Timing of Initial Cereal Exposure in Infancy and Risk of Islet Autoimmunity

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Context Dietary exposures in infancy have been implicated, albeit inconsistently, in the etiology of type 1 diabetes mellitus (DM).

Objective To examine the association between cereal exposures in the infant diet and appearance of islet autoimmunity (IA).

Design Birth cohort study conducted from 1994 to 2002 with a mean follow-up of 4 years.

Setting Newborn screening for HLA was done at St Joseph's Hospital in Denver, Colo. First-degree relatives of type 1 DM individuals were recruited from the Denver metropolitan area.

Participants We enrolled 1183 children at increased type 1 DM risk, defined as either HLA genotype or having a first-degree relative with type 1 DM, at birth and followed them prospectively. We obtained exposure and outcome measures for 76% of enrolled children. Participants had variable lengths of follow-up (9 months to 9 years).

Main Outcome Measures Blood draws for the detection of insulin autoantibody, glutamic acid decarboxylase autoantibody, or IA-2 autoantibody were performed at 9, 15, and 24 months and annually thereafter. Children with IA (n=34) were defined as those testing positive for at least 1 of the autoantibodies on 2 or more consecutive visits and who tested positive or had diabetes on their most recent visit.

Results Children initially exposed to cereals between ages 0 and 3 months (hazard ratio [HR], 4.32; 95% confidence interval [CI], 2.0-9.35) and those who were exposed at 7 months or older (HR, 5.36; 95% CI, 2.08-13.8) had increased hazard of IA compared with those who were exposed during the fourth through sixth month, after adjustment for HLA genotype, family history of type 1 DM, ethnicity, and maternal age. In children who were positive for the HLA-DRB1*03/04,DQB8 genotype, adjusted HRs were 5.55 (95% CI, 1.92-16.03) and 12.53 (95% CI, 3.19-49.23) for initial cereal exposure between ages 0 to 3 months and at 7 months or older, respectively.

Conclusion There may be a window of exposure to cereals in infancy outside which initial exposure increases IA risk in susceptible children.

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TYPE 1 DIABETES MELLITUS (DM) results from the destruction of the insulin-producing cells of the pancreas. Autoantibodies to the islet cells, or islet autoimmunity (IA), which mark this destructive process, can be present for years prior to the diagnosis of type 1 DM. Exposures in the infant diet have been implicated, albeit inconsistently, in the etiology of type 1 DM.[1] Of 4 cohort studies examining IA as the outcome, 3 showed no effect of the duration of exclusive breastfeeding[2,3] or age at exposure to cow's milk[2,4] on the risk of IA, and 1 showed that short-term exclusive breastfeeding and early exposure to cow's milk increased risk for IA.[5] One explanation for these discrepant findings is that exposure to cow's milk may be correlated with the actual diabetogenic exposure in some populations but not in others.

Studies of other foods in the infant diet also have been contradictory. While 2 studies found that type 1 DM cases had been exposed to solid foods earlier than controls,[6,7] 2 studies found no association,[8,9] and 1 study reported that type 1 DM cases had been exposed to solid foods later than controls.[10] Finally, exposure to cereals before age 6 months was not associated with IA in a study of children with a family history of type 1 DM.[2]

See also pp 1721 and 1771 and Patient Page.

While the definition of solid foods differs from country to country, cereal is one of the first solid foods to be introduced into the infant diet in the United States.[11] The purpose of this prospective study was to examine the association between exposures to cereals and cow's milk in the infant diet and appearance of islet autoantibodies in a birth cohort of children at increased risk of type 1 DM based on HLA genotype and family history of type 1 DM.

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METHODS

Study Population

Since 1994, the Diabetes Autoimmunity Study in the Young (DAISY) has been investigating the natural history of IA in infants and children who are at moderate to high risk of developing type 1 DM.[12] Newborns at increased risk for type 1 DM were identified from those born at St Joseph's Hospital in Denver, Colo, by screening umbilical cord blood samples for diabetes susceptibility alleles in the HLA region. The St Joseph's Hospital newborn population is representative of the general population of the Denver metropolitan area. We did not recruit families in which parents had difficulties understanding English, or whose newborn was in very poor health, due to either being extremely premature or having severe congenital malformation or disease, as assessed by hospital personnel. Of the families approached, 86% gave written informed consent to the genetic screening. HLA screening has been completed on more than 27 000 newborns; the details of the newborn screening have been published elsewhere.[12] Based on their HLA genotype, newborns were categorized into 3 risk groups determined by the odds of developing type 1 DM by the age of 20 years: high, odds of developing type 1 DM by the age of 20 years, 1:16; moderate, 1:75 in non-Hispanic whites or 1:230 in Hispanics; or low, less than 1:300. All newborns found to be at high risk and a sample of those found to be at moderate risk were asked to participate in the follow-up.

