Breast-feeding protects against celiac disease[1–3]

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ABSTRACT

Background: Celiac disease, or permanent gluten-sensitive enteropathy, is an immunologic disease strictly dependent on exposure to wheat gluten or related proteins in rye and barley.

Objective: The aim of this study was to explore whether breast-feeding and the mode of introducing dietary gluten influence the risk of celiac disease in childhood.

Design: A population-based incident case-referent study of Swedish children, 627 cases with celiac disease and 1254 referents, was conducted; 78% of the matched sets were included in the final analyses. A questionnaire was used to assess patterns of food introduction to infants. Models were built, based on current epidemiologic and immunologic knowledge of celiac disease, to study the potential influence of dietary patterns on disease risk and were evaluated by conditional logistic regression in multivariate analyses.

Results: The risk of celiac disease was reduced in children aged <2 y if they were still being breast-fed when dietary gluten was introduced [adjusted odds ratio (OR): 0.59; 95% CI: 0.42, 0.83]. This effect was even more pronounced in infants who continued to be breast-fed after dietary gluten was introduced (OR: 0.36; 95% CI: 0.26, 0.51). The risk was greater when gluten was introduced in the diet in large amounts (OR: 1.5; 95% CI: 1.1, 2.1) than when introduced in small or medium amounts. In older children, these risk factors were of no or only minor importance.

Conclusions: The gradual introduction of gluten-containing foods into the diet of infants while they are still being breast-fed reduces the risk of celiac disease in early childhood and probably also during the subsequent childhood period. *Am J Clin Nutr* 2002;75:914–21.

KEY WORDS Breast-feeding, case-referent study, celiac disease, childhood, diet, epidemiology, gluten, Sweden, prevention

INTRODUCTION

Celiac disease is recognized as a common but largely undiagnosed health problem in many European countries (1–4) and the United States (5, 6). This situation is challenging because untreated celiac disease is associated with high morbidity (7).

The etiology of celiac disease is not fully understood. Genetic susceptibility is a prerequisite, although the genes involved have not yet been identified (8). The condition is considered to be an immunologic disease, possibly of autoimmune type with tissue protein-glutamine γ-glutamyltransferase (transglutaminase) suggested as a major autoantigen (9). The result of the disease process on the small-intestinal mucosa (ie, villous atrophy), however, is strictly dependent on the individual's exposure to wheat gluten or related proteins in rye and barley, and efforts have been made to identify the precise structure of crucial epitopes in these cereals (10, 11).

In general, environmental exposures—including the dietary patterns of infants—might influence the immunologic process, resulting in tolerance or intolerance to a food constituent (12). There might be an age interval during which humans have a decreased ability to develop oral tolerance to a newly introduced antigen. The amount of antigen given might also influence the process. The type of food used could be important if the process of food preparation alters antigenicity (13). Furthermore, it is likely that the response of the immune system to an antigen may be modified by other exposures, eg, breast-feeding, because of its immune-modulating effect (14, 15).

The causal role of these different aspects of infant dietary patterns in the etiology of celiac disease has not been shown consistently (16–18). Sweden recently experienced an epidemic of symptomatic celiac disease in children aged <2 y, and we showed that both the abrupt increase and decline in the incidence of the disease coincided with changes in the dietary patterns of infants (19). During the peak years of the epidemic, we performed a population-based incident case-referent study to explore the risk factors for celiac disease. We analyzed whether the risk of developing celiac disease was affected by *1*) the age at which gluten was introduced in the diet, *2*) the amount of gluten introduced, 3) the type of gluten-containing foods introduced, and *4*) breast-feeding status (ie, whether the infant was still breast-feeding when dietary gluten was introduced).

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TABLE 1
Overview of participation for the celiac disease cases and referents in the 2 age groups

Criteria	Age group[1]			
	0–1.9 y		2.0–14.9 y	
	Cases	Referents	Cases	Referents
	n (%)			
Cases with a verified diagnosis of celiac disease and their referents[2]	475 (100)	950 (100)	152 (100)	304 (100)
Questionnaires				
Answered	455 (96)	856 (90)	146 (96)	268 (88)
Complete[3]	411 (86)	718 (76)	113 (74)	201 (66)
Part of a matched set of a case and 1 or 2 referents	392 (82)	626 (66)	99 (65)	155 (51)

[1] The age of the cases when the initial small-intestinal biopsy specimen was obtained.
[2] Criteria of the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition (20, 21).
[3] Information on both breast-feeding status and flour consumption.

