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Systematic review with meta-analysis: early infant feeding and coeliac disease – update 2015

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[a]*See Appendix.*

SUMMARY

Background
New evidence emerged on early feeding practices and the risk of coeliac disease.

Aim
To systematically update evidence on these practices to find out whether there is a need to revise current recommendations.

Methods
MEDLINE, EMBASE and the Cochrane Library were searched from July 2012 (end of last search) to February 2015 for studies of any design that assessed the effect of gluten consumption and breastfeeding on the development of coeliac disease and/or coeliac disease-related autoimmunity.

Results
We identified 21 publications, including two, new, large, randomised controlled trials performed in high-risk infants. Exclusive or any breastfeeding, as well as breastfeeding at the time of gluten introduction, did not reduce the risk of developing coeliac disease during childhood. For infants at high risk of developing coeliac disease, gluten introduction at 4 months of age in very small amounts, or at 6 or 12 months of age, resulted in similar rates of coeliac disease diagnosis in early childhood. Later gluten introduction was associated with later development of coeliac specific autoimmunity and coeliac disease during childhood, but not total risk reduction. Observational studies indicate that consumption of a higher amount of gluten at weaning may increase the risk for coeliac disease development.

Conclusions
Infant feeding practices (breastfeeding, time of gluten introduction) have no effect on the risk of developing coeliac disease during childhood (at least at specific timeframes evaluated in the included studies), necessitating an update of current European recommendations.

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INTRODUCTION

Coeliac disease (CD) is an immune-mediated systemic disorder elicited by the consumption of gluten and related prolamines in genetically susceptible individuals. CD is characterised by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 or HLA-DQ8 haplotypes and enteropathy.[1] The prevalence of CD in the general population varies considerably from <0.25% to >1%,[2] but may be as high as 3%.[3, 4] The only currently available treatment for CD is a lifelong gluten-free diet. Primary prevention strategies focus on early feeding practices, namely breastfeeding and the time and mode of gluten introduction into the infant's diet.

In 2008, based on the available evidence obtained exclusively from observational studies, scientific authorities such as the Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Food Safety Authority (EFSA)[5, 6] recommended that it is prudent to avoid both early (less than 4 months of age) and late (7 or more months of age) gluten introduction and to introduce gluten while the infant is still being breastfed.[6] It was considered that such a strategy may reduce not only the risk of CD but also the risks of type 1 diabetes mellitus and wheat allergy. The American Academy of Pediatrics (AAP) recommended that complementary foods can be introduced between 4 and 6 months of age; however, the AAP did not give specific guidelines with regard to gluten introduction.[7] These recommendations are still prevailing.

Prevent Coeliac Disease (PREVENTCD; www.preventcd.com) is an ongoing multinational project financed, among others, by the European Commission under the 6th FP programme (FP6-2005-FOOD-4B-36383). The purpose of this project is to investigate the influence of infant nutrition on the development of CD and related autoimmune phenomena, as well as how genetic, immunological and environmental factors relate to this development.[8] In 2012, as part of PREVENTCD, we summarised, in a systematic review, evidence on the possible relationship between early feeding practices and the risk of developing CD.[9] However, in the last few years, a number of new relevant studies have been published, including one randomised, double-blind, placebo-controlled trial conducted by the PREVENTCD Study Group.[10] These studies have prompted interest in updating current evidence. Here, our aim was to update the 2012 assessment of the effects of early gluten consumption and breastfeeding on the risk for CD to find out whether there is a need to revise current recommendations. As previously, our systematic review was designed to answer a number of clinically important questions, which are summarised below:

(i) *Breastfeeding and CD*. Does exclusive or any breastfeeding reduce the risk of developing CD? Does exclusive or any breastfeeding change the age when CD develops? Is the duration of breastfeeding related to the risk of developing CD?

(ii) *Breastfeeding at the time of gluten introduction and CD*. Is breastfeeding while gluten is introduced important for risk reduction?

(iii) *Timing of gluten introduction*. Is the age of gluten introduction important for the risk of developing CD? Is the age of gluten introduction important to the age when CD develops?

(iv) *Amount of gluten at weaning (and later) and CD*. Is the amount of gluten ingested at the time of introduction and/or later a risk factor for the development of CD? Is there a threshold level of gluten consumption for developing CD?

(v) *Type of gluten*. Does the type of cereal [wheat, rye, or barley] at gluten introduction influence CD risk? Does the type of gluten-containing food [e.g. bread, porridge, follow-on formula] used at gluten introduction influence CD risk?

(vi) *Gluten during lactation*. Does consumption of a gluten-free-diet vs. a normal diet by lactating mothers alter the risk of the offspring developing CD?

(vii) *Genetic predisposition*. Does the influence of any of the identified factors differ between infants from the general population and the infants at high risk of developing CD?

METHODS

The protocol for this systematic review was registered with PROSPERO, registration number CRD42014013865. The same methodology that has been already presented in our previous review was followed.[9] In brief, MEDLINE, EMBASE and the Cochrane Library were searched from July 2012 (end of last search) to November 2014, and again in February 2015. The principal search terms used separately for each clinical question were as follows: 'celiac or coeliac or CD or sprue or gluten enteropathy or gluten intolerance; breast feeding or breastfeeding or breast feeding or breastfed or human milk; child* or childhood or children or infant* or toddler or early;

gluten and (timing or time) and introduction; amount or quantity'. Researchers working in the field were contacted for any unpublished data. Letters to the editor, abstracts and proceedings from scientific meetings were excluded, unless a full set of data was available from the authors. No language restriction was imposed. The searches were carried out independently by three reviewers (AC, MPL, HS). Studies of any design investigating the potential association between early feeding practices and CD risk were eligible for inclusion. Special emphasis was placed on randomised controlled trials (RCTs). In the prospective studies, participants had to be infants at population risk or increased risk of developing CD (defined by either HLA status and/or first-degree relative with CD and/or type 1 diabetes mellitus). For retrospective studies, participants had to be children or adults with CD proven by small bowel biopsy or presenting with positive CD-specific autoantibodies (i.e., anti-transglutaminase or anti-endomysial antibodies) indicating CD-related autoimmunity.

Interventions eligible for assessment were those involving the consumption of gluten-containing products of any type (any food containing gluten or preparations manufactured for research purposes).

The primary outcome measure was the development of CD or the development of CD-related autoimmunity. The first step of the systematic review was the initial screening of titles, abstracts and keywords of every record identified. Then, full texts of the trials considered as relevant were obtained. The reviewers independently assessed the eligibility of each trial, and any disagreements were resolved by discussion.

Two reviewers (AC, MPL) independently, but without being blinded to the authors or journal, assessed the risk of bias in the studies that met the inclusion criteria using the tools recommended by The Cochrane Handbook for Systematic Reviews of Interventions.[11] For RCTs, risk of bias assessment includes the following criteria: adequacy of sequence generation; allocation concealment; blinding of participants, personnel and outcome assessors; incomplete outcome data and selective outcome reporting. In all cases, an answer of 'yes' indicates a low risk of bias, and an answer of 'no' indicates a high risk of bias. For nonrandomised studies, the study quality was assessed with the Newcastle-Ottawa Scale (NOS),[12] as recommended by The Cochrane Handbook for Systematic Reviews of Interventions.[11] It includes a 'star system' in which a study is judged on three domains: representativeness of study group selection (four items); comparability of groups (two items); and ascertainment of either the exposure or outcome (three items). This scale awards a maximum of four stars for the adequate selection of cases and controls, two stars for comparability of cases and controls on the basis of the design and analysis, and three stars for the adequate ascertainment of the exposure in both the case and control groups. Overall, the NOS scores vary between 0 and 9 (the highest level of quality).