In addition to the HLA-screened population, newborns were recruited (January 1994 and ongoing) from families with type 1 DM using The Barbara Davis Center for Childhood Diabetes in Denver, Colo, other diabetes care clinics, the Colorado IDDM (insulin-dependent DM) Registry,[13] and newspaper publicity. We obtained prospective exposure and outcome data on 76% of the recruited infants. The study population consists of 841 HLA-screened children without a family history of type 1 DM and 342 children with a first-degree relative (parent or sibling) with type 1 DM. For the analysis herein, the study dates were 1994 to 2002.

In the DAISY follow-up, all children were tested at 9, 15, and 24 months of age and annually thereafter for antibodies to pancreatic islet antigens (as described below). Children who are autoantibody positive were placed on an accelerated schedule in which they returned for a blood draw every 3 to 6 months. Children in this cohort were followed up from birth to a mean of 4.0 years (range, 9 months to 9 years of age). Written informed consent was obtained from the parents of each study participant at genetic screening (for the HLA-screened population) and again at enrollment in the DAISY follow-up. The Colorado multiple institutional review board approved all study protocols.

The majority of the cohort (71.0%, n=837) was non-Hispanic white. The remaining 346 children were divided between the following ethnic/racial groups: Hispanic (n=268, 77%), biracial (n=41, 12%), African American (n=26, 8%), Asian (n=5, 1%), Native American (n=1, 0.3%), and not specified (n=5, 1%). One sibling from 989 families, 2 siblings from 91 families, and 3 siblings from 4 families comprised the birth cohort of 1183 children.

Measurement of Autoantibodies

Insulin autoantibodies were measured by a micro-insulin autoantibody (IAA) assay with sensitivity of 58%, specificity of 99%, and interassay coefficient of variation of 11%.[14] The combined glutamic acid decarboxylase 65 (GAD_{65}) and IA-2 autoantibody radioassay was performed in duplicate using methods described previously.[15] The levels of both antibodies are expressed as an index=(sample cpm [counts per minute]−negative control cpm)/(positive control cpm−negative control cpm). In the 1995 Immunology of Diabetes Society Workshop, the GAD autoantibody (GAA) assay showed an 82% sensitivity and 99% specificity using sera from patients with new-onset DM who were younger than 30 years. The interassay coefficient of variation was 6%. The IA-2 assay showed a 73% sensitivity and 100% specificity and the interassay coefficient of variation was 10%.[15] Based on 198 controls without DM, aged 0.4 to 67.5 years, the 99th percentile for IAA (0.01) and GAA (0.032) and the 100th percentile (single highest value) for IA-2 (0.07) were used as the cutoffs for positivity. All serum samples that were positive for IAA, GAA, or IA-2 and a random 10% of the remaining samples were retested in a blinded manner for confirmation.

Persistent IA confers a high risk of subsequent development of type 1 DM in relatives[16] and the general population.[17] Therefore, we defined a case of IA as a child who had at least 1 autoantibody (IAA, GAA, or IA-2) above the 99th percentile on 2 or more consecutive visits and who remained positive for the autoantibody at their most recent visit (n=34); that is, children who subsequently have tested negative for the autoantibody were not included in this case definition. This definition excluded positivity due to transplacental transmission of autoantibodies, which we defined as positivity at the 9-month blood draw and negative on all subsequent visits. As of July 3, 2003, 16 of the 34 children who met this definition have converted to type 1 DM.

Dietary Measurement

Data for infant diet were collected during telephone or face-to-face interviews at 3, 6, 9, 12, and 15 months of age. At each interview, mothers were asked to report the date of introduction and frequency of exposure (ie, number of servings per day) of all milks, formulas, and foods that the infants consumed during the previous 3 months. The type and brand name of infant formulas and the types of cereal were recorded. In addition, juice, fruit, vegetables, meat, breads, other dairy products, eggs, sweets, and snack foods were recorded separately. Breastfeeding initiation and termination also were recorded.

The exposure to cow's milk was defined as intake of any formulas, milks, or foods containing cow's milk, yogurt,

cheese, or milk products of any kind. The cereal definition included both gluten-containing and gluten-free (rice) cereals. Gluten exposure was defined as an intake of foods containing oats, wheat, barley, or rye, including infant cereals, zwieback, breads, crackers, tortillas, teething biscuits, cookies, cakes, pretzels, and pasta. Rice exposure was defined as an intake of foods containing rice, such as infant rice cereal, boiled rice, rice milk, rice cakes, or rice noodles. We chose the age at initial exposure of 4 to 6 months as the reference group for the current analyses because US pediatricians generally recommend the introduction of solid foods, and in particular cereals, between the ages of 4 and 6 months, although no official American Academy of Pediatrics practice guideline exists regarding this practice.[11] The study was a purely observational study. No dietary advice was given to the participating families.