SUBJECTS AND METHODS

Subjects

A prospective register of all incident cases of celiac disease in children aged <15 y was established in 1991. The surveillance covered 40% of the Swedish population in this age group, ie, 623 439 children (19). From 1 November 1992 to 30 April 1995, a total of 714 children with suspected celiac disease were reported to the register, of whom 665 (93%) had a characteristic small-intestinal mucosal abnormality on histologic examination of a biopsy specimen taken while they were consuming a gluten-containing diet, followed by clinical remission during a gluten-free diet (20). After further diagnostic measures (described below), celiac disease was confirmed in 627 children (94%). In the diagnostic evaluation, all information available through 31 December 1998 was considered. The age at diagnosis was defined as the age when the initial small-intestinal biopsy was obtained.

All of the initially invited children (n = 665) fulfilled the established diagnostic criteria for celiac disease (20); however, the diagnosis was ascertained further (21) by obtaining a biopsy specimen from 524 of the children after they had consumed a gluten-free diet; 509 of the children showed an improvement in the small-intestinal mucosa. Subsequently, 391 children were challenged with gluten, and a third biopsy specimen was taken; 368 of the children showed a deterioration of the mucosa. Thus, a total of 38 children were excluded from the study on the basis of these additional diagnostic measures because a longer follow-up would have been necessary to confirm a diagnosis of celiac disease. Accordingly, had all 665 children initially invited to participate had a second and third biopsy specimen taken, the diagnosis may have, theoretically, been questioned in another 3% (n = 19) of the children.

Two referents matched with each celiac disease case for date of birth, sex, and area of residence (n = 1254) were selected through the national population register. A questionnaire (*see* below) was answered by 601 cases (96%) and 1124 referents (90%). Complete information on key variables concerning infant feeding was available for 524 cases (84%) and 919 referents (73%); 491 cases and 781 referents were also part of matched sets, corresponding to 78% of the eligible case-referent triplets (**Table 1**).

The Swedish Data Inspection Board and the Research Ethics Committees of all Swedish Medical Faculties approved the study, and informed consent was obtained from the families.

Questionnaire

A questionnaire about the general health and diet of the participating children was mailed to the families; our special interest in celiac disease was not indicated. The questionnaire asked whether the child had ever been breast-fed, and if so, the age (in mo) at the last breast-feeding. The food-frequency component of the questionnaire, which contained semiquantitative information on portion sizes (3 levels) was used on the basis of experience from an earlier study (22). The pattern of introduction for each food item was assessed on the basis of the age (in mo) of the infant at the time when the first portion was given, on the size of the first portion, and on the average portion size and frequency 2 wk later. Dietary intake at 7 mo of age was assessed on the basis of the frequency of consumption and portion sizes of each food item.

Definitions

Breast-feeding duration was defined as the period of time when infants were exclusively or partially breast-fed. The age at which dietary gluten was first introduced was defined as the first month postpartum, during which time flour from wheat, rye, or barley was given to the infant. Breast-feeding status during introduction of gluten-containing flour was constructed from these variables and categorized into 3 groups: breast-feeding that discontinued during the month preceding introduction of gluten into the diet, breast-feeding that continued until the month when dietary gluten was introduced or even into the following month, and breast-feeding that continued even longer than that. Furthermore, the age at introduction of gluten-containing flour was regrouped into what was the nationally recommended age at introduction (ie, age 5–6 mo) and separate categories for earlier and later introduction. The types of foods used during introduction of flour into the diet were categorized into 2 groups: *1*) solid foods, including bread, biscuits, porridge, and pasta; and *2*) gluten-containing follow-up formula, used exclusively or in combination with solid foods. In Sweden, gluten-containing follow-up formula is widely used but only after the age of 6 mo. The amount of gluten-containing flour in home-prepared foods was calculated on the basis of standard Swedish recipes, and the amount in industrially produced foods was obtained from the manufacturers. The amount of gluten-containing flour consumed during introduction was assessed on the basis of the food item contributing the largest amount of flour during the first 2 wk of consump-

TABLE 2
Characteristics of celiac disease cases and referents

	Age group[1]			
	0–1.9 y		2.0–14.9 y	
Characteristics	Cases (n = 392)	Referents (n = 626)	Cases (n = 99)	Referents (n = 155)
Sex (%)				
Boys	33	34	42	41
Girls	67	66	58	59
Age at first symptoms (mo)[2]	11 (9.0, 13)[3]	—	26 (18, 65)	—
Age at diagnosis (mo)	14 (12, 18)	—	59 (32, 97)	—
Delay between diagnosis and return of questionnaire (mo)	2.2 (1.2, 3.7)	2.4 (1.3, 4.4)	2.9 (1.6, 4.5)	3.2 (1.7, 6.0)

[1] The age of the cases when the initial small-intestinal biopsy specimen was obtained.
[2] Information missing for 21 cases (5%) in the group aged 0–1.9 y and for 36 cases (36%) in the group aged 2.0–14.9 y.
[3] Median (25th and 75th percentiles).

