Gluten Introduction to Infant Feeding and Risk of Celiac Disease: Systematic Review and Meta-Analysis

María Inés Pinto-Sánchez, MD[1], Elena F. Verdu, MD[1], Edwin Liu, MD[2], Premysl Bercik, MD[1], Peter H. Green, MD[3], Joseph A. Murray, MD[4], Stefano Guandalini, MD[5], and Paul Moayyedi, MD[1]

Objective To assess the evidence regarding the effect of time of gluten introduction and breastfeeding on the risk of developing celiac disease (CD).

Study design We included randomized controlled trials and observational studies evaluating the proper timing for introducing gluten to the infant diet, the appropriate quantity of gluten consumption at weaning, and the effect of breastfeeding on CD risk. Studies were located through the electronic databases Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (Ovid), EMBASE (Ovid), and System for Information on Grey Literature in Europe (SIGLE). Two independent authors collected the data.

Results A total of 1982 studies were identified, 15 of which were eligible for data extraction. A meta-analysis was performed on 2 randomized controlled trials, 10 cohort studies, and 1 case-control study. There was a 25% increase in CD risk with late (>6 months) vs recommended (4-6 months) gluten introduction (risk ratio [RR], 1.25; 95% CI, 1.08-1.45). There was no significant effect of breastfeeding vs no breastfeeding on CD risk (OR, 0.55; 95% CI, 0.28-1.10), with substantial heterogeneity (I^2 = 92%) among studies.

Conclusion There is currently no evidence to support that early introduction of gluten to the infant diet increases the risk of CD; however, late introduction of gluten may be associated with increased risk of CD. More studies are needed that control for potential confounders and that evaluate environmental factors in low-risk families. *(J Pediatr 2016;168:132-43)*.

Celiac disease (CD) is an autoimmune disorder triggered by gluten in genetically susceptible individuals. In CD, gluten induces a chronic inflammatory response that progressively leads to small intestinal atrophy.[1] Not everyone with genetic predisposition will develop CD; thus, additional environmental risk factors, such as the way in which gluten is introduced to infant's diet, have been proposed.[2] This has impacted European feeding recommendations, although evidence-based recommendations are scarce.[1-5]

The Nutrition Committee of the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition has recommended avoiding the introduction of gluten before age 4 months and after age 7 months.[6] Thus, the ideal time for introducing gluten to the diet would fall between the fourth and sixth months of life, when gluten should be introduced in "small quantities" and progressively, while maintaining breastfeeding whenever possible.[7] The evidence for this came from 1 systematic review of the effect of gluten introduction on the risk of CD[8]; however, owing to heterogeneity among studies, a summary estimate of risk was not provided.

This is a rapidly changing field, with new epidemiologic data emerging regularly. Thus, we conducted an updated systematic review of randomized controlled trials (RCTs) and observational studies evaluating the current evidence regarding the possible relationship between the timing and quantity of gluten introduction, breastfeeding, and the risk of developing CD. We hypothesized that the data could be synthesized as a meta-analysis to provide a risk estimate for the development of CD.

Methods

We included studies evaluating the introduction of gluten in infants in whom the development of CD was assessed. CD diagnosis used any well-defined criteria available (duodenal biopsy- and/or serology-compatible and HLA DQ2/8-positive, when performed) or at risk for CD (positive HLA DQ2/8, first-degree relative with CD or type 1 diabetes). Controls included infants in which CD diagnosis was not established or CD was excluded by duodenal biopsy or specific serology (tissue transglutaminase antibody, anti-endomysium antibody, or deaminated peptide gliadin).

CD	Celiac disease
RCT	Randomized controlled trial
RR	Risk ratio

From the [1]Department of Medicine, Farncombe Family Digestive Research Institute, McMaster University, Hamilton, Ontario, Canada; [2]Colorado Center for Celiac Disease, Children's Hospital Colorado, Aurora, CO; [3]Celiac Disease Center at Columbia University, New York, NY; [4]Department of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN; and [5]Department of Pediatrics, Section of Gastroenterology, Hepatology and Nutrition, University of Chicago, Chicago, IL

Funded by Nestle, which had no role in the design and conduct of the study, collection, management, analysis, and interpretation of the data. M.P.-S. received a CIHR-FHP Health Professional Award Fellowship. The authors declare no conflicts of interest.

http://dx.doi.org/10.1016/j.jpeds.2015.09.032

The following intervention and control groups were eligible according to the research question they were answering. For timing of gluten introduction, the intervention group included any gluten-containing product (eg, cereals, flour or any other foods containing gluten, preparations manufactured for research purposes) introduced early (<4 months) or late (>7 months) and the control group included gluten introduced between 4-6 months of age. For gluten dose and mode of introduction, the intervention group was considered as a large amount of gluten introduced in the diet and control group a standard amount as defined by the authors. The mode of introduction of gluten was considered "gradual" in the intervention group and "usual" in the control group, as defined by the authors. We considered the intervention group to be breastfed for any duration and the control group to not have had any breastfeeding. An alternative definition was an intervention group that was breastfed vs a control group that was not breastfed during weaning. The primary outcome was to assess systematically the development of CD autoimmunity (tissue transglutaminase antibody or anti-endomysium antibody) and/or biopsy.

We included observational studies (cohort or case-control studies) and randomized, double-blind, placebo-controlled trials (RCTs) up to January 2014. We considered cross-over studies only if the results were available before the cross-over, so the study could be evaluated as a parallel group. Publications were considered regardless of language and publication status. Abstracts were included only if we were able to obtain further details from the investigators. Only studies performed in a pediatric population with CD defined according to compatible biopsy and/or serology and an eligible non-CD control group were considered. If information was missing from a study, the authors were contacted to provide details. Studies were excluded if they were case reports or case series, if CD was not confirmed by serology or biopsy, if there was no non-CD control group, or if reported in duplicate publications. The search strategy is outlined in **Appendices 1-4** (available at www.jpeds.com).

Two authors screened the titles and abstracts to ensure that we captured all eligible studies. A list of studies to include for assessment of eligibility was created, and duplicate studies were removed at this initial stage. To ensure that inclusion and exclusion criteria were rigorously interpreted, full-text screening was performed by 2 blinded reviewers. For publications in a language other than English, a translator with expertise in the field was provided with specific instructions for the screening process for 8 studies. Data related to the full-text screening were collected in Excel (Microsoft, Redmond, Washington), and results were compared. Agreement was calculated after full-text screening by using kappa statistics (GraphPad Software, La Jolla, California) for categorical data and raw agreement for continuous data. Raw agreement was reported in percentage, and kappa as fair agreement (κ = 0.4-0.59), good agreement (κ = 0.6-0.74), or excellent agreement ($\kappa \geq 0.75$). In cases of disagreement, the study was discussed, and if inclusion remained unresolved, a third party with experience in the topic and systematic reviews adjudicated. All of these steps were properly documented, and a table of excluded studies was created. The previous 2 reviewers extracted the data independently. A data extraction form was developed to collect detailed information regarding study design, population, intervention, controls, and outcomes, in addition to the information provided by the screening form. Patient demographic data, treatment, outcomes, and adverse events were recorded as mean ± SD, n/N, or % as applicable. Information to identify possible risk of bias was also collected on this form. The first author (M.P.) entered the information into RevMan 5.3[9] (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen) for further analysis, and a second author checked for the consistency of data entry in this step.

Two authors independently assessed the risk of bias for each study using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions*.[10] The risk of bias for RCTs was assessed according to the following domains: (1) random sequence generation; (2) allocation concealment; (3) blinding of participants and personnel; (4) blinding of outcome assessment; (5) incomplete outcome data; (6) selective outcome reporting; and (7) other bias. The Newcastle-Ottawa scale was used to assess quality from observational studies.[11] Evidence was graded according to study design, consistency, directness, imprecision, and reporting bias. Considering the lack of evidence of adequacy of follow-up in cohort studies, we used a cutoff of 3 years based on results from a large study in which >80% of patients with CD were diagnosed within the first 2 years.[4] To explore the possibility of risk of publication bias, a funnel plot and statistical tests for asymmetry were evaluated if there were more than 10 studies in the meta-analysis.[12]

Measures of Treatment Effect

Information regarding follow-up of the study population (patients enrolled and treated) was reported as total N, and data collected were reported as number of patients over the total number of patients for each arm (n/N). The total numbers of patients who did and did not develop CD in each arm at each time point were reported as number over the total sample population (n/N) in each arm. RCTs and cohort studies were summarized with risk ratio (RR) and case-control studies were summarized with OR, all with 95% CI. For quantitative analysis, a meta-analysis was performed when appropriate, using RevMan 5.3.[9] Data were pooled using a random-effects model.[10] Statistically significant heterogeneity was assessed using both the I^2 statistic and the χ^2 test. A value of 0% for I^2 indicates no observed heterogeneity, and larger values denote heterogeneity. Significant heterogeneity was considered at an I^2 >25% or a χ^2 P value of <.10.

