



# First Infant Formula Type and Risk of Islet Autoimmunity in The Environmental Determinants of Diabetes in the Young (TEDDY) Study

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## OBJECTIVE

Studies on the introduction of infant formulas and its effect on the risk of islet autoimmunity and type 1 diabetes (T1D) have yielded inconsistent results. We investigated whether the introduction of formula based on hydrolyzed cow's milk as the first formula is associated with reduced islet autoimmunity risk in a large prospective cohort.

## RESEARCH DESIGN AND METHODS

The Environmental Determinants of Diabetes in the Young (TEDDY) study prospectively monitors 8,676 children at increased genetic risk for T1D. Autoantibodies to insulin, GAD65, and IA2 were measured regularly to define islet autoimmunity. Information on formula feeding was collected by questionnaires at 3 months of age.

## RESULTS

In survival analyses, after adjustment for family history with T1D, HLA genotype, sex, country, delivery mode, breast-feeding  $\geq 3$  months, and seasonality of birth, we observed no significant association with islet autoimmunity in infants who received extensively hydrolyzed compared with nonhydrolyzed cow's milk-based formula as the first formula during the first 3 months (adjusted hazard ratio 1.38 [95% CI 0.95; 2.01]), and a significantly increased risk for extensively hydrolyzed formula introduced during the first 7 days (adjusted hazard ratio 1.57 [1.04; 2.38]). Using a partially hydrolyzed or other formula as the first formula, or no formula, was not associated with islet autoimmunity risk.

## CONCLUSIONS

These results add to the existing evidence that islet autoimmunity risk is not reduced, and may be increased, by using hydrolyzed compared with nonhydrolyzed cow's milk-based infant formula as the first formula in infants at increased genetic risk for T1D.

Exclusive breast-feeding is recommended worldwide for infants during the first 4–6 months of life. Nevertheless, the prevalence of exclusive breast-feeding for this amount of time is lower than recommended in most countries, and breast milk is most commonly substituted with infant formulas that are based on cow's

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milk proteins (1). Numerous studies have examined the association between age at first exposure to cow's milk and type 1 diabetes, resulting in inconsistent findings. One meta-analysis suggested an increased risk of type 1 diabetes with early exposure to cow's milk (2), whereas a second meta-analysis reported only a weak association that might have been influenced by study bias (3). Moreover, most prospective cohort studies showed no association between cow's milk introduction and islet autoimmunity and/or type 1 diabetes risk (4–7).

Most of the above-mentioned studies focused on whether the formulas contained cow's milk protein or not, did not account for the use of protein hydrolysates and the degree of hydrolysis, nor did they distinguish between whether the infant formula was given as the first or subsequent formula. Moreover, the availability and use of partially or extensively hydrolyzed infant formulas differs between countries and continents, possibly as a result of inconsistencies in terminology and regulations governing infant formulas (8). Weaning to infant formulas containing hydrolyzed cow's milk proteins is recommended for infants at increased allergy risk in some countries (9) and has also been hypothesized to protect infants at increased type 1 diabetes risk from developing islet autoimmunity (10). However in the Trial to Reduce IDDM in the Genetically at Risk (TRIGR), which studied 2,159 newborn infants from 15 countries, weaning to extensively hydrolyzed formula did not reduce islet autoimmunity risk compared with conventional formula (11).

Although the evidence for an association between early cow's milk exposure or longer exclusive breast-feeding duration and type 1 diabetes risk is limited, various infant feeding policies suggest a type 1 diabetes protective effect by delayed introduction of cow's milk (12,13). As a result, health care professionals and mothers are uncertain regarding the choice of infant formula if breast-feeding is not possible or additional milk feeding is needed. This is of particular interest to mothers with type 1 diabetes, who experience difficulties with exclusive breast-feeding and have to introduce infant formula in the early postpartum period (14).

Therefore, the aim of this study was to investigate whether the introduction

of hydrolyzed cow's milk-based formula as the first formula is associated with reduced islet autoimmunity risk in a large prospective cohort, accounting for different degrees of hydrolyzation. We used data from The Environmental Determinants of Diabetes in the Young (TEDDY) study, which is unique in the number of children with genetically increased type 1 diabetes risk monitored from birth and its prospectively collected detailed information of infant diet.

## RESEARCH DESIGN AND METHODS

TEDDY is a prospective cohort study funded by the National Institutes of Health with the primary goal to identify environmental causes of type 1 diabetes. The TEDDY study enrolled children with increased genetic risk for type 1 diabetes who were recruited in six clinical research centers—three in the U.S.: Colorado, Georgia/Florida, Washington, and three in Europe: Finland, Germany, and Sweden. Detailed study design and methods have been previously published (15,16). Written informed consents were obtained for all study participants from a parent or primary caretaker separately for genetic screening and then for participation in prospective follow-up. The study was approved by local Institutional Review Boards and is monitored by an External Advisory Board formed by the National Institutes of Health.