tion, whereas the amount consumed at 7 mo of age was based on all food items consumed at that age. The amount of flour consumed in each feeding situation was initially divided into thirds on the basis of the distribution of flour intake by the referents. In the final analyses, the 2 lower thirds (ie small and medium amounts) were analyzed together because their risk estimates were comparable, whereas the upper third (ie, large amounts) was analyzed separately. This resulted in a cutoff of 7.0 g flour for the first portion of gluten-containing food introduced and in a cutoff of 16 g flour/d for the consumption of gluten-containing food consumed 2 wk after the first portion. At 7 mo of age, the analysis resulted in a cutoff of 58 g flour/d for the total amount of gluten-containing food consumed, in a cutoff of 39 g flour/d for solid foods, and in a cutoff of 17 g flour/d for follow-up formula.

Statistical analyses

Matched sets of cases and 1–2 referents were included in the final analyses if there was complete information on breast-feeding duration, age at introduction of flour into the diet, and the average daily amount of flour consumed 2 wk after the first portion was introduced.

The children were analyzed in 2 separate groups on the basis of their age at diagnosis of celiac disease (0–1.9 or 2.0–14.9 y), mainly because the epidemic of celiac disease only affected the younger age group. The basic characteristics and dietary patterns of these children as infants were described. Associations between covariates were evaluated, and stratified analyses were performed to identify possible effect-modification or confounding. Models were developed for the tentative influence of dietary patterns on the risk of celiac disease and were evaluated with the use of conditional logistic regression in bivariate and multivariate analyses. The population attributable fraction (AF_p) was estimated as follows:

$$AF_p = p_c\,(OR - 1)/OR \qquad (1)$$

where p_c is the prevalence of the studied exposure among the cases and OR is the adjusted odds ratio.

The possible effect of missing values was evaluated. Information on breast-feeding duration was missing for 1% of both cases and referents and was replaced by random draws among the observed values for cases and referents, respectively. Information on the age of the infants at the time dietary gluten was introduced in the diet was missing for 6% of the cases and for 10% of the referents, and the average daily amount of gluten-containing flour given 2 wk after the first portion was introduced was missing for 9% of the cases and for 15% of the referents. Based on a model by Rubin (23), 5 data sets were created by multiple imputations for these missing values. The final model for the influence of dietary patterns on the risk of celiac disease was assessed by analyzing each of the 5 data sets separately. The average of the 5 adjusted ORs and the 95% CIs are reported.

Version 9.0 of SPSS (24) was used for data summarization and the stratified analyses, EGRET (25) was used for the conditional logistic regression analyses, and SOLAS (26) was used to analyze the effect of missing values. Statistical significance was defined as a P value <0.05, or 95% CIs of ORs excluding 1.0.

RESULTS

Characteristics of the children

Celiac disease was diagnosed before 2 y of age in most of the cases (80%), and most of the cases were girls. The median delay between the onset of the first symptoms compatible with celiac disease and diagnosis was 3.0 mo in the group aged 0–1.9 y; however, a reliable estimate could not be made in the group aged 2.0–14.9 y (**Table 2**).

Validity of feeding information

The procedure by which cases were matched with referents on the basis of date of birth, sex, and area of residence had the potential of introducing selection bias; however, the dietary exposures did not differ significantly by sex or area of residence (data not shown). Compared with referents in the older age group, the younger age group had a longer breast-feeding duration and were introduced to dietary gluten at a later time (**Table 3**). This finding agrees with national trends in infant-feeding practices in Sweden (19). The analyses were stratified by age partly for this reason.

In the group aged 0–1.9 y, 96% of the cases and 90% of the referents answered the questionnaire (Table 1). Information on breast-feeding duration was reported for 99% of both groups. Information on the age at which dietary gluten was first introduced was available for 94% of the cases and for 90% of the referents. Information about the average daily amount of gluten-containing flour given 2 wk after the first portion was introduced was available for 91% of the cases and for 85% of the referents. The median breast-feeding duration was not significantly different between children with complete data and those with missing data for the variables described above.

TABLE 3
Dietary patterns during infancy for the celiac disease cases and referents