Subgroup analyses were performed considering the risk of CD on the following: (1) amount of gluten introduced; (2) gradual (2-3 g/100 g food) vs sudden gluten introduction; and (3) studies conducted in North America vs other countries. Sensitivity analyses were planned to address questions

on early (<2-4 months) vs late (>6-7 months) gluten introduction or to evaluate the effect of specific studies.

The current systematic review and meta-analysis were performed according the recommended Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (http://www.prisma-statement.org/statement.htm).

Results

We identified a total of 1982 studies, of which 1789 remained after removing duplicates. Twenty-six additional studies were identified by a recursive bibliography search from the identified papers. Of these studies, 1900 were excluded at the title screening stage, and 108 were excluded at the abstract screening stage; 45 studies were eligible for full-text screening (**Figure 1**; available at www.jpeds.com). Good interreviewer agreement was found at the title and abstract screening stages (κ = 0.60; SE of κ = 0.108; 95% CI, 0.39-0.81) and moderate in the full-text screening (κ = 0.43; SE of κ = 0.083; 95% CI, 0.270-0.595). After full-text review, 28 studies were excluded. The results from ongoing investigations were not published until the analysis of 5 studies identified in abstract format or clinical trials website.[13-17] Authors of these studies were contacted; however, none of the authors was able to provide additional information. Seventeen studies finally met the inclusion and exclusion criteria for quantitative and qualitative synthesis, and data were extracted. The characteristics of these 17 included studies are summarized in **Table I**.

Timing of Gluten Introduction

Fifteen studies[2-4,18-29] met the inclusion criteria and evaluated the risk of CD at different time points of gluten introduction. These included 2 RCTs[18,19] and 13[2-4,20-29] observational studies.

Introduction of Gluten at 6 Months vs 12 Months

Two RCTs[18,19] evaluated gluten introduction at 5-6 months vs 12 months; together, these trials reported 18 events in 183 patients. There was no statistically significant increased risk of CD associated with the "standard" gluten introduction (5-6 months) compared with "late" gluten introduction (12 months) (RR, 1.41; 95% CI, 0.59-3.39). No heterogeneity was observed between the studies (I^2 = 0%), although the number of events was modest. A sensitivity analysis was performed with results from a follow-up of the study from Sellito et al[18] that were provided by the authors, and the results remained unchanged. The risk of bias was assessed, and a high risk of bias owing to blinding and a high rate of noncompliance (30%) was identified for one study,[19] and a high risk of selection bias owing to an imbalance of dropouts in the study groups and unclear for randomization and allocation concealment were identified for the other study.[18] Forest plots of RCTs used in the meta-analysis and risk of bias tool are shown in **Figure 2**, A.

Introduction of Gluten at <4 Months vs the Recommended Time (4-6 Months) or Later (>6 Months)

Four cohort studies[2,3,20,21] compared early (<4 months) vs late (>6 months) gluten introduction involving a total of 50 451 children and 282 events. The pooled analysis for early gluten introduction compared with late gluten introduction from these observational studies revealed no differences in the risk of CD (RR, 1.08; 95% CI, 0.76-1.54; P = .68), with no significant heterogeneity between studies (I^2 = 0%) (**Figure 2**, B). The same 4 cohort studies[2,3,20,21] also compared early (<4 months) and recommended gluten introduction (4-6 months), and found no significant difference in the risk of CD (RR, 1.27; 95% CI, 0.86-1.86; P = .38), with no significant heterogeneity (I^2 = 3%) (**Figure 2**, C). One case control study[4] that evaluated introduction of gluten in 491 children with CD and 781 controls without known CD found no difference in gluten introduction at 1-4 months vs 5-12 months for CD cases vs controls (OR, 0.70; 95% CI, 0.48-1.03; P = .07).

Introduction of Gluten at the Recommended Time (4-6 Months) vs Later (>6 Months)

Five cohort studies[2,3,20-23] compared the recommended time for gluten introduction (4-6 months) vs later introduction. A meta-analysis of these studies demonstrated a 25% increase in the risk of CD in the population with late gluten introduction compared with the population with the recommended introduction (RR, 1.25; 95% CI, 1.08-1.45; P = .002) (**Figure 2**, D). The sensitivity analysis with sequential removal of any individual study did not influence the results, except for the removal of 1 study[22] in which the pooled data became marginally significant (RR, 1.21; 95% CI, 0.97-1.50; P = .09). One case-control study[4] evaluated introduction of gluten in 491 children with CD and 781 controls without known CD. In contrast to the cohort data, this study found that CD cases were more likely to have gluten introduced at 1-6 months compared with 7-12 months (OR, 1.42; 95% CI, 1.01-2.00; P = .04). There was, however, no association between the time of gluten introduction and the risk of CD in a multivariate analysis that controlled for breastfeeding during weaning, amount of flour introduced, and type of food given when flour was introduced.[4]

Introduction of Gluten at Other Time Points

Other studies evaluated the risk of early vs late gluten introduction on the risk of CD; however, these studies used different definitions for timing, and thus were not included in the meta-analysis. For example, Peters et al[23] reported that the age of gluten introduction (<3 months vs >3 months) had no influence on the incidence of CD (OR, 0.72; 95% CI, 0.29-1.79). A similar definition for gluten introduction was used by Auricchio et al[24] evaluating 216 children with CD and their healthy siblings, who reported that early introduction of gluten was not associated with increased risk of CD. A risk of bias related to the design was identified in that study,

however. Similar findings were reported by Greco et al,[25] who considered a lower cutoff of 2 months for the differentiation of early and late gluten introduction.

Time to Introduction of Gluten and Risk of CD

Four studies[23,26-28] reported continuous data on the time of gluten introduction in a total of 240 patients with CD compared with 534 controls. Each study found no statistically significant difference in the time of gluten introduction and odds of CD. We assumed that the median values given in 3 of those reports[26-28] were similar to the mean, and estimated the SD from the ranges given. Pooling the studies also revealed no statistically significant difference in mean difference in months of gluten introduction (mean difference, −0.10; 95% CI, −0.27 to 0.07), with little heterogeneity among the studies (I^2 = 12%) (**Figure 2**, E).

Gluten Dose and Mode of Introduction

Four studies[2,4,22,28] evaluated the amount of gluten introduced and the effect on CD risk; however, differences in the definition on amount on gluten considered by each group precluded pooling of the data from these studies. Ivarsson et al[4] reported that larger amounts of gluten at the time of first introduction (16 g) increased the risk of CD (OR, large vs small or medium amounts, 1.9; 95% CI, 1.4-2.6). A recent ecological study by the same group[22] comparing 2 populations born in 1993 and 1997 found that the population with the lower risk for CD (born in 1997) ingested significantly less gluten containing cereal compared with the population with the greater risk, that born in 1993 (24 g/day vs 38 g/day). Norris et al[2] proposed that greater amounts of gluten introduced at 7 months could be associated with increased risk of CD compared with the amount of gluten introduced at 4 months; however, the exact amounts of gluten consumed were not specified. In contrast, another study[28] found similar consumption of wheat at the time of diagnosis in patients with CD and in controls; however, that study evaluated the amount of gluten consumed at the moment of the biopsy for CD diagnosis, but not during weaning.

Breastfeeding at the Time of Gluten Introduction and Risk of CD

Three cohort studies evaluated the association between breastfeeding and CD; however, different outcomes were reported by each study, and meta-analysis was not possible. Stordal et al[20] reported an increased risk of CD in infants breastfed for >12 months compared with those breastfed for <6 months (aOR, 1.49; 95% CI, 1.01-2.21; P = .04). The authors found no difference in CD risk between children breastfed for >1 month and those breastfed for <1 month during weaning (RR, 1.04; 95% CI, 0.66-1.03). Norris et al[2] found no difference in CD risk between 1560 children breastfed during gluten introduction and those not breastfed (RR, 1.23; OR, 0.72-2.11). Finally, Ivarsson et al[4] compared breastfeeding duration in populations born in 1993 and 1997, and found that the population with lower CD risk, that born in 1997, was breastfed for a longer period than that born in 1993. Thus, prolonged breastfeeding beyond food introduction could decrease the risk of CD. Five studies[24-26,30,31] evaluated breastfeeding vs never breastfed (or breastfed for <1 month) in a total of 172 011 participants, including 851 patients with CD. Two of these studies[24,25] reported that patients with CD were less likely to have been breastfed compared with those without CD, whereas the other 3 studies[26,30,31] found no statistically significant association between breastfeeding and CD. Overall, 433 of 851 patients with CD (51%) were breastfed, compared with 119 034 of 171 160 controls (70%). The meta-analysis showed a nonsignificant trend toward lower odds of breastfeeding in the CD group (OR, 0.55; 95% CI, 0.28-1.10; P = .09) (**Figure 2**, F). There was a high degree of heterogeneity (I^2 = 92%) among the studies, but there were too few studies to enable an adequate exploration of the reasons for this.