Analyses

All analyses were performed in SAS version 8 (SAS Institute Inc, Cary, NC). Pearson correlation coefficients were used to examine the correlation between the timing of infant diet exposures. Ongoing recruitment since 1994 and continuing follow-up have resulted in variable lengths of follow-up for the children in DAISY, producing right-censored data. Some of the affected children were positive for autoantibodies on their first blood draw (at approximately 9 months of age), producing left-censored data. Finally, we have interval-censored data in that we know only the time of the last negative and first positive autoantibody blood draw, rather than the actual time of conversion to autoantibody positivity. Therefore, all unadjusted and adjusted hazard ratios (HRs) were estimated using survival analysis (SAS Proc Lifereg) with the Weibull distribution, and accounting for right, left, and interval censoring.[18] Calculations of follow-up time began at birth. The variables, HLA risk-group and family history of type 1 DM, were included in our multivariate models to account for the fact that our cohort was selected based on these risk characteristics.

To determine whether inclusion of multiple siblings per family in this cohort affected our findings, we randomly selected 1 child per family, resulting in the exclusion of 100 children, and performed the analyses again. The results were the same as those from the full cohort; therefore, we are presenting the data from the full cohort of 1183 children. The level of significance was set at .05.

Table 1. Descriptive Characteristics of Study Cohort of the Diabetes Autoimmunity Study in the Young, 1994-2002

Characteristic	Affected With IA (n = 34)	Unaffected (n = 1149)	Unadjusted Hazard Ratio (95% Confidence Interval)
Gestational age, mean (SD), wk	39.0 (2.9)	39.5 (1.9)	0.91 (0.80-1.05)
Age, mean (SD), y*	2.2 (1.5)	3.9 (2.1)	NA
Non-Hispanic white, No. (%)†	31 (91.1)	806 (70.1)	3.83 (1.17-12.5)
Male, No. (%)	18 (52.9)	604 (52.5)	1.00 (0.51-1.95)
HLA-DRB1*03/04,DQB8, No. (%)	18 (52.9)	303 (26.4)	3.17 (1.62-6.22)
First-degree relative with type 1 DM, No. (%)	22 (64.8)	320 (27.8)	5.00 (2.47-10.11)
≤12 Years maternal education, No. (%)‡	10 (30.4)	289 (27.1)	1.30 (0.62-2.72)
Maternal age at birth, mean (SD), y	31.6 (6.6)	29.6 (5.7)	1.06 (0.99-1.12)
Percentage of expected weight at first visit§	95.7	96.6	0.99 (0.96-1.02)

Abbreviations: DM, diabetes mellitus; IA, islet autoimmunity; NA, not applicable.
*For children affected with IA, age is age at visit when they first tested positive for autoantibodies. For children not affected with IA, age is age at last visit.
†Four unaffected children (0.3%) were missing ethnicity information.
‡One affected child (2.9%) and 81 unaffected children (7.0%) were missing maternal education information.
§First visit occurred between 9 and 15 months of age. Expected weight based on national statistics and child's age at first visit.

RESULTS

Dietary and Descriptive Characteristics

Of the 1183 children, 85% (n=1001) were breastfed. We examined which foods infants in our cohort first were exposed to after breast milk, if applicable. After breast milk, milks and foods containing cow's milk and cereals were the first exposures in the diet in 55% and 15% of the children, respectively, and in another 6%, exposure to cow's milk and cereals occurred at the same time. Soy-based infant formulas were the first exposure in 13% of the children, with another 4% exposed to soy formula and cow's milk (formulas) at the same time. In only 7% of the children did their first food not contain either cow's milk, cereal, or soy formula. These other foods included hydrolyzed protein formulas, fruit juices, and fruits.

Breastfeeding duration was correlated with age at first exposure to cow's milk ($r=0.33$; $P<.001$), to all cereals ($r=0.27$; $P<.001$), to rice cereals ($r=0.25$; $P<.001$), and minimally, to gluten-containing cereals ($r=0.09$; $P=.003$). In addition, the age at first exposure to cow's milk was correlated with age at first exposure to all cereals ($r=0.22$; $P<.001$), to rice cereals ($r=0.20$; $P<.001$), and to a lesser extent to gluten-containing cereals ($r=0.14$; $P<.001$). Finally, age at first exposure to gluten-containing cereals was correlated with that of rice cereals ($r=0.26$; $P<.001$).

The mean (SD) age at first visit with a positive autoantibody result for the 34 affected children was 2.2 (1.5) years, and the mean (SD) age at the last follow-up for the 1149 unaffected children in the cohort was 3.9 (2.1) years (TABLE 1). Affected children compared with unaffected children were more likely to be non-Hispanic white (91% vs 70%), positive for HLA-DRB1*03/04,DQB8 genotype (53% vs 26%), and have a first-degree relative with type 1 DM (65% vs 28%).