	Age group[1]					
	0–1.9 y			2.0–14.9 y		
Exposures	Cases (n = 392)	Referents (n = 626)	P[2]	Cases (n = 99)	Referents (n = 155)	P[2]
Breast-feeding duration (mo)	5 (3, 7)[3]	7 (4, 9)	<0.001	6 (4, 8)	6 (3, 9)	0.9
Breast-feeding status when flour was introduced (%)						
Discontinued	54	33		37	38	
Continued[4]	24	25		28	25	
Continued beyond[5]	22	42	<0.001	34	37	0.8
Age when flour was introduced (%)						
1–4 mo	8	11		11	15	
5–6 mo	82	73		73	73	
7–12 mo	10	16	0.002	16	12	0.5
Amount of flour introduced[6]						
First portion (%)						
Small to medium	60	64		70	69	
Large	40	36	0.2	30	31	0.9
Daily average amount of flour consumed 2 wk after flour was introduced (%)						
Small to medium	52	66		61	68	
Large	48	34	<0.001	39	32	0.2
Type of food given when flour was introduced (%)						
Solid foods	44	52		51	61	
Follow-up formula	56	48	0.02	49	39	0.09
Amount of flour given at 7 mo of age[6]						
Solid foods (%)						
Small to medium	70	68		75	64	
Large	30	32	0.4	25	36	0.06
Follow-up formula (%)						
Small to medium	43	68		58	61	
Large	57	32	<0.001	42	39	0.7
Total (%)						
Small to medium	53	67		64	62	
Large	47	33	<0.001	36	38	0.7

[1] The age of the cases when the initial small-intestinal biopsy specimen was obtained.
[2] The median test was used for breast-feeding duration; the chi-square test was used for all other exposures.
[3] Median (25th and 75th percentiles).
[4] Continued until the month flour was introduced or until the following month.
[5] Continued beyond the month after flour was introduced.
[6] Information on the amount of flour introduced was missing for 3 cases and 5 referents in the group aged 0–1.9 y and for 2 cases and 2 referents in the group aged 2.0–14.9 y. Information on the amount of flour consumed at 7 mo of age was missing for 16 cases and 39 referents in the group aged 0–1.9 y and for 6 cases and 9 referents in the group aged 2.0–14.9 y.

Infant feeding and risk of celiac disease

Dietary patterns in infancy differed significantly between cases diagnosed before 2 y of age and their referents (Table 3). Breast-feeding duration was significantly longer in the referents than in the cases, and more referents than cases were being breast-fed when dietary gluten was introduced and thereafter. Most children were introduced to flour at 5–6 mo of age, more so in the cases than in the referents. Cases received larger initial amounts of flour, assessed as the averaged daily amount consumed 2 wk after the first portion. Furthermore, more cases than referents had been given their first portion of flour as follow-up formula than as solid foods. At 7 mo of age, cases consumed larger amounts of flour than did referents, with follow-up formula being the major source.

Associations between the dietary exposures of the referents are shown in **Table 4**. Continued breast-feeding at the time gluten-containing flour was introduced was associated with the provision of small to medium amounts of gluten, whereas if breast-feeding had already been discontinued the amounts given at introduction ranged from small to large amounts. A small to medium amount of flour was usually given as solid foods, and a large amount was usually given as follow-up formula. A similar pattern of associations was found for the cases (data not shown). Stratified analyses showed no effect-modification (data not shown) but verified the above-described confounding. This was addressed by multivariate analyses.

The first multivariate model (model 1) was restricted to exposures characterizing introduction of gluten-containing flour into the diet (ie, the antigen) with respect to the age of the infant, the average daily amount consumed 2 wk after the first portion was introduced, and the type of food given (**Table 5**). The introduction of large amounts of flour was significantly associated with an increased risk of celiac disease. There was a significant bivariate association with higher risk of celiac disease if dietary gluten was introduced between 5 and 6 mo of age, but in this adjusted model

TABLE 4
Associations between the dietary exposures for the referents to the celiac disease cases aged <2 y

Exposures	Breast-feeding status when flour was introduced			Age when flour was introduced			Daily average amount of flour consumed 2 wk after flour was introduced	
	Discontinued	Continued[1]	Continued beyond[2]	1–4 mo	5–6 mo	7–12 mo	Small to medium	Large
	%			%			%	
Age when flour was introduced								
1–4 mo	10	10	11					
5–6 mo	77	73	70					
7–12 mo	13	17	19					
P[3]			0.4					
Daily average amount of flour consumed 2 wk after flour was introduced								
Small to medium	48	64	82	88	62	72		
Large	52	36	18	12	38	28		
P[3]			<0.001			<0.001		
Type of food given when flour was introduced								
Solid foods	40	41	67	89	48	45	66	24
Follow-up formula	60	59	33	11	52	55	34	76
P[3]			<0.001			<0.001		<0.001

[1] Continued until the month flour was introduced or until the following month.
[2] Continued beyond the month after flour was introduced.
[3] Chi-square test.

this association was no longer significant. The type of gluten-containing food given was not an independent risk factor.

In the next model (model 2), breast-feeding status at the time flour was introduced in the diet was added because of its immune-modulating potential (Table 5). Continued breast-feeding at the time gluten-containing flour was introduced had a protective effect against celiac disease. The risk pattern was similar to that with model 1, and the risk estimates changed only slightly.