For assessing the quality of evidence for outcomes reported in the included studies, we chose using the GRADE methodology[11] and GRADEProfiler software (version 3.6, 2011). The quality of a body of evidence involves consideration of within-study risk of bias (methodological quality), directness of evidence, heterogeneity, precision of effect estimates and risk of publication bias. Study quality refers to study methods and execution such as the adequacy of allocation concealment, blinding and follow-up. Consistency refers to the similarity of estimates of effect across studies. Directness refers to the extent to which the people, the interventions and outcome measures are similar to those of interest. The GRADE system offers four categories of the quality of the evidence (high, moderate, low, and very low).

The data were analysed using the Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014. Depending on the original publication, the binary measure for individual studies was reported as the risk ratio (RR), or the odds ratio (OR), or as the hazard ratio (HR), all with 95% confidence interval (CI). Continuous outcomes were given as the mean with standard deviation (s.d.) or the median with ranges. For meta-analyses of observational studies, we aimed to pool adjusted odds ratios from the primary studies; however, as these were not always available, we used raw outcome data to yield unadjusted odds ratios. If no data for pooling were available, we report the results in a narrative format only. Statistical heterogeneity was quantified by I^2. A value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity. As observational studies were included, a substantial level of heterogeneity was expected; thus, random effects models were used for pooling, if appropriate.

RESULTS

For a flow diagram documenting the identification process for eligible trials, as well as the characteristics of the excluded trials, with reasons for exclusion, see online Supporting Information (Figure S1 and Table S1). Tables 1 to 4 summarise the characteristics of the

Table 1 | Characteristics of included randomised controlled trials

Study ID (country)	Population	Intervention	Comparison	Outcomes
Vriezinga 2014 PREVENTCD (seven European Union countries & Israel)[10]	*N* = 944 HLA-DQ2/DQ8 positive and at least one 1st degree relative with CD	100 mg of immunologically active gluten from week 16 to 24 (*n* = 475)	Placebo (lactose) from week 16 to 24 (*n* = 469)	Biopsy-confirmed CD at 3 years of age.
Lionetti 2014 CELIPREV (Italy)[29]	*N* = 553 HLA-DQ2/DQ8 positive and one 1st degree relative with CD	Gluten at 6 months (*n* = 297)	Gluten at 12 months (*n* = 256)	CDA and overt CD at 5 years of age.
Sellitto 2012 (USA)[22]	*N* = 30 (infants HLA-DQ2/DQ8 positive)	Early exposure group (gluten from 6 months) (*n* = 17)	Delayed exposure group (gluten from 12 months) (*n* = 13)	CD defined by the appearance of CD anti-TTG antibodies, the onset of CD-related symptoms and/or evidence of enteropathy.
Hummel 2011 (Germany)[31] & Beyerlein 2014[32]	*N* = 150 (infants <2 months with at least one 1st degree relative with type 1 diabetes and at risk HLA)	Gluten at 6 months (*n* = 77)	Gluten at 12 months (*n* = 73)	Growth, CD autoimmunity (anti-TTG), islet autoantibodies to insulin, GAD, insulinoma-associated protein 2 and type 1 diabetes up at 3 years of age and up to 13 years.

CD, coeliac disease; CDA, coeliac disease autoimmunity; HLA, human leucocyte antigen; anti-TTG, anti-tissue transglutaminse .

included studies. In addition to the previously identified 12 studies,[13–24] 9 new publications were identified,[10, 25–32] including three RCTs described in four publications.[11, 30, 32, 33] Two publications[24, 26] described the same population at different time points. One of these publications,[26] although published in 2007, had not been identified previously, and thus, was not included in the previous systematic review. Moreover, in addition to the two previously identified systematic reviews,[33, 34] one new systematic review became available and was evaluated.[35] Included studies are described with respect to their risk for bias in Tables S2 and S3. While the risk of bias in one RCT was low,[10] three other included trials had methodological limitations such as for example unclear allocation concealment, no or unclear blinding.[22, 29, 31] The Newcastle-Ottawa scores ranged from six points (six studies) through seven points (eight studies) to the maximum of eight points (two studies). Thus, the overall quality of the observational studies was moderate (mean score 6.75 of a total possible of nine).

The GRADE assessment for outcomes related to breastfeeding and the timing of introduction of gluten and the risk of developing CD is presented in Tables S4 and S5. Using the GRADE, the overall quality of evidence for all assessed outcomes was rated as high or moderate in case of RCTs, and very low in case of observational studies.

The new, RCTs investigated whether the age when an infant is first exposed to dietary gluten affects his or her risk of developing CD. The PREVENTCD[10] family study was a double-blind, placebo-controlled RCT, carried out in eight countries (Croatia, Germany, Hungary, Israel, Italy, the Netherlands, Poland and Spain), involving 944 children with HLA-DQ2 or HLA-DQ8 positivity who had at least one-first-degreerelative with CD. Children were randomly assigned to receive daily placebo (*n* = 469) or 100 mg of immunologically active gluten (*n* = 475) from 16 to 24 weeks of age. The primary outcome was the frequency of biopsy-confirmed CD at 3 years of age. The Risk of Celiac Disease and Age at Gluten Introduction (CELIPREV) trial was a multicenter (20 centres in Italy), randomised, interventional trial that compared early (at 6 months of age; *n* = 297) and delayed (at 12 months of age; *n* = 256) introduction of gluten to the diet of infants at risk for CD (first-degree relative with CD; tested later

Table 2 | Characteristics of included case-control studies

Study ID (country) (*N*)	Cases	Controls	Age	Exposure measurement	Confounding factors considered
Decker 2010 (Germany)[15] (*n* = 1534)	*N* = 157/123 CD patients at out-patient GI clinics (plus 931 with IBD and other GI diseases)	*N* = 862 Patients at out-patient orthodontic, dental and ophthalmologic clinics	Mean, years (s.d.): Cases: 9.1 (4.5) Controls: 10.0 (4.5)	Questionnaire	Potential risk factors that influence breastfeeding: mode of delivery, post-natal complications
Roberts 2009 (UK)[21] (*n* = 248 521)	*N* = 90 (children with CD) Record linkage study investigating perinatal risk factors for CD	*N* = 248 521	Mean, cases: 4.3 years	Maternity records	Number of maternal and perinatal risk factors such as maternal age, marital status, smoking, etc.
Ivarsson 2002 (Sweden)[18] (*n* = 1272)	*N* = 491 (392 < 2 years; 99 > 2 years) CD patients consecutively reported to a CD national register	*N* = 781 (626 < 2 years; 155 > 2 years) children from national population register	Range 0–14.9 years	Questionnaire	Age, sex, area of residence, other infant feeding practices
Peters 2001 (Germany) (*n* = 280)[20]	*N* = 143 CD patients from an incidence national study	*N* = 137 Healthy children from a population registry	Mean: 6.4 years Median: 6.2 years	Questionnaire	Age, sex, number of inhabitants in area, family pre-disposition to CD, age at gluten introduction
Ascher 1997 (Sweden)[13] (*n* = 81)	*N* = 8 Siblings of known CD patients found to have CD and HLA genotype DQA1*0501-DQB1*02 and CD on screening	*N* = 73 Siblings of known CD patients with the HLA genotype who did not have CD on small intestinal biopsy	Median Cases: 7.9 years Controls: 7.4 years	Questionnaire	HLA genotype, socioeconomic factors
Falth-Magnusson 1996 (Sweden)[16] (*n* = 336)	*N* = 72 Children with CD	*N* = 264 Healthy children from the same region	Median (range): 3.1 years (1.4–5.1)	Questionnaire	Age, area of residence
Greco 1988 (Italy)[17] (*n* = 2150)	*N* = 201 Children with CD	*N* = 1949 Healthy children from the same region	Mean, years (s.d.): Cases: 2.14 (2.6) Controls: 2.34 (2.93)	Interview	Age and area of residence, age at gluten introduction, father's occupation
Auricchio 1983 (Italy)[14] (*n* = 505)	*N* = 216 CD patients who had healthy siblings	*N* = 289 Siblings of cases without symptoms of CD	Age at diagnosis, median (range): 15 months (6 months–14 years).	Interview	Unclear

CD, coeliac disease; CDA, coeliac disease autoimmunity; GI, gastrointestinal; HLA, human leucocyte antigen; IBD, inflammatory bowel disease; s.d., standard deviation.

for HLA-DQ2 or HLA-DQ8 positivity). The primary outcome was the prevalence of CD autoimmunity and overt CD at 5 years of age.[29] The BABYDIET RCT was a single centre (Germany), open study that compared early (at 6 months of age; *n* = 77) and delayed (at 12 months of age; *n* = 73) introduction of gluten to the diet of infants

with a first-degree family history of type 1 diabetes and a risk HLA genotype who were followed until the age of 3 years,[31] and then up to 13 years.[32] The CD-related outcome was the development of CD autoimmunity.