Six studies,[2,4,20,23,27,28] with a total of 48 845 participants, including 926 patients with CD, evaluated whether participants were breastfed at weaning. Three of these studies[4,23,27] reported that patients with CD were less likely to have been breastfed during weaning compared with those without CD, whereas the other 3 studies found no difference between the 2 groups.[2,20,28] Overall 479 of 926 patients with CD (52%) were breastfed during weaning, compared with 40 789 of 47 919 controls (85%). The meta-analysis showed a nonsignificant trend toward lower odds of breastfeeding in the CD group (OR, 0.70; 95% CI, 0.45-1.10; P = .12) (**Figure 2**, G). There was a high degree of heterogeneity (I^2 = 78%) among the studies, but there were too few studies for an adequate exploration of this. **Table II** summarizes outcomes and results.

Discussion

CD is a common and serious disease, and parents need advice on the best approach to introducing gluten into their child's diet. Breastfeeding is beneficial in many ways, but whether it also helps reduce the risk of CD is unclear. It has been hypothesized that early introduction of gluten, usually defined as before 4 months of age, may increase the risk of CD,[20,30-32] and that breastfeeding may be protective, particularly at the time of gluten introduction. In addition, a high gluten content at the time of introduction has been suggested as another important risk factor. These issues were addressed in a previous systematic review,[8] but evidence was not conclusive. We have updated that review with additional evidence, and have also synthesized the data in an attempt to provide a clearer evidence base.

Our review included 5 studies of children at increased risk (family members of those with CD or diabetes mellitus, or HLA-compatible),[2,18,19,21,28] and 12 studies of children at general risk.[3,4,20,22-27,29-31] Our results indicate that late introduction of gluten to the infant diet may increase the risk of CD compared with introduction at age 4-6 months. Our best estimate of the increase in risk is 25%, which is

Table I. Characteristics of included studies

Study	Year	Methods	Participants	Intervention	Outcome	Notes
RCTs						
Sellitto et al[18] (US)	2012	CD was defined as the presence of CD tTG, the onset of CD-related symptoms, and/or evidence of autoimmune enteropathy.	34 infants HLA-DQ2/-DQ8 positive; 17 were randomized to gluten introduction at 6 mo, and 13 were randomized to gluten introduction at 12 mo.	Gluten introduction at 6 mo vs 12 mo	Development of CD autoimmunity (tTG) and changes in gut microbiota	
Hummel et al[19] (Germany)	2011	Children with a first-degree family member with DBT1 or HLA-positivity were randomly assigned to gluten introduction at 6 mo or 12 mo (late exposure)	150 children; 77 in the control group (6 mo) and 73 in the late exposure group (12 mo)	Gluten introduction at 6 and 12 mo	Development of CD autoimmunity (tTG) and islet autoimmunity in children at genetic risk (HLA-positive, DBT1, or family member)	Follow-up at 3 y
Observational studies						
Norris et al[2] (US)	2005	Data from the DAISY study in children at increased risk for CD, defined as having either HLA-DR3 or -DR4 alleles (measured in cord blood) or a relative with type 1 diabetes. Mother was asked about infant diet through telephone interview or face to face at 3, 6, 9, 12, and 15 mo. CD was defined as autoimmunity-positive tTG.	1560 children, 51 with CD and 1501 controls	Gluten introduction at <4 mo vs 4-6 mo and >7 mo	To examine whether the timing of gluten exposure in the infant diet was associated with the development of CD autoimmunity	Mean follow-up of 4.8 y
Welander et al[3] (Sweden)	2010	Data were collected from a population-based database (ABIS project), infants born in 1997-1999 in southeast Sweden, with data available on breastfeeding and gluten introduction. CD was diagnosed through duodenal biopsy and tTG.	9408 children, 44 with CD and 9364 controls	Gluten introduction at 4 mo vs 4-6 mo, 7-8 mo, 9-10 mo, and 11-12 mo	Impact of gluten introduction on CD risk	
Ivarsson et al[4] (Sweden)	2002	A prospective register of all incident cases of CD in children aged <15 y was established in 1991. A questionnaire was mailed and answered by 601 cases (96%) and 1124 referents (90%). CD was defined according to ESPGHAN criteria.	601 children with CD (455 aged 0-1.9 y and 146 aged 2-14.9 y) and 1124 referents (856 aged 0-1.9 y and 268 aged 2-14.9 y)	Gluten introduction at <4 mo, 5-6 mo, and >7 mo	To analyze whether the risk of developing CD was affected by the age at which gluten was introduced in the diet, the amount of gluten introduced, the type of gluten-containing foods introduced, and breastfeeding status at the time of dietary gluten introduction	The study population covered 40% of the Swedish population. Mean duration of follow-up was 6 y.
Stordal et al[20] (Norway)	2013	Data were collected from the Norwegian Mother and Child Cohort Study (MoBa) from 1999 to 2008. CD was diagnosed according to ESPGHAN criteria. Controls were children without known CD from the same population.	Overall population: 82 167 children, 324 with CD and 81 843 controls	Gluten introduction at <4 mo vs 5-6 mo and >6 mo	To study the association between timing of gluten introduction modified by breastfeeding and the risk of CD in childhood in a prospective birth cohort	The study population comprised 24 750 children; 23% were excluded from analysis. The duration of follow-up was 10 years.

(*continued*)

Table I. Continued

Study	Year	Methods	Participants	Intervention	Outcome	Notes
Hummel et al[21] (Germany)	2007	Prospective observational study with follow-up from birth between 1989 and 2000. Mean follow-up was 7.6 years. Children were tested for tTG and islet autoantibodies. Data on diet were collected via questionnaires and telephone interviews at 9 mo and 2 y.	1511 children at risk for DBT1 or CD	Gluten introduction at <3 mo vs 3.1-6 mo and >6 mo	Development of CD autoimmunity (tTG) and islet autoimmunity in children at genetic risk in first-degree relatives of patients with type 1 diabetes	
Ivarsson et al[22] (Sweden)	2013	Two-phase cross-sectional study (ETICS). Screening was done in 2 cohorts of students aged 12 y in 2005-2006 and 2009-2010. The first cohort represents the epidemic birth cohort (born in 1993); the second, the postepidemic birth cohort (born in 1997).	13 279 children, 5712 in the 1997 cohort and 7567 in the 1993 cohort	Gluten introduction at <6 mo vs >6 mo	Total prevalence of clinically detected CD in 2 birth cohorts of 12-y-olds, with the findings related to each cohort's ascertained infant feeding	The response rate was 75% for the 1993 cohort and 69% for the 1997 cohort.
Peters et al[23] (Germany)	2001	Case control study with prospective recruitment in 1995-1996. Patients were enrolled from 2 sources: a previous incidence study in northern Germany and the German CD Society. Parents of all recruited children completed self-administered questionnaires. CD was diagnosed according to ESPGHAN criteria.	280 children, 143 with CD and 137 healthy children from the same population registry	Duration of breastfeeding >2 mo vs <2 mo; age at gluten introduction <4 mo vs >4 mo; percentage breastfeeding when gluten introduced	To investigate the associations between duration of breastfeeding and the age of gluten introduction and the incidence and age at onset of CD	
Auricchio et al[24] (Italy)	1983	Population was recruited at 3 different centers in Italy. Data related to feeding practices and breastfeeding were collected through interviews with mothers.	Total population 545, including 188 patients with CD and 366 siblings as controls.	Gluten introduction at <3 mo vs >3 mo	To investigate the frequency and duration of breastfeeding and the time of gluten introduction in patients with CD compared with non-CD controls	
Greco et al[25] (Italy)	1998	Case-control study. Data related to feeding practices and breastfeeding were collected.	2150 patients, including 201 patients with CD and 1949 controls matched by age and geographic area	Gluten introduction at <3 mo vs >3 mo	To evaluate the possible role of early gluten introduction and interruption of breastfeeding as risk factors for CD	
Challacombe et al[26] (UK)	1997	Population study conducted in Somerset from 1971 to 1992. Two periods were considered: 1971-1980 and 1981-1992. CD was diagnosed according to ESPGHAN criteria.	26 patients with CD and 13 with transient gluten intolerance. Controls with children admitted to the hospital with CD ruled out.	Gluten introduction at 3 mo vs 5.5 mo	To investigate the influence of changing infant feeding practices on the incidence of childhood CD and transient gluten intolerance	