Infant Diet Exposures and Hazard of IA

In unadjusted survival analyses, children initially exposed to any cereals before 4

Table 2. Infant Diet Characteristics of Study Cohort

Characteristic	Affected With IA (n = 34)	Unaffected (n = 1149)	Unadjusted Hazard Ratio (95% Confidence Interval)
Age at any cereal exposure, No. (%), mo*			
1-3	13 (38.2)	246 (21.5)	3.03 (1.42-6.44)
4-6	14 (41.2)	804 (70.0)	1.00
≥7	7 (20.5)	98 (8.5)	3.86 (1.56-9.56)
Age at rice cereal exposure, No. (%), mo†			
1-3	12 (35.2)	225 (19.5)	2.74 (1.28-5.85)
4-6	15 (44.1)	770 (67.0)	1.00
≥7	7 (20.5)	154 (13.5)	2.31 (0.94-5.68)
Age at gluten-containing cereal exposure, No. (%), mo‡			
1-3	4 (11.8)	78 (6.7)	2.76 (0.88-8.66)
4-6	11 (32.3)	586 (51.1)	1.00
≥7	19 (55.8)	485 (42.2)	1.95 (0.93-4.11)
Age at cow's milk exposure, No. (%), mo§			
1-3	19 (55.8)	706 (61.4)	0.77 (0.31-1.92)
4-6	6 (17.6)	163 (14.1)	1.00
≥7	9 (26.5)	280 (24.3)	0.89 (0.32-2.49)
Breastfeeding duration, mean, mo	5.86	6.08	0.99 (0.94-1.04)
Breastfed when first exposed to cow's milk, No. (%)	23 (67.6)	710 (61.7)	1.23 (0.60-2.53)
Breastfed when first exposed to cereals, No. (%)	15 (44.1)	585 (50.9)	0.74 (0.37-1.45)

*Any cereal exposure includes intake of cereals, foods, and milks containing rice, oats, wheat, barley, and rye.
†Rice cereal exposure includes intake of any infant cereals, foods, and milks containing rice.
‡Gluten-containing cereal exposure includes intake of infant cereals and foods containing oats, wheat, barley, and rye.
§Cow's milk exposure includes milk-based infant formulas, cow's milk, and all milk-containing foods.

months or after 6 months of age showed an increase in the hazard of IA compared with those exposed to cereals between ages 4 and 6 months (1-3 months: unadjusted HR, 3.03; 95% confidence interval [CI], 1.42-6.44; ≥7 months: unadjusted HR, 3.86; 95% CI, 1.56-9.56) (TABLE 2). We examined whether this association was due to exposure to a certain type of cereal by performing additional analyses using restricted definitions of exposure based on whether the cereals contained gluten. Similar associations between the hazard of IA and age at exposure to gluten-containing cereals and age at exposure to rice cereals were observed (1-3 months: unadjusted HR, 2.76; 95% CI, 0.88-8.66 vs unadjusted HR, 2.74; 95% CI, 1.28-5.85, respectively; ≥7 months: unadjusted HR, 1.95; 95% CI, 0.93-4.11 vs unadjusted HR, 2.31; 95% CI, 0.94-5.68, respectively). Neither age at initial exposure to cow's milk (1-3 months: unadjusted HR, 0.77; 95% CI, 0.31-1.92; ≥7 months: unadjusted HR, 0.89; 95% CI, 0.32-2.49) nor breastfeeding duration (unadjusted HR, 0.99; 95% CI, 0.94-1.04) was associated with risk of IA.

We initially explored the independent associations between IA and exposures to gluten-containing cereals and rice cereals by placing both variables in the model (TABLE 3, model 1). Adjusting for HLA genotype, family history of type 1 DM, ethnicity, and maternal age, children first exposed to rice cereals before age 4 months and those who were first exposed at 7 months or older had increased hazard of IA compared with those who were exposed between ages 4 and 6 months (1-3 months: adjusted HR, 3.20; 95% CI, 1.40-7.34; ≥7 months: adjusted HR, 2.77; 95% CI, 1.07-7.20). This result was independent of the association between IA risk and gluten-containing cereals, which was increased but nonsignificant (1-3 months, adjusted HR, 2.65; 95% CI, 0.76-9.33 and ≥7 months: adjusted HR, 1.70; 95% CI, 0.79-3.66).