Four other studies are ongoing, prospective, birth cohort studies. The first study was the Generation R Study.[28] This was a prospective cohort study conducted in 1679 Dutch children who were positive for HLA-DQ2/DQ8. The aim of the study was to determine whether the timing of gluten introduction and breastfeeding duration are associated with CD autoimmunity in children at 6 years of age. The second study was the Norwegian Mother and Child Cohort Study, a prospective birth cohort study including 107 000 children.[30] Complete information was available up to 6 months and up to 18 months in subset of children (77% and 63%, respectively). In this study, CD being the main outcome measure, was identified by questionnaires and by linkage to the Norwegian Patient Register. To prevent misclassification due to unconfirmed CD, at least two entries of a CD diagnosis in the register were needed. The third study, the BABYDIAB prospective cohort study,[26] a follow-up of a previously reported study,[24] examined the natural history of islet autoimmunity and CD autoimmunity in offspring of parents with type 1 diabetes. In total, 1511 subjects were followed up until the mean age of 7.6 (up to 14) years of age. The fourth study,[25] the Environmental Determinants of Diabetes in the Young (TEDDY) study, was a prospective birth cohort study. This study included 6436 newborns in Finland, Germany, Sweden and the USA who were screened for high-risk HLA-genotypes for CD and then followed up until the median age of 5 years. Information about infant feeding practices was collected at clinical visits every third month. The primary outcome was persistent positivity for tissue transglutaminase autoantibodies. The secondary outcome was CD, which was defined as either a diagnosis based on intestinal biopsy or on persistently high levels of tissue transglutaminase autoantibodies.

Finally, one study is the ongoing Exploring the Iceberg of Celiacs in Sweden (ETICS) project,[27] known also as the PREVENTCD population study. With a quasi-experimental design, it takes advantage of Sweden's changes in infant feeding over time, resulting in birth cohorts that differ with respect to infant feeding exposure. In this study, a 2-phase cross-sectional screening of over 13 000 children in two birth cohorts of 12-year-olds investigating the total prevalence of CD in children born during the coeliac epidemic (in 1993) and those born after the epidemic (in 1997) was performed. For children who presented with previously diagnosed CD, the diagnosis was reported and confirmed. All other children were screened for serological markers. If positive, these children were then referred for a small intestinal biopsy to confirm the diagnosis. Differences between the cohorts infant feeding practices were ascertained via questionnaires. A total of 67% of participants responded with complete information on breastfeeding duration and age of gluten introduction into the diet.

Breastfeeding and CD

For the characteristics of the included studies, see Table S6.

Interventional trials. The PREVENTCD study showed that exclusive, as well as any, breastfeeding did not significantly influence the development of CD (Figure 1). The CELIPREV study reported that breastfeeding duration was similar for children in whom CD developed and in those who did not develop the disease. However, the PREVENTCD study was designed to compare introduction of small amounts of gluten at age 4 months compared to 6 months and not to evaluate the role of breastfeeding. Likewise, the CELIPREV study was not designed to address the issue of breastfeeding and CD0.

Observational studies. Previously, the results of retrospective studies indicated that there was no evidence to suggest that exclusive breastfeeding compared with formula or mixed feeding either reduces the risk of CD or delays the onset of symptoms. However, some studies, albeit not all of them, showed an association between the duration of breastfeeding and decreased risk of CD.

New data from the Norwegian Mother and Child Cohort Study[30] showed that breastfeeding for longer than 12 months was associated with a modest increase in the risk of CD. However, no screening was performed, and only patients who visited their paediatricians because of complaints were evaluated for CD. The Generation R, prospective, population-based cohort study[28] found that breastfeeding ≥6 months did not decrease the risk of CD autoimmunity in children at the age of 6 years. The results from the BABYDIAB prospective cohort study revealed no association between the duration of breastfeeding and the risk of CD autoimmunity.[26] In the TEDDY study,[25] the mean breastfeeding duration was reported, but it was not compared between children who developed CD autoimmunity or CD and those who did not. The ETICS study[27] compared duration of breast feeding, age of gluten introduction, amounts of

Table 3 | Characteristics of included prospective cohort studies

Study ID (country) (*N*)	Cases	Controls	Age	Exposure measurement	Confounding factors considered
Aronsson 2014 TEDDY Study (Finland/ Germany/ Sweden, USA)[25] (*n* = 6436)	*N* = 307 who developed CD *N* = 773 who developed CDA from enrolled children with high risk HLA types	*N* = 5663 Seronegative children with high-risk HLA types	Median 5 years (1.7–8.8)	Booklet (questionnaire) reviewed at each visit (every 3 months)	Country, sex, HLA status, family history of CD, maternal education level and age at delivery, season of birth, smoking during pregnancy
Jansen 2014 Generation R Study (The Netherlands)[28] (*n* = 1679)	*N* = 43 who developed CDA	*N* = 1636 seronegative children with positive HLA	6 years	Questionnaire	Sex, gestational age, birth weight, caesarean section.
Størdal 2013 MoBa (the Norwegian Mother and Child Cohort Study) (Norway)[30] (*n* = 82 167)	*N* = 324 with clinically diagnosed CD	*N* = 81843 children without symptomatic CD	Mean. Cases 6.8 years; controls 5.9 years.	Questionnaire plus linkage to the Norwegian Patient Register.	Child's age and gender, breastfeeding and maternal CD.
Welander 2010 ABIS (Sweden)[23] (*n* = 9408)	*N* = 44 Children with CD from population-based project exploring factors for developing immune-mediated diseases (All infants in Southeast Sweden, ABIS project)	*N* = 9364 Children from ABIS cohort with no diagnosis of CD	Mean. Cases: 8.4 years Controls: 8.3 years	Questionnaire handed-out at birth	Age at gluten introduction, age at the end of breastfeeding and age at infection (or gastroenteritis)
Norris 2005 DAISY Study (USA)[19] (*n* = 1560)	*N* = 51 who developed CDA from DAISY prospective cohort study: at increased risk for CD or DM1 (HLA-DR3 or DR4 alleles, or 1st degree relative with DM1) followed in mean of 4.8 years	*N* = 1509 seronegative children from the DAISY cohort	Mean, years (s.d.): CDA (+): 4.7 (1.5) CDA (−): 4.8 (2.9)	Interview/ Questionnaire (for children recruited at birth or at age 2–3 years, respectively)	Race/ethnicity, HLA-DR3 status, family history of DM1
Ziegler 2003[24] – same cohort as Hummel 2007 BABYDIAB (Germany)[26] (*n* = 1610)	*N* = 27 who developed CDA	*N* = 1610 (children at risk of DM1 [parent (s) with DM1]	Median in years: 6.5 (9 months–12.5 years)	Questionnaire and interview by phone	Maternal DM1, gestational age <36 weeks, birth weight <2700 g, region of residence

Table 3 | (Continued)

Study ID (country) (*N*)	Cases	Controls	Age	Exposure measurement	Confounding factors considered
Hummel 2007[26] BABYDIAB – same cohort as Ziegler 2003 (Germany)[24] (*n* = 1511)	*N* = 63 who developed CDA children at risk of DM1 [parent(s) with DM1]	*N* = 1448 ? seronegative children at risk of DM1 [parent(s) with DM1]	Mean follow-up in years: 7.6 (to 14)	As above (Ziegler 2003)	As above (Ziegler 2003)

CD, coeliac disease; CDA, coeliac disease autoimmunity; DM1, type 1 diabetes mellitus; GI, gastrointestinal; HLA, human leucocyte antigen; IBD, in_ammatory bowel disease; s.d., standard deviation.

