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

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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

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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