These analyses suggest that both rice and gluten-containing cereals contribute to IA risk. We more efficiently examined the effect of cereal exposure by performing all subsequent analyses using the combined "any cereal" definition. Adjusting for covariates and confounders, children exposed to any cereals before 4 months of age and those who were first exposed at 7 months or older had increased hazard of IA compared with those who were exposed between ages 4 and 6 months (adjusted HR, 4.32; 95% CI, 2.00-9.35 and adjusted HR, 5.36; 95% CI, 2.08-13.77, respectively) (Table 3, model 2). Being breastfed when first exposed to cereals was associated with a lower hazard of IA (adjusted HR, 0.50; 95% CI, 0.25-0.99). Adjusting this model for age at exposure to cow's milk did not change the HRs for cereal exposure (data not shown), suggesting that these 2 dietary variables showed no confounding. We also examined other solid foods in the infant diet that did not contain cereal or cow's milk (eg, fruit, vegetables, meat, and eggs). The age at introduction of these foods did not differ by affected status, nor did it alter the association between cereal introduction and IA (data not shown).

Cereal Exposure by HLA-Defined Risk

We stratified our cohort on whether the children had the HLA-DR3/4,DQ8 genotype (321 positive and 862 negative for the genotype) to examine whether the association between IA risk and cereal exposure was consistent by genetic risk status. The association between age at exposure to any cereal and IA was stronger in children who were HLA-DRB1*03/04,DQB8 positive than in children who were HLA-DRB*03/04,DQB8 negative, although the trend of the association was similar in both (1-3 months: adjusted HR, 5.55; 95% CI, 1.92-16.03 vs adjusted HR, 2.93; 95% CI, 0.95-9.07, respectively; >7 months: adjusted HR, 12.53; 95% CI, 3.19-49.23 vs adjusted HR, 2.42; 95% CI 0.62-9.44, respectively). We examined whether HLA modified the effect of cereal exposure by including 2 interaction terms in the model (representing the interaction between the dichotomous and trichoto-

mous variables). The *P* values for the interaction terms were .34 and .10, suggesting marginal evidence for effect modification of cereal exposure by HLA status (TABLE 4). The FIGURE displays the proportion of children who became IA-positive by age at exposure to cereals overall, and in children who were HLA-DRB1*03/04,DQB8 positive and HLA-DRB1*03/04,DQB8 negative, respectively.

Cereal Exposure by Positivity for IA-2

A previous study suggested that infant diet associations may only be seen when the affected children in the analysis are positive for at least the IA-2 autoantibody,[5] presumably because IA-2 is one of the last autoantibodies to appear before clinical onset of type 1 DM and thus may be the most predictive of disease.[19,20] We therefore limited our affected population to only those who were positive for at least the IA-2 autoantibody (n=21 affected children) to address this hypothesis. The HRs for age at exposure to cereals were very similar to those found in the analysis of all children (Table 4).

Table 3. Age at Exposure to Cereals in Infancy and Risk of Islet Autoimmunity in Diabetes Autoimmunity Study in the Young Cohort*

Risk Factors	Exposure Categories	Adjusted Hazard Ratio (95% Confidence Interval)
Model 1. Examining the Independent Effects of Rice and Gluten-Containing Cereal Exposures		
Age exposed to rice cereal, mo	1-3	3.20 (1.40-7.34)
	4-6	1.00
	≥7	2.77 (1.07-7.20)
Age exposed to gluten-containing cereals, mo	1-3	2.65 (0.76-9.33)
	4-6	1.00
	≥7	1.70 (0.79-3.66)
HLA genotype	HLA-DRB1*03/04,DQB8 vs other genotypes	7.30 (3.53-15.10)
First-degree relative with type 1 diabetes mellitus	Yes vs no	7.36 (3.42-15.84)
Race/ethnicity	Non-Hispanic white vs other	2.66 (0.79-8.99)
Maternal age	1-Year increase	1.05 (0.98-1.13)
Model 2. Combining Rice and Gluten-Containing Exposures Into an Any Cereal Variable		
Age exposed to any cereals, mo	1-3	4.32 (2.00-9.35)
	4-6	1.00
	≥7	5.36 (2.08-13.77)
Breastfed when first exposed to cereal	Yes vs no	0.50 (0.25-0.99)
HLA genotype	HLA-DRB1*03/04,DQB8 vs other genotypes	8.69 (4.15-18.16)
First-degree relative with type 1 diabetes mellitus	Yes vs no	7.64 (3.55-16.46)
Race/ethnicity	Non-Hispanic white vs other	2.83 (0.83-9.70)
Maternal age	1-Year increase	1.05 (0.98-1.12)

*For each model, all variables were included in the survival analysis model simultaneously.