Table 4 | Characteristics of included cross-sectional study

Study ID (country) (*N*)	Cases Controls	Age	Exposure measurement	Confounding factors considered
Ivarsson 2013 (Sweden)[27] (*n* = 13 288)	Cohort born in 1993 *N* = 7567 Cohort born in 1997 *N* = 5721	12 years old	Questionnaire	Unclear

gluten introduced and breast feeding during introduction. In that study, the duration of breastfeeding was 7 and 9 months in the 1993 and the 1997 cohorts, respectively, comparable to the general Swedish population. The median age at gluten introduction was 5 months in both cohorts. However, the cohorts differed in the amount of gluten containing flours ingested during weaning (38 g/child/day vs. 24 g/child/day, respectively). Moreover, women in the 1997 cohort, compared with the 1993 cohort, breastfed for significantly longer after they had introduced gluten (9 vs. 7 months, respectively, $P < 0.001$), and a significantly larger proportion of women continued breastfeeding beyond gluten introduction (78% vs. 70%, respectively; $P < 0.001$). The findings of the ETICS study suggest that introduction of gluten in smaller amounts during breastfeeding affects the risk of developing CD, at least up until 12 years of age.

The pooled results for five observational studies showed that any breastfeeding compared with no breastfeeding had no effect on the risk of developing CD (OR: 0.69, 95% CI: 0.30–1.59). Considerable heterogeneity across the studies was found (I^2 = 93%, Figure 2).

Breastfeeding at the time of gluten introduction and CD

For the characteristics of the included studies, see Table S7.

Interventional trials. The PREVENTCD study showed that breastfeeding during gluten introduction did not significantly influence the development of CD (Figure 1). Similarly, the CELIPREV study did not show a protective effect of introducing gluten during breastfeeding. In at-risk children who developed CD compared with at-risk children who did not develop CD, the mean duration of breastfeeding was similar (5.6 vs. 5.8 months, respectively).

Observational studies. Previously, the results from a meta-analysis of four observational case–control studies suggested that breastfeeding at gluten introduction is associated with a lower risk of CD compared with formula feeding.[33] One prospective study, conducted in children at high risk for CD, found no statistically significant difference between the case and control groups.[19]

New data from the Norwegian study do not support a protective effect of breastfeeding at the time of gluten introduction on the risk of developing CD.[30] The results of the TEDDY Study also showed no difference in the development of CD autoimmunity or CD, irrespective of a long-term vs. short-term (>1 vs. ≤1 month) continuation of breastfeeding after gluten introduction or discontinuation of breastfeeding before gluten introduction.[25] No difference in developing anti-tissue transglutaminase antibody positivity, irrespective of whether the

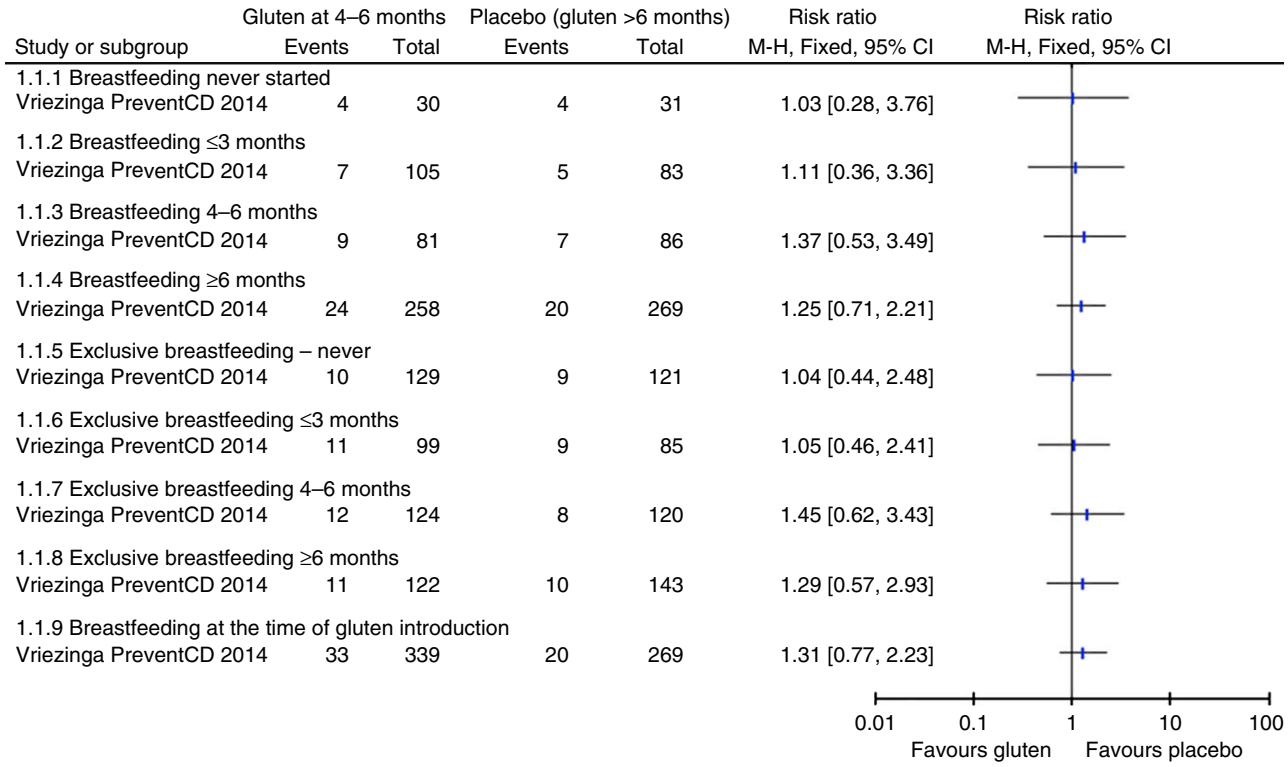

Figure 1 | Effect of breastfeeding on the risk of coeliac disease (randomised controlled trials).

children were still being breast-fed at gluten introduction or not, was reported; however, data were not shown.

The pooled results for seven observational studies showed that breastfeeding at gluten introduction has no effect on the risk of developing CD compared with formula feeding (OR: 0.88, 95% CI: 0.52–1.51). Considerable heterogeneity across the studies was found (I^2 = 89%, Figure 2).

Timing of gluten introduction

For the characteristics of the included studies, see Table S8.

Interventional trials. In the PREVENTCD study, as compared with placebo, the introduction of 100 mg of immunologically active gluten at 16–24 weeks of age resulted in a similar risk of CD at 3 years of age.[10] Three RCTs compared introduction of gluten at 6 months and 12 months of age. In the largest, CELIPREV randomised trial, the introduction of gluten at 6 months of age, compared with the introduction at 12 months of age, increased the risk of CD autoimmunity and overt CD at 2 years but had no effect on the risk of CD autoimmunity and overt CD at 5 years of age (the primary outcome).[29] Two other interventional studies, conducted in small groups of subjects, reported no difference in the risk of CD and/or CDA at various ages in children exposed to gluten at the age of 6 months compared to first exposure at 12 months at various time intervals (Figures 3 and 4).[22]

Observational studies. Out of six previously identified studies,[16, 18–20, 23, 24] only one prospective, observational, cohort study by Norris *et al.*[19] showed that both early (less than 3 months) and late (more than 7 months of age) introduction of gluten to children at increased risk of CD and type 1 diabetes mellitus was associated with an increased risk of CD autoimmunity. The remaining studies did not show a relationship between the timing of gluten introduction and the risk of developing CD.