The final model (model 3) included all variables of potential importance (Table 5). A protective effect was found if the infant was still being breast-fed when gluten-containing foods were introduced (OR: 0.59; 95% CI: 0.42, 0.83), and this effect was

TABLE 5
Dietary patterns during infancy and the risk of celiac disease before 2 y of age[1]

Exposures	Bivariate analyses	Multivariate analyses			
		Model 1[2]	Model 2	Model 3 (final)	Model 4[3]
Breast-feeding status when flour was introduced					
Discontinued	1.0		1.0	1.0	1.0
Continued[4]	0.55 (0.40, 0.77)		0.59 (0.42, 0.85)	0.59 (0.42, 0.83)	0.63 (0.46, 0.86)
Continued beyond[5]	0.30 (0.22, 0.42)		0.35 (0.25, 0.50)	0.36 (0.26, 0.51)	0.35 (0.25, 0.48)
Age when flour was introduced					
1–4 mo	1.0	1.0	1.0	1.0	1.0
5–6 mo	1.8 (1.1, 2.9)	1.5 (0.93, 2.5)	1.5 (0.89, 2.5)	1.4 (0.87, 2.4)	1.2 (0.76, 1.9)
7–12 mo	0.86 (0.48, 1.6)	0.74 (0.40, 1.4)	0.79 (0.43, 1.5)	0.76 (0.41, 1.4)	0.71 (0.41, 1.3)
Daily average amount of flour consumed 2 wk after flour was introduced					
Small to medium	1.0	1.0	1.0	1.0	1.0
Large	2.0 (1.5, 2.7)	1.9 (1.4, 2.6)	1.6 (1.1, 2.3)	1.5 (1.1, 2.1)	1.4 (1.1, 1.9)
Type of food given when flour was introduced					
Solid foods	1.0	1.0	1.0		
Follow-up formula	1.4 (1.1, 1.9)	1.0 (0.75, 1.4)	0.89 (0.64, 1.2)		

[1] Conditional logistic regression using 392 matched sets of cases and referents. Values are odds ratios (OR) (and 95% CIs).
[2] Model 1: likelihood ratio statistics (LRS) on 4 df = 37, $P < 0.001$; model 2: LRS on 6 df = 76, $P < 0.001$; model 3: LRS on 5 df = 76, $P < 0.001$.
[3] Model 4 was based on 447 matched sets of cases and referents with missing values handled by a multiple imputation procedure; for details *see* Subjects and Methods.
[4] Continued until the month flour was introduced or until the following month.
[5] Continued beyond the month after flour was introduced.

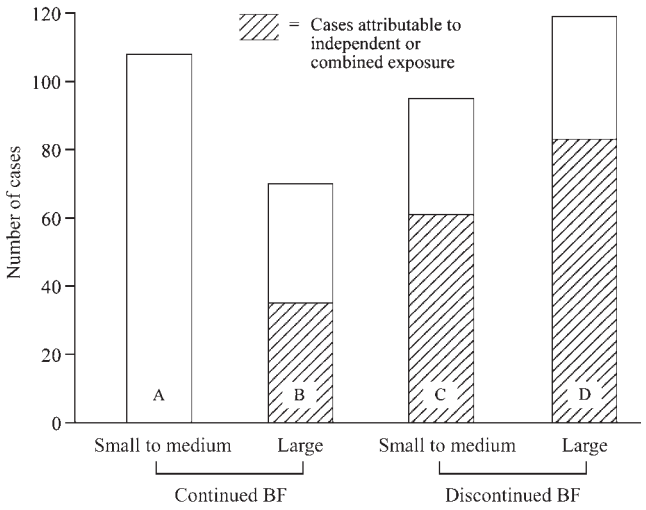

FIGURE 1. The number of celiac disease cases aged <2 y with respect to breast-feeding (BF) status (continued or discontinued) at the time gluten-containing flour was introduced into their diets, the amount of flour given (small to medium or large amounts), and the number of cases attributable to the independent or combined effect of these exposures. Risk estimates were based on conditional logistic regression with 392 matched sets of cases and referents and were adjusted for the age of the infant at the time flour was introduced in the diet. The ORs (and 95% CIs) were as follows: A, 1.0; B, 2.0 (1.4, 3.0); C, 2.8 (1.9, 4.0); and D, 3.3 (2.3, 4.8).

even more pronounced if breast-feeding continued beyond the time of gluten introduction (OR: 0.36; 95% CI: 0.26, 0.51). Furthermore, there was a greater risk of celiac disease associated with a large average daily consumption of flour 2 wk after the first portion was introduced (OR: 1.5; 95% CI: 1.1, 2.1) than with consumption of a small to medium amount. The risk estimates were largely unchanged after adjustment for the amount of flour consumed at 7 mo of age (data not shown).