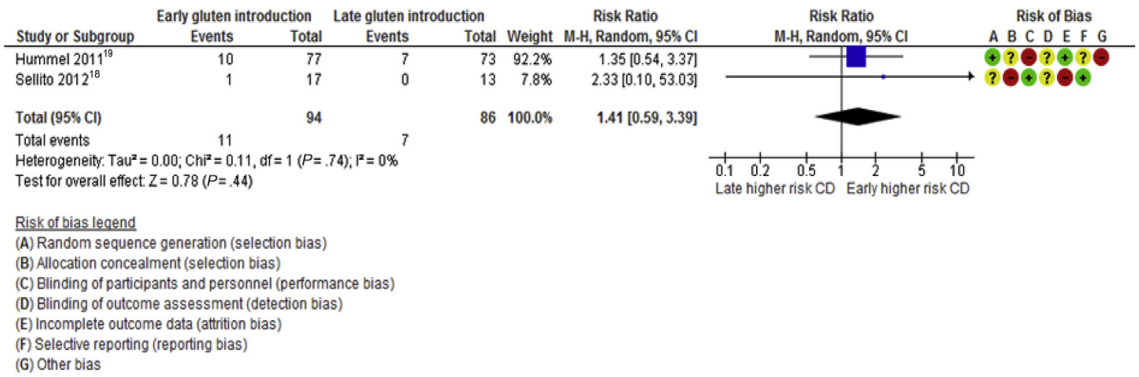
(*continued*)

Table I. Continued

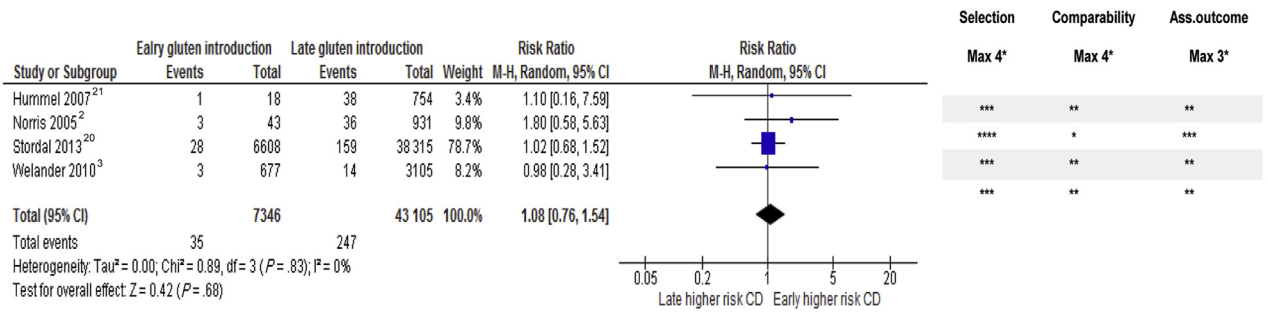
Study	Year	Methods	Participants	Intervention	Outcome	Notes
Fälth-Magnusson et al[27] (Sweden)	1996	Questionnaires on feeding practices were mailed to a CD population diagnosed between 1989 and 1991 and controls. Children age <2 y were diagnosed with CD through duodenal biopsy according to Alexander criteria. Four-age matched controls from the general population were included.	72 patients with CD and 288 age-matched controls from the same geographic area	Mean age and mode of gluten introduction; breastfeeding duration and concomitant breastfeeding at the time of gluten introduction	To clarify the feeding practices in infants in 2 different populations	
Ascher et al[28] (Sweden)	1997	268 families from 272 CD patients (ESPGHAN) were invited to participate. Siblings and parents were tested for HLA and CD serology.	85 siblings of patients with genetic-positive CD	Gluten introduction at 4-6 mo vs at >6 mo	Impact of early infant feeding and gluten introduction on the risk of CD in individuals with positive HLA-DQ2	Population initially had increased risk of CD determined by genetic compatible
Stevens et al[29] (Ireland)	1987	Retrospective review of a cohort of patients from County Galway over a 33-year period (1960-1981), divided into five 5-year quarters. Data related to breast feeding and gluten introduction were extracted from a national database and patient records for the earlier period. CD was defined by biopsy (Watson capsule).	69 463 births overall; 16 313 born in 1960-1965, 15 874 born in 1965-1970, 18 005 born 1971-1976, and 19 271 born in 1976-1981	Mean age of gluten introduction and percentage of patients breastfed for >1 mo in each period	Unclear; possibly to evaluate the risk of CD and the influence of changes in feeding practices	
Decker et al[30] (Germany)	2010	Retrospective, multicenter case-control study. Information on intestinal disease manifestation, mode of delivery, gestational age at birth, postnatal complications, and breastfeeding was collected by the physician from children and their parents who were visiting a gastrointestinal outpatient clinic.	123 patients with CD and 862 controls, plus 931 patients with irritable bowel disease and other gastrointestinal disorders	Potential risk factors that influence breastfeeding, including mode of delivery and postnatal complications; breastfeeding likelihood and duration in each group	To analyze a possible association between cesarean delivery and enteric inflammatory diseases in children	
Roberts et al[31] (UK)	2009	"Record linked abstracts of birth registrations, maternity, in-patient and day case records in a defined population of southern England. CD in the mothers was identified by record linkage to hospital admissions for the mother before and after the pregnancy from 1970 to 1999."	248 521 children, including 60 with CD	Breastfeeding vs not breastfeeding	"To investigate the relationship between perinatal risk factors and subsequent coeliac disease among offspring."	

DAISY, Diabetes Autoimmunity Study in the Young; *DBT1*, Type 1 Diabetes; *ESPGHAN*, European Society of Paediatric Gastroenterology, Hepatology, and Nutrition; *ETICS*, Exploring the Iceberg of Celiacs in Sweden; *tTG*, tissue transglutaminase antibody.

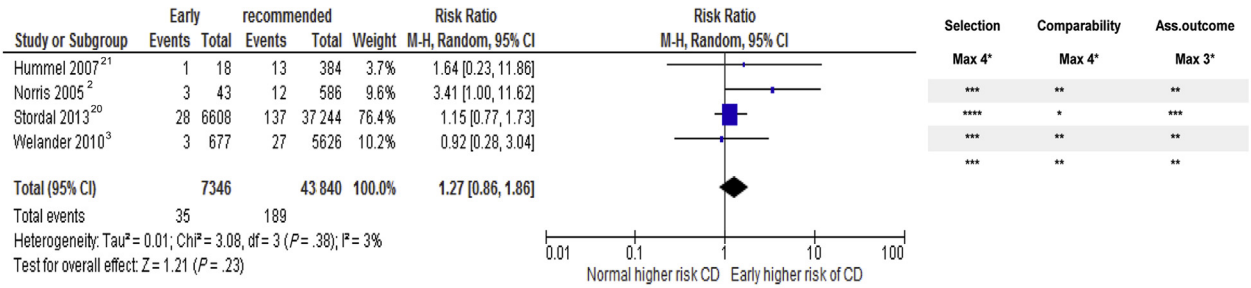
A



B



C



D

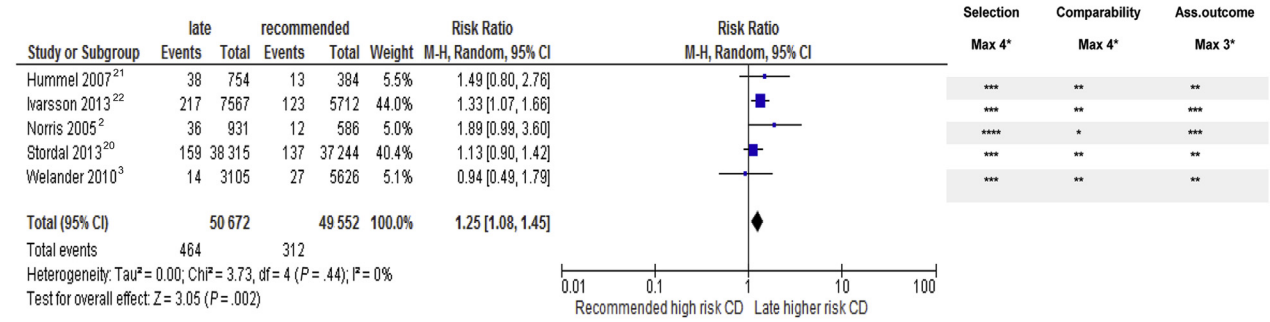


Figure 2. Forest plots of **A,** RCT standard (5-6 months) vs late (12 months); **B,** comparison of cohort studies early (<4 months) vs late (>6 months) gluten introduction, **C,** comparison of cohort studies early introduction (<4 months) vs recommended (4-6 months), **D,** comparison of cohort studies late (>6 months) vs recommended gluten introduction, **E,** mean time of gluten introduction in CD compared with controls, **F,** comparison case control studies breastfed (BF) vs never BF, and **G,** comparison case control studies BF during weaning vs not BF during weaning. *,**,***,****Number of stars attributed by the Castle Ottawas classification for risk of bias from observational studies; *IV*, independent variable. *(Continues)*