New data from the Generation R Study found that the introduction of gluten from the age of 6 months onward, compared to earlier exposure, was not significantly associated with positive anti-tissue transglutaminase concentrations (CD autoimmunity) (adjusted OR: 0.64, 95% CI: 0.31–1.31).[28] The Norwegian, prospective, birth cohort study found that gluten introduction at >6 months of age, compared to <6 months, was associated with an increased risk of CD; however, this was of borderline significance (adjusted OR: 1.27, 95% CI: 1.01–1.65).[30] In

the BABYDIAB cohort, whether gluten was introduced at <3 months or >3 months of life did not influence the risk of CD autoimmunity.[26] The design of the TEDDY Study referred to the timeframes for gluten introduction recommended by ESPGHAN, comparing first exposure to gluten occurring <17 weeks, between 17 and 26 weeks, or >17 weeks. No difference was found in the risk of developing CD autoimmunity or CD between the three groups differing in age of exposure.[25] The ETICS cross-sectional study comparing two birth cohorts of 12-year-olds found significant difference in the total prevalence of CD in children born during the CD epidemic (in 1993; gluten introduction from 6 months of age) and those born after the epidemic (in 1997; gluten introduction in small amounts, from age 4 to 6 months).

Figures 5 and 6 presents results of observational studies comparing various timing of gluten introduction on CD or CDA. With the exception of the ETICS study, no significant differences were found. Of note, considerable heterogeneity across the studies was found (I^2 from 26% to 82%).

Amount of gluten at weaning (and later) and CD

For the characteristics of the included studies, see Table S9.

Interventional trials. The PREVENTCD study reported on the mean daily gluten intake after the dose escalation in a subset of participants (596 children from the Dutch, German, Italian and Spanish cohorts) and reported that the amount of gluten at weaning was not related to the development of CD: hazard ratio per increase in gram/day 0.98 ($P = 0.74$), 1.1 ($P = 0.44$), 1.1 ($P = 0.32$) and 1.2 ($P = 0.09$) at 12, 18, 24 and 36 months of age, respectively.[10] However, this was not a planned study outcome, and amounts were not compared between the two groups.

Observational studies. Only one study analysed the amount of gluten that children received. In children younger than 2 years of age, the risk of developing CD was greater when gluten was introduced into the diet in large amounts than when introduced in small or medium amounts (adjusted OR: 1.5, 95% CI: 1.1–2.1). However, the gluten ingestion was only assessed as 'large' or 'small' quantity without quantification in grams per day. In older children, there was no effect.[18]

Type of gluten

Interventional trials. No RCTs were identified that examined the effect of the type of gluten at introduction on the risk of CD.

Observational studies. One study[18] analysed whether the risk of developing CD was affected by the type of gluten-containing foods introduced. The types of foods used during introduction of flour into the diet were categorised into two groups. These were either solid foods, including bread, biscuits, porridge and pasta, or gluten-containing follow-up formula, used exclusively or in combination with solid foods. Both bivariate analysis and multivariate analyses showed that the type of gluten-containing food given was not an independent risk factor for developing CD.

Gluten during lactation

In the PreventCD study,[10] of the 455 mothers with CD, 431 were consuming a gluten-free diet during pregnancy and lactation. This study reported that maternal diet during pregnancy and lactation had no effect on the risk of their offspring developing CD (gluten-free vs. normal, cumulative incidence 5.0% vs. 5.4%, $P = 0.71$).

Genetic predisposition

We found no studies designed to detect the effect of feeding practices on the risk of children carrying different HLA types and in homozygotes versus heterozygotes (HLA dosage). In the PREVENTCD study,[10] the different HLA types did not influence the effect of gluten or placebo on the development of CD. However, this trial was only powered to detect differences in the whole population at risk, and was not designed to detect differences between different HLA subtypes or HLA dosage.

DISCUSSION

Main findings

To date, this is the largest systematic review on the effect of early feeding practices on the risk of developing CD during childhood, and it includes data from two, recent, large, RCTs. Higher quality evidence suggests that the introduction of gluten at a specific timeframe (at 4 months of age vs. 6 months of age and at 6 months of age vs. 12 months of age) has no effect on the risk of developing CD at the age of 3 and 5 years, respectively. Moreover, there is no evidence that the duration of breastfeeding or continuation of breastfeeding at the time of gluten introduction influences the CD risk, at least during the study periods. Data on the amount of gluten at weaning and CD development are not conclusive. While one observational Swedish study found a modestly increased risk for CD in infants consuming large amounts of gluten, compared with small or medium amounts, this

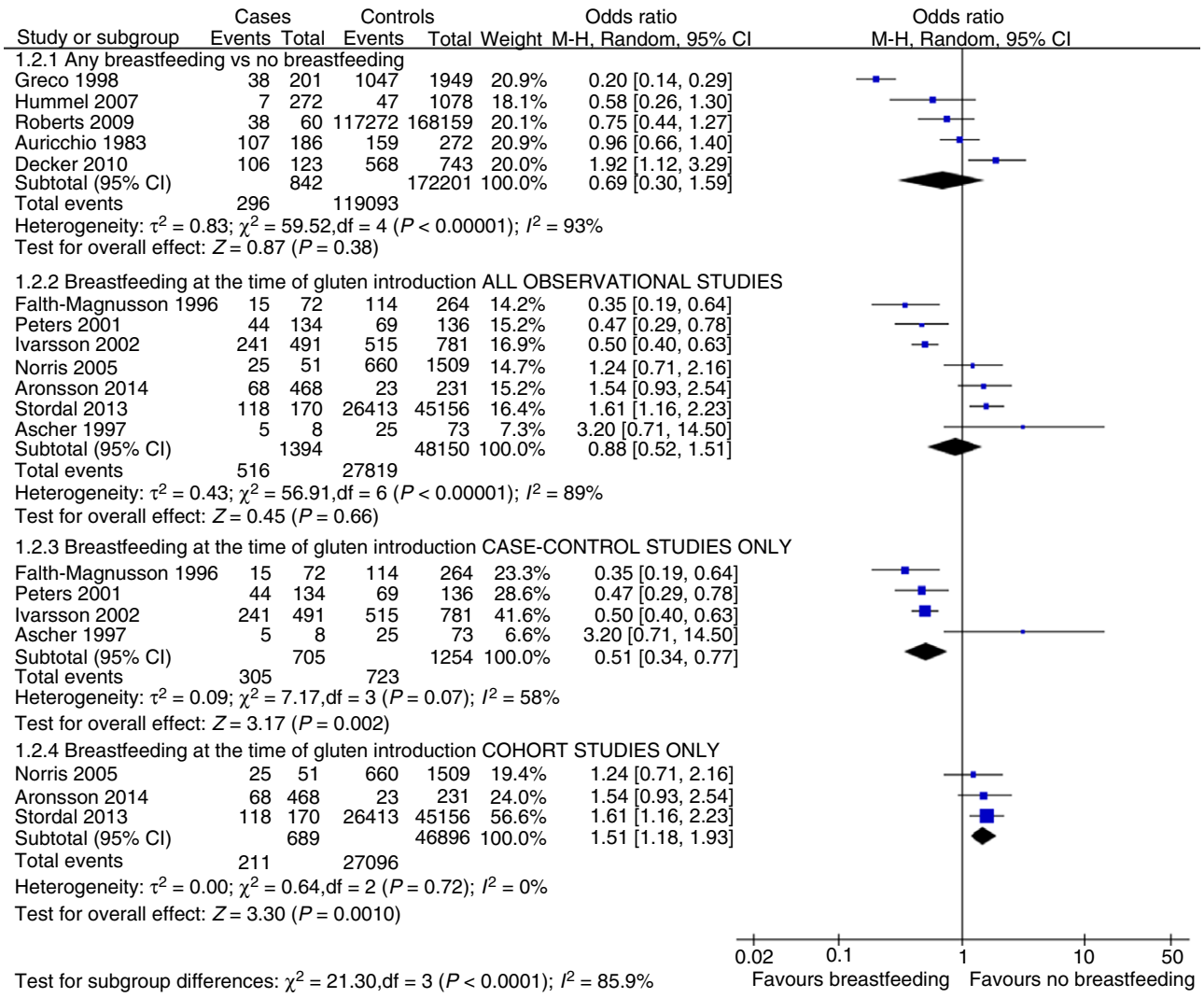

Figure 2 | Effect of breastfeeding on the risk of coeliac disease (observational studies)