Cereal Exposure by Family History of Type 1 DM

Our study cohort consisted of children with and without a family history of type 1 DM. We examined whether we would see different associations in these 2 groups (Table 4). Exposure to cereals before 4 months of age or after 7 months of age was associated with an increased risk of IA in both children with and without a family history of type 1 DM (1-3 months: adjusted HR, 3.08; 95% CI, 1.11-8.51 vs adjusted HR, 8.51; 95% CI, 2.14-33.83, respectively; ≥7 months: unadjusted HR, 4.21; 95% CI, 1.37-13.00 vs adjusted HR, 8.40; 95% CI, 1.35-52.37, respectively) (Table 4).

Cow's Milk Exposure

We also examined the association of cow's milk in these subgroups to explore possible explanations as to why our findings regarding cow's milk were negative and inconsistent with a similar cohort study.[5] No associations were observed between hazard of IA and age at first exposure to cow's milk in any of these subgroups (Table 4).

COMMENT

Our finding of an association between age at exposure to cereals and the development of IA has not been reported previously. We chose age at exposure of 4 to 6 months as the reference group for the current analyses because US pediatricians generally recommend the introduction of solid foods, and in particular cereals, between the ages of 4 and 6 months, although no official American Academy of Pediatrics practice guideline exists regarding this practice.[11] Our data suggest that introducing cereals before age 4 months may increase a child's risk of IA. Interestingly, waiting until age 7 months or older to first introduce cereals also may increase the risk for IA. This finding suggests a window of exposure to cereals outside which an increase of IA risk exists in susceptible children.

The bimodal nature of this association would make it easy to miss with conventional analyses that compare mean age at exposure or use just 1 age cutoff for exposure. When we performed our analyses again using cutoffs used in other studies (eg, exposed before vs after 6 months of age), we saw no association between exposure to cereal or cow's milk and IA hazard (data not shown). This finding may explain why we did not find this association in our previous analysis of a separate DAISY cohort.[2]

The reason why IA risk is increased when cereal exposure occurs both early or late is not entirely clear, and may be due to a combination of factors. The risk associated with early exposure might

suggest a mechanism involving an aberrant immune response to cereal antigens in an immature gut immune system in susceptible individuals. The risk associated with late exposure to cereals may be related to the larger amount of exposure at initial introduction in the older children. Ivarsson et al[21] found that children with celiac disease were exposed to a larger amount of gluten-containing foods at first exposure than children without celiac disease, and that this amount may increase the later in life gluten is introduced. In our cohort, infants exposed to cereals at 7 months or older were more likely to be given 1 or more servings per day in the first month of exposure compared with children who were exposed between 4 and 6 months or before 4 months (52%, 43%, and 31%, respectively), suggesting that the overall frequency of exposure at initial introduction differs by age.

An argument may be made that individuals who follow pediatricians' guidelines regarding the timing of cereal introduction may be different than those who do not. Indeed, in our cohort, mothers who introduced cereals in their infant's diet between ages 0 and 3 months were younger and less educated, and those who introduced cereals at age 7 months or older were older and more educated than mothers who introduced cereals during the 4- to 6-month period. However, adjusting for these factors in the analyses did not change the estimate of the HR for the cereal variable, suggesting that these differences were not responsible for the observed associations.

We found that if cereals were introduced while the child was still breastfeeding, the risk of IA was reduced, independent of the age at exposure to cereals. This finding is similar to that of Ivarsson et al[21] in that children with celiac disease were less likely to have been breastfed when gluten was introduced than controls (odds ratio, 0.59). Similarly, Scott et al[22] showed that the high rates of DM seen in BBdp rats weaned onto a wheat gluten-based diet could be substantially reduced by first exposing the rats to small amounts of gluten during the neonatal period. The authors hypothesized that early exposure to cereal while the pup was still breastfeeding had a modulating effect over the detrimental effect of weaning onto cereals directly.

Because of the similarities between the epidemiological features of celiac disease and type 1 DM and the coexistence of the 2 diseases in the same individuals or families, gluten exposure is a strong candidate for a risk factor for type 1 DM.[23] Ventura et al[24] found that at diagnosis patients with celiac disease were positive for type 1 DM autoantibodies, which disappeared after the initiation of a gluten-free diet, suggesting a potentially common etiology of these 2 diseases. However, elimination of dietary gluten for 12 months in 7 IA-positive relatives of patients with type 1 DM did not affect levels of the diabetes autoantibodies, suggesting that dietary gluten may not be the diabetogenic antigen,[25] or at least not at that point in the pathogenesis of disease. MacFarlane et al[26] identified a wheat storage globulin protein, Glb1, which may be associated with islet cell damage, and showed that antibodies to the Glb1 protein were detected in serum samples from patients with but not in

Table 4. Subgroup Analysis of Age at Initial Dietary Exposures in Infancy and Risk of Islet Autoimmunity (IA) in the Diabetes Autoimmunity Study in the Young Cohort, by Genetic Risk, Definition of Affected Children, and Family History of Type 1 Diabetes Mellitus (DM)