Model 4 was developed by reanalyzing the data from model 3 with a multiple imputation procedure to account for missing values (Table 5). The risk estimates were only slightly different from those with model 3, and the conclusions did not change.

In the older age group, no significant differences between the cases and referents were found with respect to the discussed dietary variables. Furthermore, the final model, as described above for the younger children (model 3), was not significant (model likelihood ratio statistics on 5 df = 4.4, $P = 0.5$).

Public health effect

AF_p was estimated as 31% for the independent effect of discontinued breast-feeding at the time flour was first introduced into the diet compared with continued breast-feeding at the time flour was introduced. The AF_p for introduction of flour in large compared with small to medium amounts was estimated at 16%. The combined effect resulted in an AF_p of 45%, suggesting that almost one-half of the cases would have been spared had the infant-feeding practices been as favorable as possible according to the results obtained in the present study (**Figure 1**).

DISCUSSION

A major finding of this study was the lower risk of celiac disease in infants who were still being breast-fed than in infants who had discontinued breast-feeding at the time when gluten-containing foods were introduced into the diet. The risk was even lower in those infants who continued breast-feeding also beyond the time at which gluten was introduced. Larger amounts of gluten at the time of gluten introduction increased the risk, but for any given amount the type of food given was not important. The effect of age at the time of gluten introduction on the risk of celiac disease was not conclusive.

This is the first study of the association between celiac disease and environmental risk factors in which an attempt was made to develop an analytic model for causality based on immunologic and epidemiologic knowledge. Multivariate analyses were used to adjust risk estimates for confounding and to suggest causal relations. A multiple imputation approach was used to assess the possible effect of missing data on the risk estimates, and the conclusion was that missing data had no significant effect. Because the study was population based and had a high participation rate, we are confident that the results represent the country of Sweden at large. Although the use of gluten-containing follow-up formula is typical in Sweden, the findings should be relevant to any country because the type of food used could be excluded as an independent risk factor.

Moreover, the accuracy of the diagnosis of celiac disease was ascertained by well-established criteria (20, 21); however, a small number of cases still may have been misclassified, resulting in a marginal underestimation of risks. Referents were chosen from the same population that generated the cases and were matched to the cases for date of birth and sex to decrease confounding and for area of residence to make another substudy feasible. Because dietary exposures did not differ significantly on the basis of sex or area of residence, no selection bias was introduced. To reduce differential misclassification of exposure, the study protocol was introduced to the participants without explicitly mentioning the focus on celiac disease. Only incident cases were included to reduce the recall period; however, inevitably, this was longer for older children. Moreover, the relatively few participants in the older age group reduced the statistical power.

It was suggested as early as the 1950s that breast-fed infants have a later onset of celiac disease (27), and this view was later shared by others (28, 29). Furthermore, it was shown in case-referent studies based on prevalent cases that children with celiac disease had been breast-fed for a significantly shorter duration than had referents (30–32). However, the question was raised of whether the association of celiac disease with breast-feeding is direct and causal or indirect through postponed introduction of infant formula (30) or a reduced amount of dietary gluten consumed (32).

In the present study, we showed that breast-feeding had an independent protective effect against celiac disease if the infants were breast-fed at the time when gluten-containing foods were introduced. This effect was even more pronounced in infants who continued to be breast-fed also beyond the time of gluten introduction. Confounding by other known dietary factors was unlikely because the risk estimates were influenced only slightly by adjustments for covariates, eg, the amount of gluten consumed. Furthermore, note that our study was performed in a population in which most infants were breast-fed for ≥6 mo. Thus, most of the infants were introduced to cow milk while they were still being breast-fed, and consequently through sources other than infant formula. Furthermore, the cessation of breast-feeding did not usually coincide with the introduction of infant formula. It is also biologically likely that the presence of breast milk at the

time gluten is introduced increases the chance of developing oral tolerance for the antigens of importance (14, 15).

A larger consumption of wheat gluten was reported in healthy infants in Sweden and Italy than in infants in Finland, Denmark, and Estonia, and the former countries reported a higher occurrence of celiac disease than did the latter country (17, 33, 34). In addition, a dose-related response on the small-intestinal mucosa was noted when individuals were given increasing amounts of dietary gluten (35–37). Our study showed that introduction of gluten-containing foods in large amounts rather than in small to medium amounts, was an independent risk factor. The cases consumed larger amounts of flour than did the referents also at 7 mo of age.

In most countries, the recommended age for introducing gluten-containing foods has gradually increased over time to delay the manifestations of celiac disease and possibly also to reduce the risk of disease (38). Previous case-referent studies reported that the age of the infant at the time dietary gluten is introduced has no bearing on the risk of celiac disease (30–32). In our study the bivariate association between age at introduction of dietary gluten and the risk of celiac disease was not significant in the adjusted models. Thus, it is still unclear whether the ability to develop oral tolerance to gluten is decreased if the antigen is introduced into the diet during a certain period in infancy.