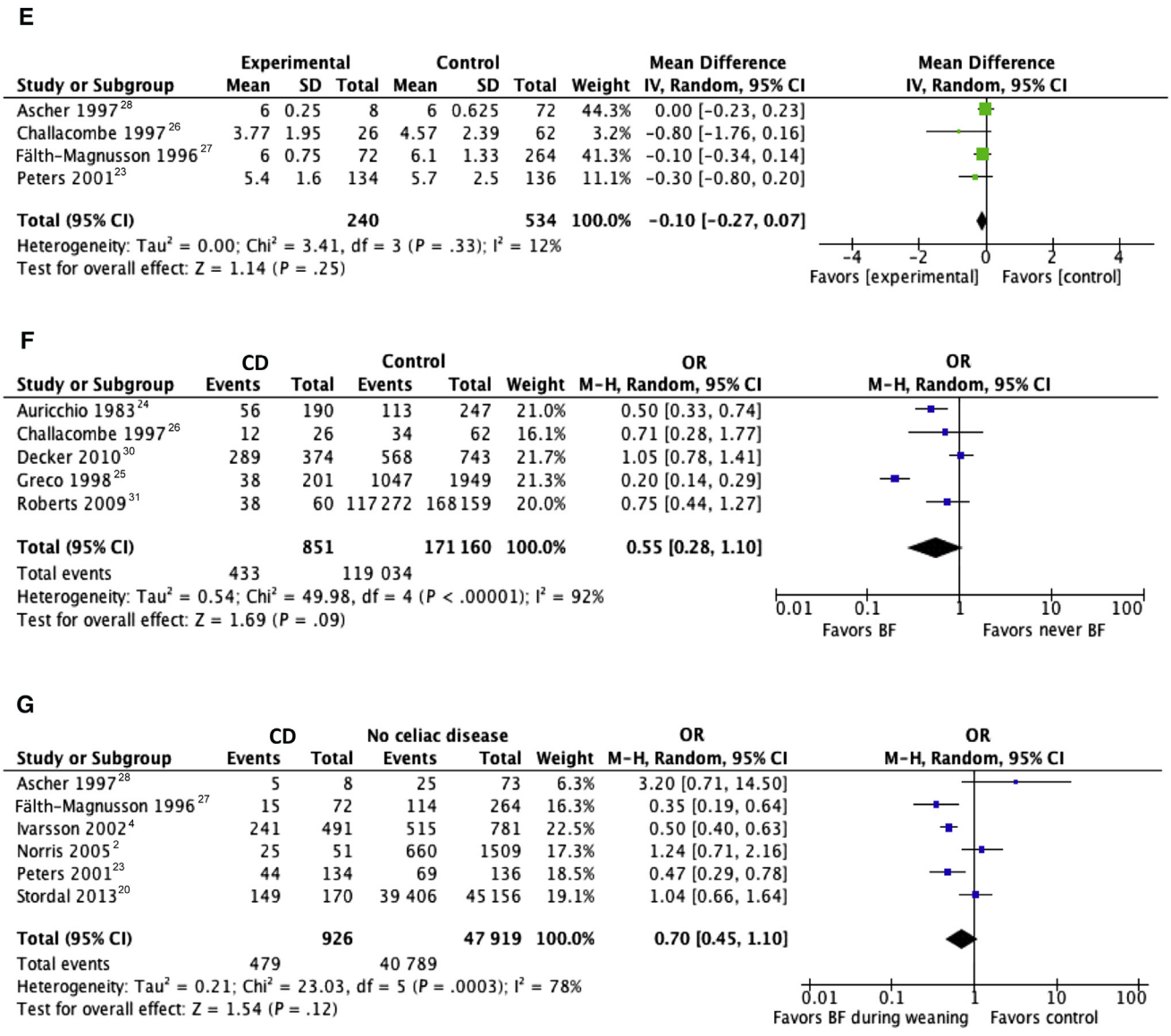


Figure 2. Continued.

statistically significant. The data related to the amount of gluten introduced are scarce, but large amounts of gluten started at weaning could be associated with an increased risk of developing CD. These data are not robust, and it is particularly difficult to evaluate the role of confounding factors in these associations. Studies included in this review rarely controlled for all possible confounding factors, such as breastfeeding, type and amount of gluten ingested, and other feeding patterns; thus, it is therefore possible that the associations identified are related to other factors in the early upbringing of children.

After this meta-analysis was concluded, 2 relevant RCTs including data from almost 1000 children each,[33,34] and an additional prospective birth cohort study,[35] were published. Although we could not include these studies in our present analysis, we believe it necessary to summarize their main conclusions and also to base our final recommendations on all of the analytically reviewed papers and these 3 newly published ones as well.

The first study, by Vrezinga et al,[33] was a multicenter, randomized, double-blind, placebo-controlled dietary intervention study involving 944 children who had at least 1 first-degree relative with CD and had an HLA status compatible with CD. From age 4 to 6 months, 475 participants received 100 mg of vital gluten daily, and 469 received placebo. After 24 weeks, intake of gluten was liberalized in both groups. CD serology was measured periodically. The study found no significant between-group difference in the risk of CD after 3 years. The presence of a placebo control removes the possibility of bias, and the randomized design reduces the possibility of confounding; however, this does not completely resolve all questions. This trial evaluated only children at high risk

Table II. Summary of outcomes and results

Outcome or subgroup	Studies	Participants	Statistical method	Effect estimate
1.1 RCT early (6 mo) vs late (12 mo)	2	184	RR (M-H, random, 95% CI)	1.61 (0.70 to 3.71)
1.2 Cohort study: early (<4 mo) vs late (>6 mo) gluten introduction	4	50 451	RR (M-H, random, 95% CI)	1.08 (0.76 to 1.54)
1.3 Cohort study: early (<4 mo) vs recommended (4-6 mo) gluten introduction	4	51 186	RR (M-H, random, 95% CI)	1.27 (0.86 to 1.86)
1.4 Cohort study: late (>6 mo) vs recommended gluten introduction	5	100 224	RR (M-H, random, 95% CI)	1.25 (1.08 to 1.45)
1.5 Mean gluten introduction	5	875	Mean difference (IV, random, 95% CI)	−0.25 (−0.59 to 0.08)
1.6 Case-control study: early (<2-3 mo) vs late (>3 mo) gluten introduction	2	2540	RR (M-H, random, 95% CI)	0.80 (0.17 to 3.73)
1.7 Case-control study: early (<4 mo) gluten introduction	1	1018	OR (M-H, fixed, 95% CI)	0.69 (0.44 to 1.08)
1.8 Case-control study: recommended (4-6 mo) gluten introduction	1	1018	RR (M-H, random, 95% CI)	1.12 (1.05 to 1.20)
1.9 Case-control study: late (>6 mo) gluten introduction	1	1018	OR (M-H, fixed, 95% CI)	0.58 (0.39 to 0.86)

IV, independent variable.

for CD, and the results might not apply to those at average risk. Furthermore, a significant proportion was lost to follow-up, and another 10% did not adhere to the recommended diet.[33] Regarding this latter point, it is also impossible to accurately determine the dietary intake of infants, and it is possible that some participants in the placebo group may have ingested gluten at 4-6 months, which contaminates the results. Finally, whether 100 mg is the optimal amount of gluten to introduce at age 4 months is unclear. It is also possible that a greater amount or a gradually increasing amount starting at 4 months may be needed as "protective." To emphasize this uncertainty, we included this randomized trial in our systematic review as a post hoc analysis (because it is outside the time period of our search) using a random-effects model, and found no differences in results (RR, 1.19; 95% CI, 0.99-1.43; $P = .06$).

