [44], is intended to be applicable to all kinds of intervention studies in autism research. Nevertheless, several specific issues emerged when applying the guidelines to the evaluation of the GFCF diet, which were resolved by applying minor modifications. As an example, for single case studies, the original guidelines require that the experimental effect is shown at least three times in the same individual [44]. In the context of the GFCF diet, this appeared, for practical reasons, to be too difficult to operationalize and therefore this criterion was lowered to a minimum of two demonstrations of the experimental effect. In respect of group studies, the guidelines require the blindness of raters (e.g. parents), who should be motivated and should implement the diet with as few errors as possible (fidelity), having been randomly assigned to either a GFCF diet or a control group (randomization). It would seem almost impossible to implement a dietary group study fulfilling all these criteria in normal conditions. The only study providing some solution to this practical problem is that of Elder et al. [43], who employed a study kitchen from which participating families were supplied with daily food regimens on a regular basis. Thus, meals could be prepared as either GFCF or regular diet without knowledge of the parents. This procedure allowed for randomized treatment application with high treatment fidelity, while at the same time ensuring the blindness of raters (parents). While this methodological approach would appear to be in keeping with core requirements of interventional science, the question remains whether parents would be willing to use such a study kitchen for longer follow-up periods (e.g. 12 months or more). In this respect, the costs incurred by the invasiveness of a highly controlled dietary treatment condition would have to be weighed against the benefits that could reasonably be obtained, given the study protocol. In view of the strictness of the evaluation guidelines used, one may reasonably expect some compromising of long-term dietary intervention trials due to the impracticability of strict adherence to sound methodological principles.

4.2 Recommendations for future studies of GFCF dietary effects

The scientific evaluation of long-term and onerous dietary interventions such as the GFCF diet has not proved to be an easily managed endeavor. There are many methodological problems, which need to be addressed with adequate research designs. Studies are often limited by practical or financial constraints. In light of the above-mentioned methodological shortcomings of existing studies and conceptual issues related to the GFCF diet, some recommendations aimed at improving the methodological quality of future dietary studies seems advisable. The following recommendations do not claim to be comprehensive or to provide the only research strategy to investigate GFCF dietary effects, but are intended to caution researchers as to potential pitfalls when conducting dietary trials. Table 5 provides several solutions to the problems of previous GFCF dietary studies, such as the implementation of adequate control procedures for single case or group research studies. It gives examples of standardized assessment instruments, which could be used with the aim of establishing comparability of results across studies as well as increasing the validity of study results obtained. Other recommendations pertain to trial duration or the use of a broader range of measures in order to gain insight into additional relevant aspects (moderator/mediators of treatment effects, potential risks of GFCF diet).

Table 5. List of recommended solutions to selected methodological problems of dietary studies

Methodological problem	Recommended solutions
Implementation of control condition	• Comprehensive assessment of additional treatments • Single case: establishing adequate measurement baseline (percentile ranks) • Control group: control for attention effects (e.g. nutritionist counseling)
Assessment of interobserver agreement	• Use of (blinded) clinician ratings in addition to parent ratings of symptoms • Complementation with test data and behavioral observations conducted in natural settings
Trial duration	At least 12 months, multiple follow-ups for trend analysis
Diversity of assessment methods	• Use of standardized rating scales for parents, teachers, clinicians (CARS, BRIEF, ASRS etc.) • Use of established coding schemes for behavioral observations (e.g. ADOS) • Use of standardized clinical measures (e.g. ADI-R) • Use of normed and standardized cognitive/linguistic/neuropsychological test procedures (RPM, K-ABC, ITPA, Bayley Scales, EVT/PPVT etc.) → Multimethod-multirater assessments
Treatment fidelity	Cooperation with nutritionists for ensuring/maintaining diet adherence
Blindness of raters	• Study kitchen supplying families with control/study foods (blindness of parents) • Ensure that involved clinicians and coders are blind to treatment status
Sample size	Use of larger sample sizes (n per group: 30); control and report of attrition
Risk measures	Integration of risk measures into data collection protocol (nutritional status, bone density, physical development etc.)
Moderators/mediators of treatment	Integration of suspected moderating/mediating variables into data collection protocol (e.g. attention/hyperactivity symptoms, food allergies, gastrointestinal symptoms, parental beliefs/ expectations prior to diet implementation etc.)

Abbreviations. CARS: Childhood Autism Rating Scale [89]; BRIEF: Behavior Rating Inventory of Executive Function [90]; ASRS: Autism Spectrum Rating Scales [91]; ADOS: Autism Diagnostic Observation Schedule [92]; ADI-R: Autism Diagnostic Interview-Revised [93]; RPM: Ravens Progressive Matrices Test [94]; K-ABC: Kaufman Assessment Battery for Children [95]; ITPA: Illinois Test of Psycholinguistic Abilities [96]; Bayley Scales: Bayley Scales of Infant Development [97]; EVT/PPVT: Expressive Vocabulary Test & Peabody Picture Vocabulary Test [98, 99]

4.3. Use of the GFCF diet in autism

Taking the findings of this review together, the scientific basis supporting the effectiveness of the GFCF diet in the treatment of autism is very weak and cannot even be judged as promising. Despite the popularity of this diet as a supplementary treatment, its widespread use (as indicated by prevalence studies) and the positive views of parents regarding its effects, several rigorous scientific evaluations failed to confirm these observations. The few methodologically acceptable studies conducted to date do not allow for any firm conclusions concerning the diet's effectiveness. The results of these studies contrast with those of a large number of flawed and unsound research studies. As can be seen from time analysis, however, there is a positive trend towards sound methodological quality (see Tables 3 and 4). As stated above, the establishment of a clear link between diet implementation and positive effects on autistic symptomatology can be regarded as one very important yardstick in the evaluation of the "opioid excess theory". On the basis of current evidence, such an evaluation cannot be performed and should be postponed until a sufficient number of methodologically sound studies have been performed.

Recent studies conducted within the framework of the "opioid excess theory" have provided inconsistent evidence regarding some of the theory's major predictions, e.g. the detection of heightened urinary (opioid) peptide levels [43,69,100–104]. These inconsistent scientific observations also weaken the underlying rationale for the recommendation and use of the GFCF diet as a direct and compensatory treatment of the hypothesized etiology of autism. Nevertheless, future dietary studies in this field of inquiry should implement methodologically sound designs in order to establish more convincing evidence regarding dietary effects (see Table 5). Studies should implement data collection strategies sensitive to assessing both the benefits and potential harms of such a dietary approach.

4.4. Concluding remarks

Although the dietary studies conducted thus far do not appear to confirm the predictions of the "opioid excess theory" and the conceptualization of autism as a metabolic disorder, the case for nutrition in autism should not be closed prematurely. As there is increasing evidence for possible links between gut anomalies and the brain in individuals with autism [14], which also point to the importance of immunological factors and their role in the frequently observed gastrointestinal disturbances in children with autism, the consideration of gluten/casein and other dietary factors should not yet be disregarded in autism research. This conceptualization of autism as an

immunological disorder could open up avenues for the explanation of multiple environmentally mediated pathways leading to autistic symptoms. In such a theoretical model, gluten/casein and other food-derived proteins may play a role in triggering allergic responses, which could influence brain development and function by exerting a direct influence on neuronal functions (see reference 14). Although the literature regarding possible links between allergic reactions to gluten/casein and autism is promising (e.g. references 105–107), there is a clear need for more scientific studies investigating such a possibility in order to more comprehensively judge the role of nutrition in the etiology and treatment of autism.

Conflict of interest

The authors declare no conflict of interest**.**

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