During the Swedish epidemic of celiac disease in children aged <2 y, the incidence increased 3-fold to levels that were higher than previously reported from any country, followed by an abrupt decline to the preceding level (19). Using an ecologic study approach, we found that the increase in incidence was preceded by an increase in the amount of gluten consumed, by a postponement of dietary gluten introduction, and by no change in breast-feeding habits. The decrease in incidence was preceded by several years of an increasing average breast-feeding duration and coincided with a decrease in the amount of gluten consumed. Thus, both the increase and decrease in incidence were most likely accompanied by a change in the proportion of infants introduced to gluten in small amounts while the infants were still being breast-fed. In other words, and on the basis of the findings in the present study, Swedish infant-feeding practices have shifted over time from a favorable to an unfavorable pattern and back to a favorable pattern. On the basis of the AF_p, about one-half of the cases during the epidemic might have been spared celiac disease if the favorable dietary pattern had been followed.

It has been suggested that environmental exposures, including the dietary patterns of infants, merely affect the clinical expression of celiac disease and not the process resulting in the small-intestinal celiac lesion (18). However, there is increasing evidence to suggest that the etiology of celiac disease is multifactorial, both with regard to genetic (8) and environmental (39–41) factors. Furthermore, the immune-modulating potential of breast milk is supported by studies of the composition of breast milk (14, 15) and by the increasing number of epidemiologic studies suggesting an inverse association between breast-feeding and the risk of autoimmune diseases, such as type 1 diabetes and inflammatory bowel disease (15, 42), and of other diseases, such as leukemia (43). Thus, it seems likely that the dietary patterns of infants, in addition to the mere presence of gluten in the diet, interact with an individual's genetics, resulting in an immunologic process that may or may not result in the small-intestinal celiac lesion.

An important question is whether favorable infant dietary patterns postpone the onset of celiac disease until later in life, or in fact reduce the overall lifetime risk of the disease. In the present study, the dietary patterns of the infants had an effect on the risk of celiac disease in the children aged <2 y but had no or only a minor effect on risk in the older children. Thus, introduction of small to medium amounts of gluten-containing foods in the diet of infants while they are still being breast-fed clearly reduces the risk of celiac disease in early childhood and probably also reduces the risk during the remaining childhood period. It is tempting to speculate that this dietary pattern also reduces the lifetime risk of celiac disease; however, further studies are needed to confirm this notion.

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REFERENCES

1. Grodzinsky E, Franzen L, Hed J, Ström M. High prevalence of celiac disease in healthy adults revealed by antigliadin antibodies. Ann Allergy 1992;69:66–70.
2. Catassi C, Rätsch I-M, Fabiani E, et al. Coeliac disease in the year 2000: exploring the iceberg. Lancet 1994;343:200–3.
3. Johnston SD, Watson RGP, McMillan SA, Sloan J, Love AHG. Coeliac disease detected by screening is not silent—simply unrecognized. QJM 1998;91:853–60.
4. Ivarsson A, Persson LA, Juto P, Peltonen M, Suhr O, Hernell O. High prevalence of undiagnosed coeliac disease in adults: a Swedish population-based study. J Intern Med 1999;245:63–8.
5. Not T, Horvath K, Hill ID, et al. Celiac disease risk in the USA: high prevalence of antiendomysium antibodies in healthy blood donors. Scand J Gastroenterol 1998;33:494–8.
6. Hill I, Fasano A, Schwartz R, Counts D, Glock M, Horvath K. The prevalence of celiac disease in at-risk groups of children in the United States. J Pediatr 2000;136:86–90.
7. Murray JA. The widening spectrum of celiac disease. Am J Clin Nutr 1999;69:354–65.
8. Sollid LM. Molecular basis of celiac disease. Annu Rev Immunol 2000;18:53–81.
9. Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997;3:797–801.
10. Arentz-Hansen H, Körner R, Molberg O, et al. The intestinal T cell response to alpha-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. J Exp Med 2000;191:603–12.
11. Anderson RP, Degano P, Godkin AJ, Jewell DP, Hill AV. In vivo antigen challenge in celiac disease identifies a single transglutaminase-modified peptide as the dominant A-gliadin T-cell epitope. Nat Med 2000;6:337–42.
12. Strobel S, Mowat AM. Immune responses to dietary antigens: oral tolerance. Immunol Today 1998;19:173–81.
13. Sjöström H, Lundin KEA, Molberg O, et al. Identification of a gliadin T-cell epitope in coeliac disease: general importance of gliadin deamidation for intestinal T-cell recognition. Scand J Immunol 1998;48:111–5.
14. Goldman AS. Modulation of the gastrointestinal tract of infants by human milk. Interfaces and interactions. An evolutionary perspective. J Nutr 2000;130(suppl):426S–31S.