In a large multicenter Italian study reported by Lionetti et al,[34] 832 newborns with a first-degree relative with CD and a determined HLA genotype were randomized to introduction of dietary gluten at age 6 or at 12 months. There was a delay in the development of CD by age 2 years in those introduced to gluten at 12 months, but no difference between the 2 groups at age 5 years. This trial did not directly address the question regarding the amount of gluten at the time of introduction.[34]

Aronsson et al[35] analyzed dietary risk factors for the development of CD autoimmunity in a multinational birth cohort study of 6436 children at risk for the development of CD. They found no associated increased risk of CD autoimmunity with early or late feeding of gluten compared with the reference group with gluten introduction at age 4-6 months.

Based on the foregoing studies, early-life gluten feeding practice does not seem to influence CD risk in children at genetic risk. Moreover, the results of this meta-analysis support only a moderate increase in risk with late, but not early, gluten introduction. The question of gluten dose as a risk factor remains unresolved, however. Furthermore, a recently published meta-analysis with similar objectives, methodology, and results as our present analysis[36] included 2 of the most recent 2 RCTs,[33,34] but failed to identify 2 large earlier studies[26,29] that were included in our analysis. Therefore, our analysis complements that recently published meta-analysis.

Studies on breastfeeding have yielded conflicting results, with some suggesting that breastfeeding is protective[4,27] and others finding an increased risk under certain conditions (eg, after 12 months)[20] or no association.[2] Our meta-analysis suggests a nonsignificant trend toward a benefit of breastfeeding and shows similarities with the 2 recent large studies that did not find any association between breastfeeding and risk of CD.[33,34] However, when those 2 studies were added in a post hoc analysis, 1 study[33] provided data for the analysis on breastfeeding vs no breastfeeding, and there remained a nonsignificant trend toward a benefit of breastfeeding (OR, 0.60; 95% CI, 0.33-1.10; $P = .10$), whereas the other study[34] provided data on breastfeeding during weaning, and again this did not change the conclusions of our review (OR, 0.74; 95% CI, 0.50-1.11, $P = .15$). Overall, these trends are insufficient to allow any conclusions regarding breastfeeding and the risk of CD.

One challenge in synthesizing this literature lies in how breastfeeding is defined. We took the approach of defining this as breastfed vs not breastfed, or not breastfed at the time of weaning. Even though we tended to homogenize the characterization of breastfeeding, as explained in the Methods section, there is substantial heterogeneity in the definition. For example, there is the issue of exclusive breastfeeding vs some breastfeeding supplemented by bottle feeding, an issue not addressed in this systematic review. Another challenge is again this issue of confounding factors. Although some positive studies[4] adjusted for potential confounders, others[27] did not.

Finally, we acknowledge our disappointment that the data are not sufficiently robust to allow definitive evidence-based recommendations for infant feeding to prevent CD. Additional studies[34,35] published since the completion of our systematic review did not help in this regard. Those studies were performed in high-risk families, and the results might not apply to the general population; thus, future studies that include infants at lower genetic risk for CD are desirable. Nonetheless, even in the absence of solid evidence, it is important to reach a consensus for common practice guidelines, in particular taking into account the fact that the recent studies considered a specific population at high risk for CD, and whether the results apply to the general population is unclear.

We believe that breastfeeding is the natural and preferred form of infant feeding, as supported by robust evidence and endorsed by major international bodies for a number of well-proven benefits outside of CD, including prevention of obesity. In regard to CD development, even without solid evidence of a protective effect of breastfeeding, we still support its use whenever possible in infants at risk for CD, in consideration of its general benefits. In this sense, we endorse the recommendation of the American Academy of Pediatrics to support exclusive breastfeeding for roughly the first 6 months of life.[37] As a corollary to this, we also feel it is now safe to assume that a short duration of breastfeeding, or introduction of gluten outside breastfeeding, carries no significant risk of later development of CD.

Overall, we can provide no solid recommendations regarding the most favorable timing for gluten introduction; however, based on our meta-analysis, the best time appears to be between 4 and 6 months. Another important point that remains unresolved is whether the amount of gluten introduced to infant diet influences CD risk. Future prospective studies evaluating the effect of gluten-free diet vs a normal diet or even the effect of a gluten overload on CD risk, as seen during the Swedish epidemic,[38] are needed as well. Unfortunately, given the lack of evidence on optimal amounts of gluten to introduce in infants born into families at risk for CD, for now this practice will remain based on expert opinion. Considering that infants ingest an average of ~5 g of gluten daily between age 7 and 12 months,[39] it seems reasonable to introduce gluten in the amount of ~1.2 g/day at age 6 months and continue this dose for roughly 4 weeks, then increase the consumption to 2.5 g/day at 7-8 months and finally to ~5 g/day up to age 12 months. After age 12 months, a full, "regular" amount of gluten might be acceptable; however, in a child at high risk for CD based on genetic determinations, the evidence suggests that limiting gluten quantity may delay the development of CD.

In conclusion, there is currently insufficient evidence supporting an association between early introduction of gluten to an infant's diet and increased risk of CD development. In contrast to a recent meta-analysis, the results from the present study suggest that late introduction of gluten may be associated with an increased risk of CD. In addition, the evidence is insufficient to determine whether breastfeeding has any affect on the risk of CD. There is a need for future large observational studies that carefully control for potential confounding factors and that evaluate these factors in both the general population and low-risk families. ■

Submitted for publication Jun 9, 2015; last revision received Jul 30, 2015; accepted Sep 9, 2015.

Reprint requests: María Inés Pinto-Sánchez, MD, 1280 Main St West, MUMC 3N51 E, Hamilton, ON, Canada L8S 4K1. E-mail: pintosm@mcmaster.ca

References

1. Setty M, Hormaza L, Guandalini S. Celiac disease: risk assessment, diagnosis, and monitoring. Mol Diagn Ther 2008;12:289-98.
2. Norris JM, Barriga K, Hoffenberg EJ, Taki I, Miao D, Haas JE, et al. Risk of celiac disease autoimmunity and timing of gluten introduction in the diet of infants at increased risk of disease. JAMA 2005;293:2343-51.
3. Welander A, Tjernberg AR, Montgomery SM, Ludvigsson J, Ludvigsson JF. Infectious disease and risk of later celiac disease in childhood. Pediatrics 2010;125:e530-6.
4. Ivarsson A, Hernell O, Stenlund H, Persson LA. Breast-feeding protects against celiac disease. Am J Clin Nutr 2002;75:914-21.
5. Akobeng AK, Ramanan AV, Buchan I, Heller RF. Effect of breast feeding on risk of coeliac disease: a systematic review and meta-analysis of observational studies. Arch Dis Child 2006;91:39-43.
6. Agostoni C, Decsi T, Fewtrell M, Goulet O, Kolacek S, Koletzko B, et al. Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 2008;46:99-110.
7. Olives JP. When should we introduce gluten into the feeding of the newborn babies? Arch Pediatr 2010;17(Suppl 5):S199-203 (in French).
8. Szajewska H, Chmielewska A, Pieścik-Lech M, Ivarsson A, Kolacek S, Koletzko S, et al. Systematic review: early infant feeding and the prevention of coeliac disease. Aliment Pharmacol Ther 2012;36:607-18.
9. Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration; 2014.
10. Cochrane Handbook for Systematic Reviews of Interventions. http://community.cochrane.org/handbook. Accessed December 2014.
11. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al; Ottawa Hospital Research Institute. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm. http://www.ohri.ca/programs/clinical_epidemiology/nosgen.pdf. Accessed July 2015.
12. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-34.
13. Roman E, Celiac Disease Working Group. Environmental factors in celiac disease: a nationwide case-control study. J Pediatr Gastroenterol Nutr 2010;50(Suppl 2):E74-5.
14. Catassi C. Risk of celiac disease and age at gluten introduction (CELIPREV). ClinicalTrials.gov identifier: NCT00639444.
15. Ilonen J, et al. Celiac disease prevention. ClinicalTrials.gov identifier: NCT00617838.
16. Krischer J, et al. TEDDY: The Environmental Determinants of Diabetes in the Young. ClinicalTrials.gov identifier: NCT00279318.
17. Fasano A. Infant nutrition and risk of celiac disease. ClinicalTrials.gov identifier: NCT00819819.
18. Sellitto M, Bai G, Serena G, Fricke WF, Sturgeon C, Gajer P, et al. Proof of concept of microbiome-metabolome analysis and delayed gluten exposure on celiac disease autoimmunity in genetically at risk infants. PLoS One 2012;7:e33387.
19. Hummel S, Pflüger M, Hummel M, Bonifacio E, Ziegler AG. Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. Diabetes Care 2011;34:1301-5.
20. Stordal K, White RA, Eggesbo M. Early feeding and risk of celiac disease in a prospective birth cohort. Pediatrics 2013;132:e1202-9.
21. Hummel S, Hummel M, Banholzer J, Hanak D, Mollenhauer U, Bonifacio E, et al. Development of autoimmunity to transglutaminase C in children of patients with type 1 diabetes: relationship to islet autoantibodies and infant feeding. Diabetologia 2007;50:390-4.
22. Ivarsson A, Myléus A, Norström F, van der Pals M, Rosén A, Högberg L, et al. Prevalence of childhood celiac disease and changes in infant feeding. Pediatrics 2013;131:e687-94.
23. Peters U, Schneeweiss S, Trautwein EA, Erbersdobler HF. A case-control study of the effect of infant feeding on celiac disease. Ann Nutr Metab 2001;45:135-42.
24. Auricchio S, Follo D, de Ritis G, Giunta A, Marzorati D, Prampolini L, et al. Does breast feeding protect against the development of clinical symptoms of celiac disease in children? J Pediatr Gastroenterol Nutr 1983;2:428-33.
25. Greco L, Auricchio S, Mayer M, Grimaldi M. Case control study on nutritional risk factors in celiac disease. J Pediatr Gastroenterol Nutr 1988;7:395-9.