	Genetic Risk of Type 1 DM		Definition of Affected Children	Family History of Type 1 DM	
	HLA-DRB1*03/04,DQB8 Children	Non–HLA-DRB1*03/04,DQB8 Children	Cases Limited to Those Positive for IA-2	Screened General Population (ie, No Family History)	First-Degree Relatives of a Type 1 DM Individual
Affected with IA, No.	18	16	21	12	22
Unaffected with IA, No.	303	846	1149	829	320
	Adjusted Hazard Ratio (95% Confidence Interval)				
	Any Cereal*				
Age at exposure, mo					
1-3	5.55 (1.92-16.03)†	2.93 (0.95-9.07)†	3.78 (1.38-10.39)‡	8.51 (2.14-33.83)§	3.08 (1.11-8.51)§
4-6	1.00	1.00	1.00	1.00	1.00
≥7	12.53 (3.19-49.23)	2.42 (0.62-9.44)	7.10 (2.23-22.63)	8.40 (1.35-52.37)	4.21 (1.37-13.00)
	Cow's Milk*				
Age at exposure, mo					
1-3	1.16 (0.31-4.33)‖	0.94 (0.26-3.44)‖	1.53 (0.43-5.39)¶	1.93 (0.24-15.53)#	0.88 (0.30-2.54)#
4-6	1.00	1.00	1.00	1.00	1.00
≥7	1.29 (0.32-5.17)	0.68 (0.14-3.39)	1.14 (0.27-4.78)	1.76 (0.18-16.99)	0.83 (0.25-2.73)

*Note: Separate models were run for cereal exposure and for cow's milk exposure.
†Adjusted for family history of type 1 DM, breastfeeding status when cereal introduced, ethnicity, and maternal age.
‡Adjusted for HLA genotype, family history of type 1 DM, breastfeeding status when cereal introduced, ethnicity, and maternal age.
§Adjusted for HLA genotype, breastfeeding status when cereal introduced, ethnicity, and maternal age.
‖Adjusted for family history of type 1 DM and breastfeeding status when cow's milk introduced.
¶Adjusted for HLA genotype, family history of type 1 DM, and breastfeeding status when cow's milk introduced.
#Adjusted for HLA genotype and breastfeeding status when cow's milk introduced.

patients without DM. While the aforementioned studies focused on gluten as the sole risk factor, our findings suggest that rice cereal, a nongluten cereal, also contributes to risk for IA. The observation that all cereals and not just gluten-containing cereals are associated with IA risk has implications with regard to development of mechanistic hypotheses as well as effective prevention strategies.

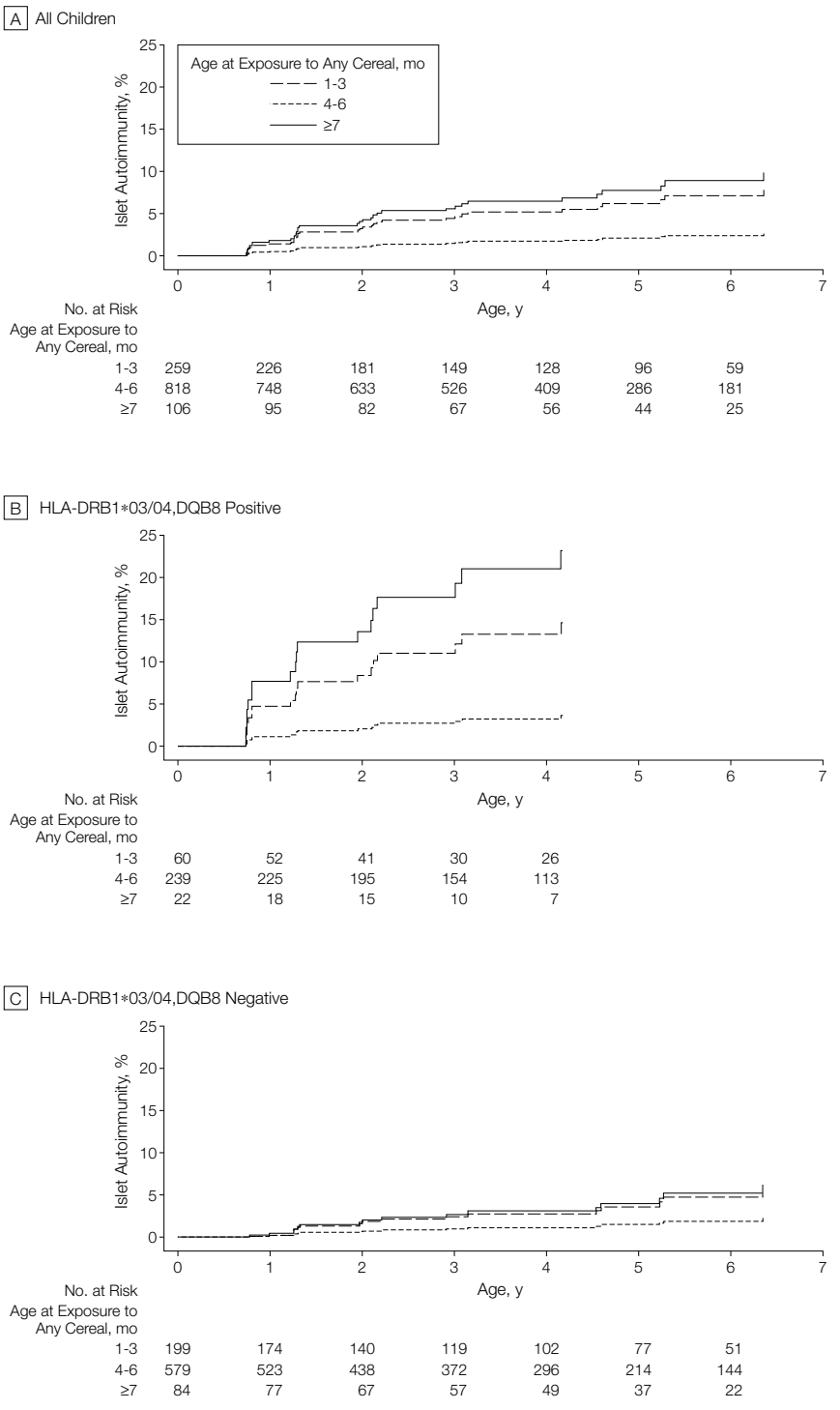
Most commercial infant cereals are supplemented with iron, zinc, thiamin, riboflavin, nicotinic acid, and vitamin E. While our study could not directly examine this, it is possible that delaying the introduction of infant cereals could result in low levels of these nutrients (in the absence of other sources) at a critical time period of development, thus increasing the risk of IA. For example, studies suggest that vitamin E[27,28] and zinc[29-31] are associated with a decreased risk of type 1 DM.