finding was not later confirmed in a subset of the PREVENTCD population study. However, in the latter study, the assessment of the amount of gluten was not a planned study outcome, and amounts were not compared between the two groups; and in the other one the amount of gluten was no quantified in grams per day; hence, caution is needed when interpreting these findings. Hypothetically, the type of gluten-containing food introduced may influence the risk of CD. Available data are scarce, but suggest that the type of gluten (solid foods or cereal-containing formula) does not appear to be a risk factor for CD. Scarce data suggest that maternal diet during pregnancy and lactation had no effect on the risk of their offspring to develop CD. No studies were found that were designed to evaluate the effect of feeding practices on the risk of children carrying different HLA types and in homozygotes vs. heterozygotes. Although the findings of the PREVENTCD suggest that diferent HLA types did not influence the effect of gluten or placebo in the development of CD, cautionis needed. This trial was not powered to detect differences within subpopulations, let alone to detect differential effects of intervention between HLA groups. Nevertheless, the high prevalence of CD in young children homozygous for HLA DQ 2.5[36] suggests that feeding practices would have a minor role, if at all, in children with high genetic predisposition.

Comparison with earlier studies

Current findings did not confirm previous evidence from observational studies suggesting that the age at gluten introduction and the effect of breastfeeding influence the occurrence of CD during early childhood. Specifically,

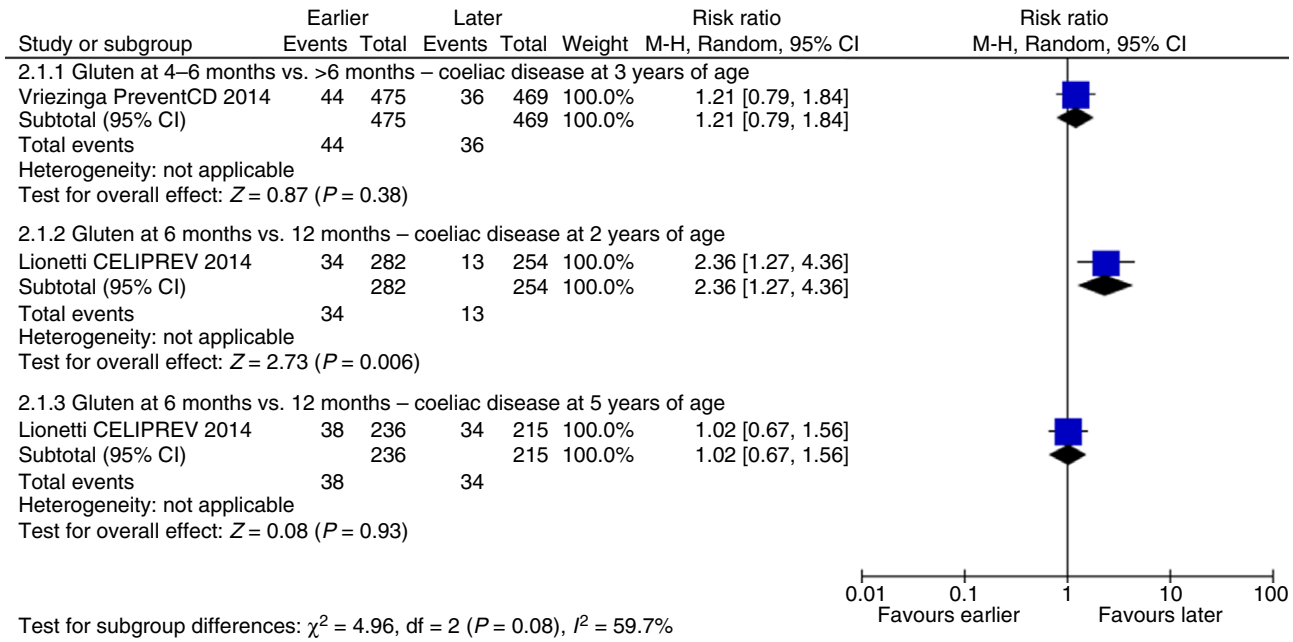

Figure 3 | Timing of gluten introduction and the risk of coeliac disease (randomised controlled trials).

earlier evidence has suggested that it is reasonable to avoid both early (<4 months) or late (≥7 months) introduction of gluten and to introduce gluten while the infant is still being breastfed. While we found no evidence that breastfeeding reduces the risk of CD, it is important to emphasise that scientific organisations currently recommend that exclusive breastfeeding for the first 6 months is a desirable goal[37] and our findings are in line with these recommendations. Moreover, although our data suggest that there is no reason to expose infants to gluten at a different age compared to other food items, introduction of other foods while breastfeeding could provide a beneficial effect. For example, the exact timing of the introduction of potentially allergenic foods is still under discussion. Worldwide, research projects are underway to resolve these controversies [e.g. EAT (Enquiring About Tolerance; www.eatstudy.co.uk) study]. Thus, while our results may serve as a basis for revising the recommendations for gluten introduction, these guidance for gluten, should not serve as a basis to change recommendations on breastfeeding and introduction of other food items.

Strengths and limitations

The major strengths of our systematic review are the inclusion of two most recent RCTs, which specifically addressed the age of gluten introduction as the primary outcome, and that it collates the largest number of studies available in the literature. Another strength is the use of the GRADE profile to rate the overall quality of evidence, which can be useful for future guideline development.

For completeness, we included both interventional and observational studies. One major disadvantage of the latter design is that the observed associations usually do not allow one to establish causality, and potential biases and confounding can only be partially considered. However, these observational studies are more likely to include much bigger and broader study populations. For pooling data from the observational studies, we used the raw data, with no correction for baseline differences or confounding factors. The latter pose particular challenge for observational studies. However, in those trials, which reported adjusted RR/OR/HR, no major difference between unadjusted and adjusted ratios were found.