15. Hanson LA. Breastfeeding provides passive and likely long-lasting active immunity. Ann Allergy Asthma Immunol 1998;81:523–37.
16. Logan RFA. Epidemiology of coeliac disease. In: Marsh MN, eds. Coeliac disease. Oxford, United Kingdom: Blackwell Scientific Publications, 1992:192–214.
17. 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 JK, eds. Common food intolerances. 1: Epidemiology of coeliac disease. Basel, Switzerland: Karger, 1992:76–82.
18. Ascher H. The role of quantity and quality of gluten-containing cereals in the epidemiology of coeliac disease. In: Mäki M, Collin P, Visakorpi JK, eds. Coeliac disease. Proceedings of the seventh international symposium on coeliac disease. Vammala, Finland: Vammalan Kirjapaino Oy, 1997:15–22.
19. Ivarsson A, Persson LÅ, Nyström L, et al. Epidemic of coeliac disease in Swedish children. Acta Paediatr 2000;89:165–71.
20. Walker-Smith JA, Guandalini S, Schmitz J, Shmerling DH, Visakorpi JK. Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. Arch Dis Child 1990;65:909–11.
21. Meeuwisse GW. Diagnostic criteria in coeliac disease. European Society for Paediatric Gastroenterology, Interlaken, 1969. Acta Paediatr Scand 1970;59:461–3.
22. Persson LÅ, Johansson E, Samuelson G. Dietary intake of weaned infants in a Swedish community. Hum Nutr Appl Nutr 1984;38:247–54.
23. Rubin DB. Multiple imputation after 18+ years. J Am Stat Assoc 1996;91:473–89.
24. SPSS Inc. Statistical package for the social sciences, version 9.0. Chicago: SPSS Inc, 1999.
25. Cytel Software Corporation. EGRET for Windows. Epidemiological graphics, estimation and testing package. Cambridge, MA: Cytel Software Corporation, 1999.
26. Statistical Solutions Ltd. SOLAS for missing data analysis 1.0. Cork, Ireland: Statistical Solutions Ltd, 1997.
27. Andersen DH, Di Sant'Agnese PA. Idiopathic celiac disease: I. Mode of onset and diagnosis. Pediatrics 1953;11:207–22.
28. 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.
29. Mäki M, Kallonen K, Lähdeaho ML, Visakorpi JK. Changing pattern of childhood coeliac disease in Finland. Acta Paediatr Scand 1988;77:408–12.
30. Auricchio S, Follo D, De Ritis G, 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.
31. 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.
32. 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.
33. Weile B, Cavell B, Nivenius K, Krasilnikoff PA. Striking differences in the incidence of childhood celiac disease between Denmark and Sweden: a plausible explanation. J Pediatr Gastroenterol Nutr 1995;21:64–8.
34. 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.
35. Doherty M, Barry RE. Gluten-induced mucosal changes in subjects without overt small-bowel disease. Lancet 1981;1:517–20.
36. Ferguson A, Blackwell JN, Barnetson RS. Effects of additional dietary gluten on the small intestinal mucosa of volunteers and of patients with dermatitis herpetiformis. Scand J Gastroenterol 1987;22:543–9.
37. Catassi C, Rossini M, Ratsch IM, et al. Dose dependent effects of protracted ingestion of small amounts of gliadin in coeliac disease children: a clinical and jejunal morphometric study. Gut 1993;34:1515–9.
38. ESPGAN committee on nutrition. Guidelines on infant nutrition. III. Recommendations for infant feeding. Acta Paediatr Scand Suppl 1982;302:16–20.
39. Logan RFA, Rifkind EA, Busuttil A, Gilmour HM, Ferguson A. Prevalence and "incidence" of celiac disease in Edinburgh and the Lothian region of Scotland. Gastroenterology 1986;90:334–42.
40. Ferguson A. New perspectives of the pathogenesis of coeliac disease: evolution of a working clinical definition. J Intern Med 1996;240:315–8 (editorial).
41. Ivarsson A, Persson LÅ, Hernell O. Does breast-feeding affect the risk for coeliac disease? In: Koletzko B, Michaelsen KF, Hernell O, eds. Short and long term effects of breast feeding on child health. New York: Kluwer Academic/Plenum Publishers, 2000:139–49.
42. Villalpando S, Hamosh M. Early and late effects of breast-feeding: does breast-feeding really matter? Biol Neonate 1998;74:177–91.
43. Shu XO, Linet MS, Steinbuch M, et al. Breast-feeding and risk of childhood acute leukemia. J Natl Cancer Inst 1999;91:1765–72.