26. Challacombe DN, Mecrow IK, Elliott K, Clarke FJ, Wheeler EE. Changing infant feeding practices and declining incidence of coeliac disease in West Somerset. Arch Dis Child 1997;77:206-9.
27. Fälth-Magnusson K, Franzén L, Jansson G, Laurin P, Stenhammar L. Infant feeding history shows distinct differences between Swedish celiac and reference children. Pediatr Allergy Immunol 1996;7:1-5.
28. Ascher H, Krantz I, Rydberg L, Nordin P, Kristiansson B. Influence of infant feeding and gluten intake on coeliac disease. Arch Dis Child 1997;76:113-7.
29. Stevens FM, Egan-Mitchell B, Cryan E, McCarthy CF, McNicholl B. Decreasing incidence of coeliac disease. Arch Dis Child 1987;62:465-8.
30. Decker E, Engelmann G, Findeisen A, Gerner P, Laass M, Ney D, et al. Cesarean delivery is associated with celiac disease but not inflammatory bowel disease in children. Pediatrics 2010;125:e1433-40.
31. Roberts SE, Williams JG, Meddings D, Davidson R, Goldacre MJ. Perinatal risk factors and coeliac disease in children and young adults: a record linkage study. Aliment Pharmacol Ther 2009;29:222-31.
32. Kelly DA, Phillips AD, Elliott EJ, Dias JA, Walker-Smith JA. Rise and fall of coeliac disease 1960-85. Arch Dis Child 1989;64:1157-60.
33. Vriezinga SL, Auricchio R, Bravi E, Castillejo G, Chmielewska A, Crespo Escobar P, et al. Randomized feeding intervention in infants at high risk for celiac disease. N Engl J Med 2014;371:1304-15.
34. Lionetti E, Castellaneta S, Francavilla R, Pulvirenti A, Tonutti E, Amarri S, et al. Introduction of gluten, HLA status, and the risk of celiac disease in children. N Engl J Med 2014;371:1295-303.
35. Aronsson CA, Lee HS, Liu E, Uusitalo U, Hummel S, Yang J, et al. Age at gluten introduction and risk of celiac disease. Pediatrics 2015;135:239-45.
36. Szajewska H, Shamir R, Chmielewska A, Pieścik-Lech M, Auricchio R, Ivarsson A, et al. Systematic review with meta-analysis: early infant feeding and coeliac disease–update 2015. Aliment Pharmacol Ther 2015;41:1038-54.
37. Eidelman AI, Schanler RJ. Breastfeeding and the use of human milk. Pediatrics 2012;129:e827-41.
38. Ivarsson A, Persson LA, Nyström L, Ascher H, Cavell B, Danielsson L, et al. Epidemic of coeliac disease in Swedish children. Acta Paediatr 2000;89:165-71.
39. Hopman EG, Kiefte-de Jong JC, le Cessie S, Moll HA, Witteman JC, Bleeker SE, et al. Food questionnaire for assessment of infant gluten consumption. Clin Nutr 2007;26:264-71.

50 Years Ago in *The Journal of Pediatrics*

The Diagnostic Value of Serum Enzyme Measurements

Howell, RR. J Pediatr 1966;68:121-34

Dr Howell reviews the (at that time) 36-year history of the value of serum enzymes in clinical practice. He traces the first diagnostic use of an enzyme, alkaline phosphatase (ALP), back to 1930. The methods used in 1966 were not standardized, and each enzyme had its own unit of measurement. The enzymes reviewed by Dr Howell are glutamic oxaloacetic and glutamic pyruvate transaminases, aldolase, creatine kinase, ALP, lactic dehydrogenase, acidic dehydrogenase, and amylase.

Now 50 years later almost all of these analytes are still in clinical use; some have changed names, and other biomarkers have been discovered. We no longer use transaminase levels to diagnose myocardial infarction, but aldolase and creatine kinase remain the serum markers for muscle disease. Today, we have more accurate and time saving equipment for analysis and standardization of measurement units across laboratories, but normal values continue to be reported differently.

Serum enzyme measurements play a crucial role in modern medicine now as they did in 1966, and we rely now on these markers more than ever. Dr Howell points out that vitamin B6 deficiency leads to low levels of transaminases. Low serum concentration of alanine transaminase has recently been shown to be predictive of all-cause mortality in adults.[1] ALP is an enzyme that normally is higher in growing children; however, this continues to raise questions among clinicians about the potential for underlying liver disease. The availability to fractionate bone and liver derived ALP enzyme helps solve this question. Low levels, however, could indicate zinc deficiency, which might be ignored as all of us are accustomed to looking for elevation as marker of disease.

Dr Howell's final comment remains true today: "there is no single change in serum enzyme which is absolutely specific for a given disease." We continue to aim for the development of a truly disease specific and sensitive biomarker. For children, this is especially needed as some of our diagnostic procedures may affect our patients in the long term (eg, the effect of general anesthesia on neurodevelopment).

Einar Thor Hafberg, MD
Division of Pediatric Gastroenterology, Hepatology, and Nutrition
Cincinnati Children's Hospital Medical Center
Cincinnati, Ohio

http://dx.doi.org/10.1016/j.jpeds.2015.07.034

Reference

1. Ramaty E, Maor E, Peltz-Sinvani N, Brom A, Grinfeld A, Kivity S, et al. Low ALT blood levels predict long term all-cause mortality among adults: a historical prospective cohort study. Eur J Intern Med 2014;25:919-21.

Appendix 1

Studies were identified from the following databases: Cochrane Database of Systematic Reviews (CDSR), the Database of Abstracts of Reviews of Effectiveness (DARE), the Medline, EMBASE and CINAHL, and the gray literature (eg, conference reports, technical reports, and dissertations) was searched using SIGLE. Different search terms will be proposed; ie, for MEDLINE, patients with CD were identified with the medical subject heading and text term

"celiac disease" "celiac" "coeliac" "CD" "sprue" "gluten enteropathy" "gluten-sensitive enteropathy" "sprue nontropical" together with text words for other terms such as "gluten sensitivity," "gluten," "child," "childhood," "children" child*, infant*, "toddler" "early" "Gluten" "glutens" "gluten proteins" and "timing or time" and "introduction" "Weaning" "Amount" or "quantity".

These were previously identified with medical subject heading (MESH) terms for gluten, CD, children, and weaning.