Not surprisingly, the pooled observational findings were heterogeneous. This may reflect differences in study quality and/or the population studied. Data from various countries were included. Despite the many positive aspects, this calls for caution in interpretation. For example, compared to many other countries, in Sweden there is a higher prevalence of CD in children.[4, 36] Often, discrepancies in findings between the Swedish and non-Swedish populations are being observed. For example, in our systematic review, the ETICS study suggested that gradual introduction of gluten in small amounts during

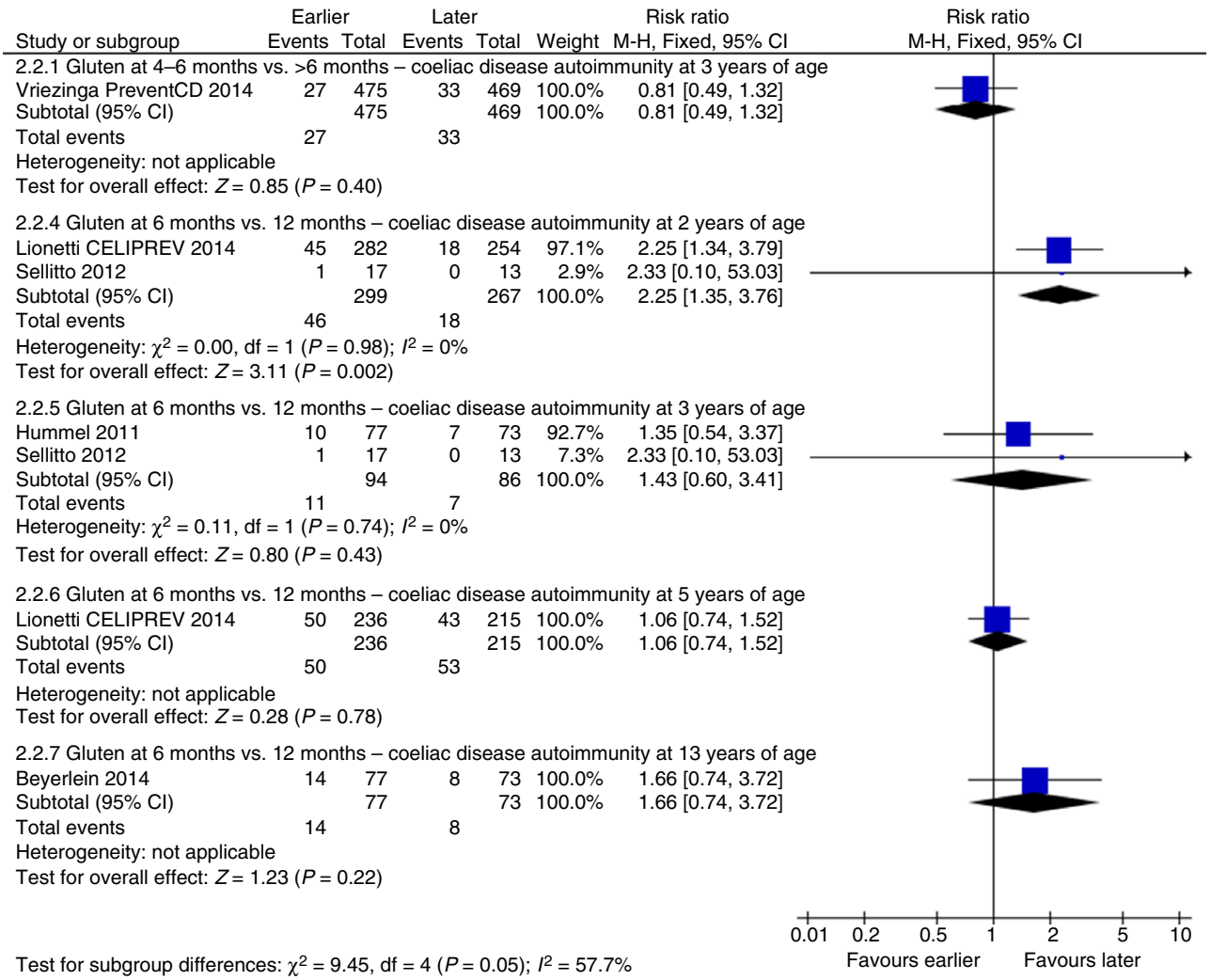

Figure 4 | Timing of gluten introduction and the risk of coeliac disease autoimmunity (randomised controlled trials).

ongoing breastfeeding is favourable, but this was not confirmed in the interventional trials. Reasons for these discrepancies may include methodological issues (e.g. the response rate of 67% might have exposed the study to response bias) as well as country-specific issues such as genetic susceptibility or environmental factors.

The shortcoming of our systematic review is that the two, new, RCTs were conducted in Europe and Israel only. Moreover, there was a mixture of genetic risks (different HLA patterns and no assessment of non-HLA genes). Environmental factors other than gluten consumption, age at introduction and breastfeeding were not controlled for and were not stratified for the genetic risk. Nevertheless, with regard to other potentially important environmental factors that may play a role in CD development, the PREVENTCD study showed that country of origin and the number, type, or members of affected family (sibling, father or mother) were not related to the development of CD, nor were rotavirus vaccination, gastrointestinal or respiratory tract infection, and mean daily gluten intake.[10] However, the PREVENTCD was not designed and powered sufficiently to demonstrate a difference if one actually exists. Thus, future research is needed.

The exposure in the PREVENTCD cohort was to a small amount of immunogenic active gluten (100 mg) that represents approximately only 2% of the amount normally introduced at weaning.[38] Nevertheless, this quantity was able to induce an immunological response, as shown in the PREVENTCD cohort with the early antibody response;[10] this suggests that the PREVENTCD exposure, although lower than the usual exposure in other studies and in practice, is immunogenic. Regardless, based on the available evidence, especially from

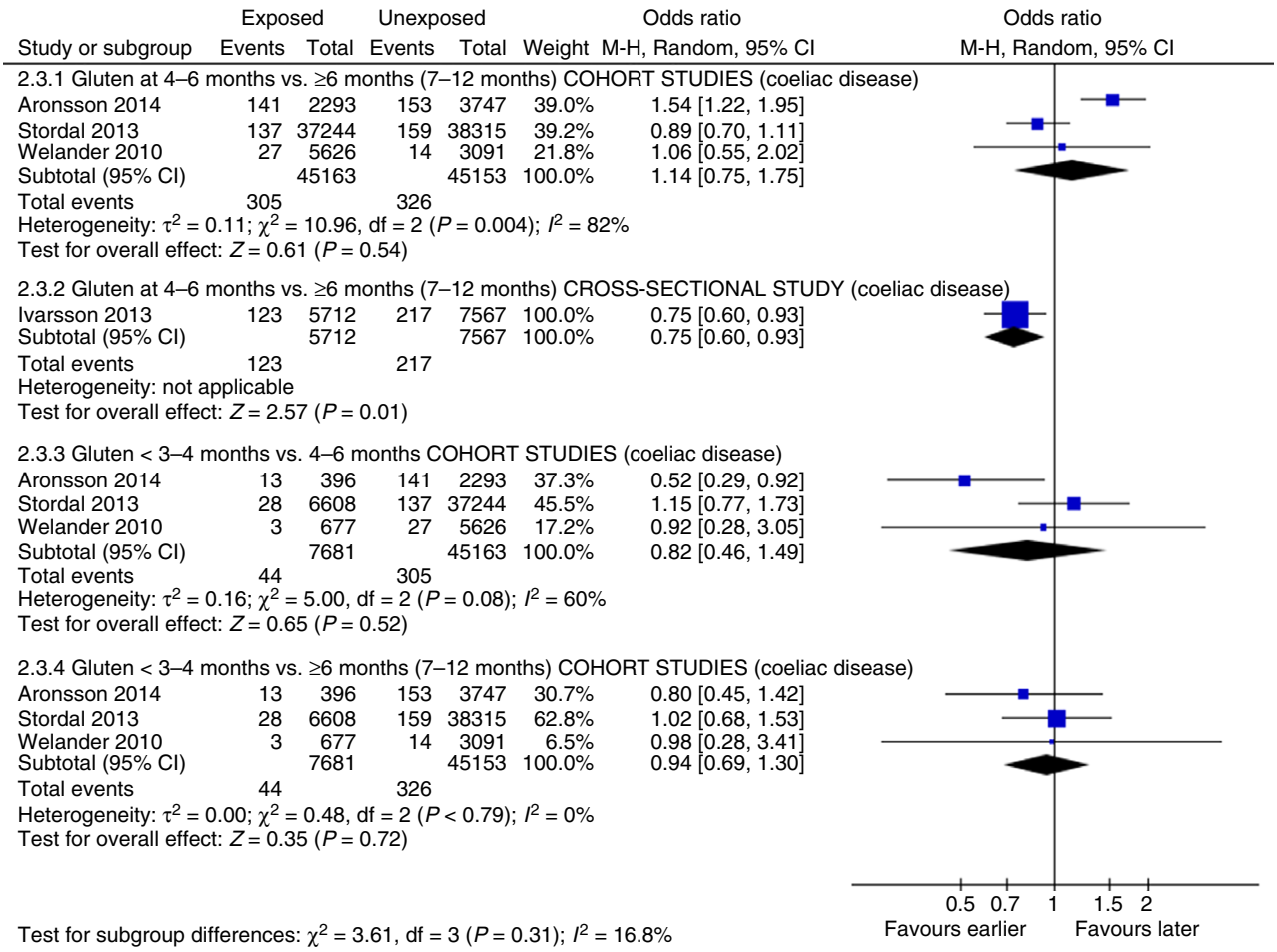

Figure 5 | Timing of gluten introduction and the risk of coeliac disease (observational studies).