Cereals differ from breast milk and infant formulas in the amount of carbohydrates per calorie that an infant consumes at each feeding. Therefore, a hypothesis could be explored about increased carbohydrate load in infancy and its impact on the pancreas and the immune system. The rate of insulin release has been correlated with expression of GAD,[32] suggesting that carbohydrate loading in infancy may stimulate the pancreas to secrete more insulin, resulting in an increase in the expression of the autoantigens, which ultimately may increase the risk of islet cell destruction.

We found no association between exposure to cow's milk and risk of IA overall or in any of the risk subgroups. We also ruled out cow's milk as a confounder of the association between cereal exposure and IA risk. Our inability to reproduce the results of Kimpimäki et al[5] may be due to factors (foods or behaviors) in the infant diet that may be correlated with breastfeeding and infant formula choices, with these correlations differing across the 2 populations.

The DAISY birth cohort, which consists of both children from a screened

Figure. Infant Diet Exposures by HLA-Defined Risk



A, Proportion becoming positive for islet autoimmunity by age at first exposure to any cereals in all 1183 children. B, Proportion becoming positive for islet autoimmunity by age at first exposure to any cereals in 321 children who are HLA positive. C, Proportion becoming positive for islet autoimmunity by age at first exposure to any cereals in 862 children who are negative.

general population and those with a family history of type 1 DM, gives us the unique ability to observe that the effect of age at cereal introduction was similar in each group. Moreover, the independent effects of HLA and family history of type 1 DM on risk of IA are large and about equal, with HRs greater than 7. We also observed a suggestion of a gene-environment interaction in our analyses, in that the association between IA and age at first exposure to cereals was stronger in individuals who possessed the high-risk genotype, HLA-DRB1*03/04,DQB8.

Of our cohort, 31% were exposed to cereals outside of the 4- to 6-month age time window, yielding an adjusted HR of 4.3 for IA. Using this to calculate the population percent attributable risk, we found that 50% of IA would be eliminated in this population of children at moderate and high risk for type 1 DM if cereals were first introduced to the infant's diet between 4 and 6 months of age. While this population percent attributable risk is not directly applicable to the general population because it was derived from a population that was selected for being at increased risk for type 1 DM, it does indicate that manipulation of this infant diet exposure could have a strong impact on risk in children at increased risk for type 1 DM and potentially in the general public as well. We recommend that these results be confirmed in other prospective cohorts of children at risk for type 1 DM before any interventions are implemented. Additional studies may shed light on the importance of quantity of exposure and/or whether the risk is related to exposure to specific antigens or to other components of cereals. Our results do not suggest any need to change the current US infant feeding guidelines with regard to cereal introduction.

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Administrative, technical, or material support: Norris, Barriga, Erlich, Rewers.
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