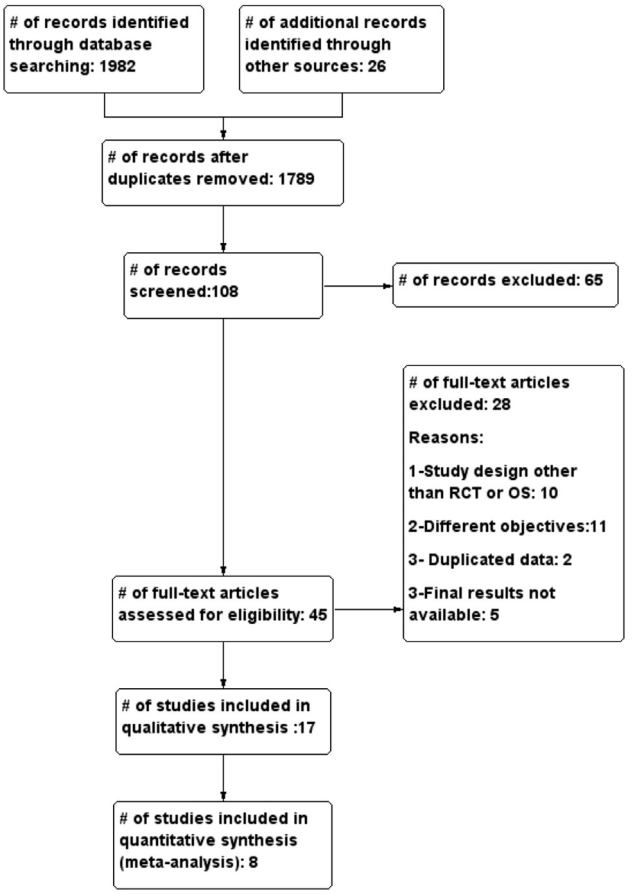


Figure 1. Flow chart of the included studies. *OS*, observational studies.

Appendix 2. Search strategy

	Searches	Results, n
1	celiac.mp. [mp = title, abstract, full text, caption text]	17 295
2	celiac disease.mp. [mp = title, abstract, full text, caption text]	8462
3	celiac sprue.mp. [mp = title, abstract, full text, caption text]	740
4	coeliac disease.mp. [mp = title, abstract, full text, caption text]	5160
5	gluten sensitiv.mp. [mp = title, abstract, full text, caption text]	2
6	gluten sensitiv*.mp. [mp=title, abstract, full text, caption text]	1541
7	gluten enteropathy.mp. [mp = title, abstract, full text, caption]	194
8	gluten.mp. [mp = title, abstract, full text, caption text]	7439
9	glutens.mp. [mp = title, abstract, full text, caption text]	73
10	gluten proteins.mp. [mp = title, abstract, full text, caption text]	288
11	time.mp. [mp = title, abstract, full text, caption text]	2 686 968
12	timing.mp. [mp = title, abstract, full text, caption text]	223 086
13	weaning.mp. [mp = title, abstract, full text, caption text]	31 254
14	child*.mp. [mp = title, abstract, full text, caption text]	975 736
15	infant*.mp. [mp = title, abstract, full text, caption text]	283 937
16	probiotic.mp. [mp = title, abstract, full text, caption text]	7104
17	2 and 8 and 11	1925
18	1 and 8 and 12	226
19	3 and 8 and 11	218
20	19 and 4 and 8 and 11	18
21	4 and 8 and 13 and 14	63

Appendix 3. Studies excluded after full text review and primary reason for exclusion

Excluded study	Year	Reason
Roman et al[13]	2010	Abstract; no information obtained from authors
Catassi[14]		Ongoing study; no information provided by the authors
Ilonen[15]		Ongoing study; no information provided by the authors
Krischer[16]		Ongoing study; no information provided by the authors
Fasano[17]		Ongoing study
Lionetti et al[34]	2014	Post hoc study from the BABYDIET study; different objective: factors influencing potential and over CD; no control group
Vriezinga et al[33]	2014	Not an original study; commentary
Fasano et al[37]	2012	Not an original study; review
Wesol-Kucharska et al[38]	2000	Not an original study; review
Szaflarska-Poplawska et al[39]	2007	Different objective; time to symptom onset
Radlovic et al[40]	2010	Different objective; time to symptom onset
Pittschieler et al[41]	2003	Different objective; not time of gluten introduction
Persson[42]	2002	Not an original study; review
Bouguerra et al[43]	1998	Different objective; time of onset of symptoms
Lindberg[44]	1996	Not original study; review
Ansaldi et al[45]	1991	Different objective; not CD risk
Juto[46]	1990	Not an original study; commentary
Jos et al[47]	1969	Case report; gluten challenge
Greco et al[48]	1985	Different objective; time of symptom onset
Cataldo et al[49]	1991	Different objective; not risk of CD
Matek et al[50]	2000	Different objective; time of symptom onset
D'amico et al[51]	2005	Different objective; time of symptom onset
Mitt et al[52]	1998	Different objective; not time of gluten introduction
Maèki et al[53]	1992	Not an original study; book chapter
Agostoni et al[54]	2008	Not an original study; review
Anderson et al[55]	1985	Letter to editor; no data provided
Ziegler et al[56]	2003	Preliminary data from Hummel et al 2007[21]
Carlson et al[57]	2006	Preliminary data from Ivarsson et al 2013[22]

References 40-57 available as Appendix 4 (available at www.jpeds.com).

Appendix 4

40. Radlovic NP, Mladenovic MM, Lekovic ZM, Radlovic VN. Influence of early feeding practices on celiac disease in infants. Croat Med J 2010;51: 417-22.
41. Pittschieler K, Gentili L, Niederhofer H. Onset of coeliac disease: a prospective longitudinal study. Acta Paediatr 2003;92:1149-52.
42. Persson LA, Ivarsson A, Hernell O. Breast-feeding protects against celiac disease in childhood–epidemiological evidence. Adv Exp Med Biol 2002;503:115-23.
43. Bouguerra F, Hajjem S, Guilloud-Bataille M, Khlat M, Khaldi F, Bennaceur B. Breast feeding effect relative to age of onset of celiac disease. Arch Pediatr 1998;5:621-6.
44. Lindberg T. Celiaki - vilken betydelse har tidpunkt och sätt för glutenintroduktion?. [Celiac disease - effect of time and mode of gluten introduction?] Scandinavian Journal of Nutrition 1996;40:123-4.
45. Ansaldi N, Tavassoli K, Dell'Olio D, Bramante L, Faussone D, Balocco L, et al. Clinical data on celiac disease with an early or late onset. Minerva Pediatr 1991;43:377-81.
46. Juto P. Delayed introduction of gluten in the diet is a possible cause of increased incidence of celiac disease. Lakartidningen 1990;87:4175-6.
47. Jos J, Rey J, Frézal J. Early effects of the reintroduction of gluten on the intestinal mucosa in celiac disease in remission. Arch Fr Pediatr 1969; 26:849-59.
48. Greco L, Mayer M, Grimaldi M, Follo D, De Ritis G, Auricchio S. The effect of early feeding on the onset of symptoms in celiac disease. J Pediatr Gastroenterol Nutr 1985;4:52-5.
49. Cataldo F, Maltese I, Paternostro D, Traverso G, Albeggiani A. Celiac disease and dietary habits in the 1st year of life. Minerva Pediatr 1991;43:7-10.
50. Matek Z, Jungvirth-Hegedus M, Kolacek S. Epidemiology of coeliac disease in children in one Croatian county: possible factors that could affect the incidence of coeliac disease and adherence to a gluten-free diet (Part II). Coll Antropol 2000;24:397-404.
51. D'Amico MA, Holmes J, Stavropoulos SN, Frederick M, Levy J, DeFelice AR, et al. Presentation of pediatric celiac disease in the United States: prominent effect of breastfeeding. Clin Pediatr (Phila) 2005;44: 249-58.
52. Mitt K, Uibo O. Low cereal intake in Estonian infants: the possible explanation for the low frequency of coeliac disease in Estonia. Eur J Clin Nutr 1998;52:85-8.
53. Mäki M, Holm K, Ascher H, Greco L. Factors affecting clinical presentation of coeliac disease: role of type and amount of gluten-containing cereals in the diet. In: Auricchio S, Visakorpi J, eds. Common food intolerances 1: epidemiology of coeliac disease; 1992;. p. 76-82. Karger, Basle.
54. Agostoni C, Shamir R. Can a change in policy of complementary infant feeding reduce the risk for type 1 diabetes and celiac disease? Pediatr Endocrinol Rev 2008;6:2-4.
55. Anderson CM, Brueton MJ. Does breast feeding protect against development of clinical symptoms of celiac disease in children? J Pediatr Gastroenterol Nutr 1985;4:507-8.
56. Ziegler AG, Schmid S, Huber D, Hummel M, Bonifacio E. Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. JAMA 2003;290:1721-8.
57. Carlsson A, Agardh D, Borulf S, Grodzinsky E, Axelsson I, Ivarsson SA. Prevalence of celiac disease:before and after a national change in feeding recommendations. Scand J Gastroenterol 2006;41:553-8.