Sweden,[4, 18, 27, 39, 40] introduction of large amounts of gluten should be discouraged.

Available evidence does not allow one to form conclusions on the consequences of very early (i.e. earlier than currently recommended for complementary foods) introduction of gluten. Earlier data has shown clearly that exposure to gluten before 4 months of age increases the risk for CD autoimmunity and is not recommended.[6, 24] In the current systematic review, one cohort study (BABYDIAB) reported no difference in the risk of CD autoimmunity in the case of gluten introduction before 3 months of age. However, the number of subjects exposed to gluten at that early age was very small. The lack of scientific data on the safety of too early (i.e. before 3 months of age) introduction of gluten is sufficient to discourage such practice, as undesirable health consequences cannot be excluded.

Ultimately, early introduction of gluten in genetically at-risk children may lead to earlier development of CD autoimmunity and CD, without changing the absolute risk for CD at the specific time points studied. The early occurrence of CD may have an adverse effect on children in the absence of a screening programme, because growth velocity and nutritional status may be adversely affected to a larger extent during the first 2 years of life compared to the effect of CD on growth at a later age. Furthermore, given the opportunity to influence the age of CD occurrence, parents may prefer to see their children diagnosed later rather than earlier. This, however, should be weighed against the possibility that without a screening programme, a late diagnosis of CD may expose the subject to long duration of autoimmunity and its possible complications, and may even be missed due to mild symptoms.

CONCLUSIONS

This updated systematic review did not confirm previous evidence from observational studies suggesting that the

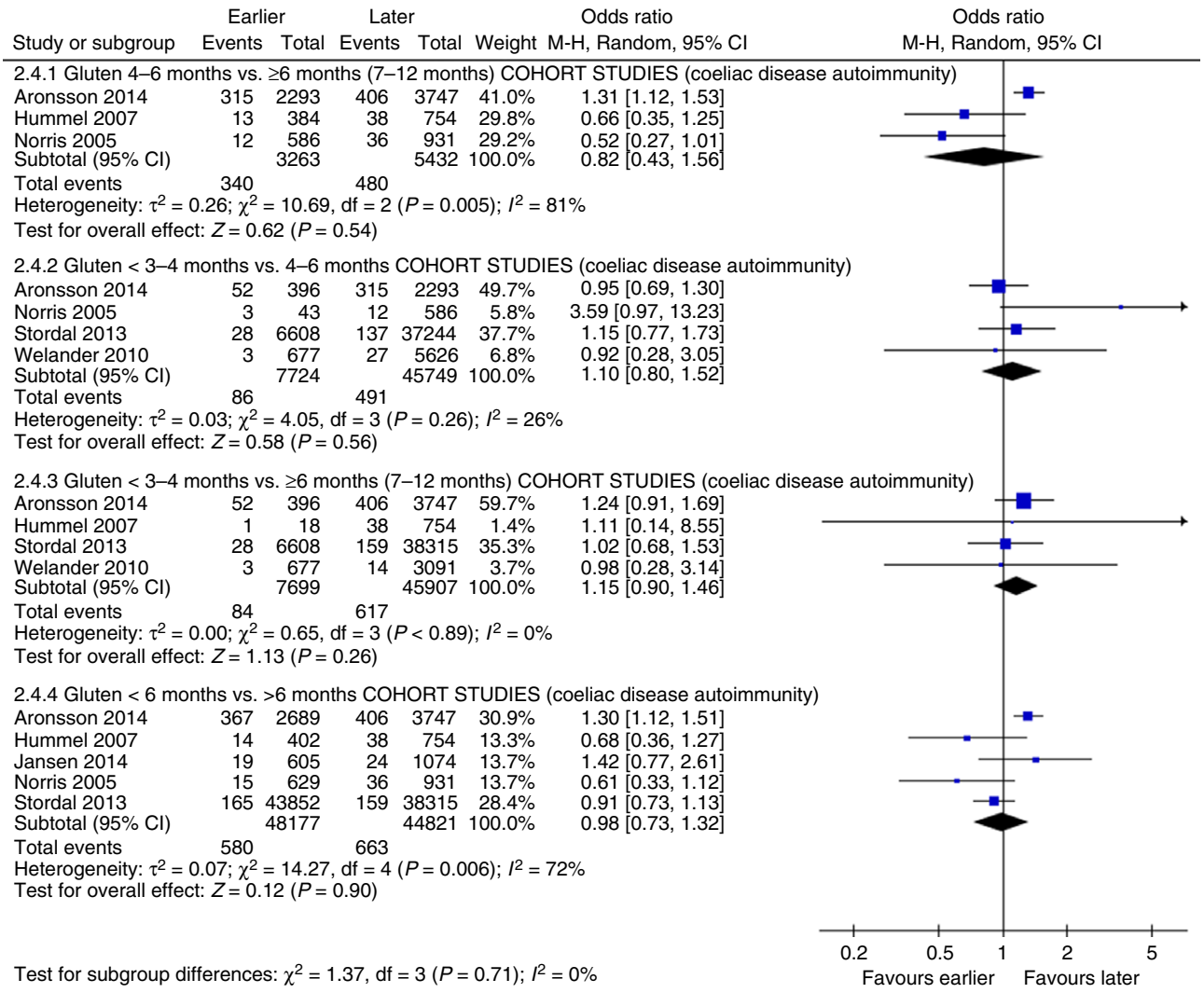

Figure 6 | Timing of gluten introduction and the risk of coeliac disease autoimmunity (observational studies).

age of gluten introduction (at least at specific timeframes evaluated in the included studies) and/or breastfeeding influence the occurrence of CD. On the contrary, current evidence suggests that infant feeding practices (breastfeeding, time of gluten introduction) have no effect on the risk of developing CD during childhood. An update of current European recommendations regarding gluten exposure in young children is needed.

AUTHORSHIP

Guarantor of the article: H. Szajewska.

Author contributions: HS and RS initially conceptualised this study. AC, MPL and HS were responsible for data collection, data analysis, data interpretation and preparation of the report. HS, RS, AC, MPL assumed the main responsibility for the writing of this manuscript. All authors contributed to (and agreed upon) the final version.

All authors approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow chart of study identification process.

Table S1. Characteristics of excluded studies and reasons for exclusion.

Table S2. Assessment of risk of bias in interventional trials.

Table S3. Assessment of risk of bias in observational studies assessed using Newcastle-Ottawa Quality Assessment Scale.

Table S4. GRADE evidence profile summarising the effect of breastfeeding and the risk of coeliac disease (CD) and/or coeliac disease autoimmunity (CDA).

Table S5. GRADE evidence profile summarising the effect of timing of gluten introduction and the risk of coeliac disease (CD) and/or coeliac disease autoimmunity (CDA).

Table S6. Duration of breastfeeding and coeliac disease.

Table S7. Breastfeeding at the time of gluten introduction and coeliac disease.

Table S8. Time of gluten introduction and coeliac disease.

Table S9. Amount of gluten at weaning and coeliac disease.

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