### Study Population

Between September 2004 and February 2010, 424,788 newborn infants were screened for HLA genotypes associated with type 1 diabetes (17). The initial screening identified 21,589 eligible infants, of whom 8,676 were enrolled in the follow-up study before the age of 4 months. From the total cohort of 8,676 children, 170 were excluded from this analysis because of HLA ineligibility ( $n = 116$ ) or indeterminate islet autoantibody status ( $n = 54$ ), leaving a sample size of 8,506.

### Assessment of Study End Point

The primary outcome was the development of persistent islet autoimmunity, assessed in serum samples obtained during a clinical visit every 3 months starting at 3 months of age. Persistent autoimmunity was defined by the presence of at least one islet autoantibody among autoantibodies to GAD (GADA),

insulinoma-associated protein 2 (IA-2A), or insulin (IAA) on two or more consecutive visits confirmed by two laboratories. Date of persistent autoimmunity was defined as the draw date of the first sample of the two consecutive samples that deemed the child persistent confirmed positive for an autoantibody. The presence of persistent multiple islet autoantibodies was defined by the presence of at least two persistent and confirmed islet autoantibodies. Date of persistent multiple islet autoantibodies was defined as the draw date of the first sample when the second persistent and confirmed islet autoantibody was detected. Children with positive islet autoantibody results that were a result of maternal IgG transmission were not considered to be positive for that autoantibody unless the child had a negative sample before the first positive sample or the autoantibody persisted beyond 18 months of age (18).

### Assessment of Infant Diet

Information on infant diet in the first 3 months of life was collected by questionnaire from the primary caretaker during the first clinical visit at 3 to 4 months after birth. The age at introduction, duration of intake, and type of infant formulas used, breast-feeding status and duration, and the age at introduction of all new foods were recorded. Primary caretakers were asked during the first interview whether the baby was still receiving any breast milk. If they responded "no," they were asked the age of the child when breast-feeding was cancelled or whether the child had never been breast-fed. A further question collected similar information for the use of banked/donated breast milk. Concerning the use of infant formulas, primary caretakers were asked whether the baby had been given infant formula(s). Caretakers were asked to remember and include small amounts of formula, such as when it was mixed into food. Caretakers who responded yes were provided a list of formulas so that they could choose the formula(s) the baby had been given. The child's age when formula use was started and stopped was recorded by study nurses. For this analysis, information about the type and use of the first and subsequent infant formulas fed during the first 3 months of life was categorized and coded by the type of

protein source (e.g., cow's milk, casein, soy, synthetic amino acids, whey, other) and by the degree of processing (non-hydrolyzed, partially hydrolyzed, extensively hydrolyzed). The TEDDY study did not provide any recommendations or advice on infant feeding to the families.

### Assessment of Covariates

Information about basic demographic characteristics and family history of diabetes was received from the infant screening form. Perinatal variables, such as mode of delivery, were obtained by structured interviews during the first study visit.

### Statistical Analyses

The first formula introduced for each child was classified according to the protein source and type of processing (nonhydrolyzed cow's milk, partially hydrolyzed cow's milk, extensively hydrolyzed cow's milk, other than cow's milk protein, no formula but regular cow's milk, or no formula and no cow's milk), independent of breast-feeding continuation.

Kaplan-Meier analysis was used to estimate (unadjusted) cumulative risks of development of any islet autoantibodies by first infant formula type until age 3 months. In addition, we fitted Cox regression models to assess hazard ratios (HRs) and corresponding 95% CIs of the subsequent development of any islet autoantibodies with first infant formula use until age 3 months as a predictor. Time to event was defined as time from birth to the first visit of the two consecutive visits that deemed the subject persistent confirmed positive antibodies. Models were adjusted for the potential confounders sex, country, HLA genotype (DR3/4, DR4/4, DR3/3 or other), having a first-degree relative with type 1 diabetes (yes/no), having a mother with type 1 diabetes (yes/no), delivery mode (cesarean [C]-section, yes/no), any breast-feeding  $\geq 3$  months (yes/no), and seasonality of birth (i.e., whether the child was born in spring [March to May], summer [June to August], fall [September to November], or winter [December to February]). Interaction terms of the respective predictor variables with time were calculated to check the proportional hazards assumption for each model, which was not rejected for any of these models. In addition, we explored potential effect

modification by HLA genotype and country by including interaction terms in the model and evaluating significance based on the  $\chi^2$  test.

We performed three sensitivity analyses. First, because extensively hydrolyzed formula had frequently been introduced already shortly after birth, we restricted the time interval of introduction to first formula exposure to the first 7 days of life. Second, we assessed associations with outcome multiple islet autoantibodies and with the appearance of IAA autoantibodies only or GADA autoantibodies only at seroconversion (18). Third, because children who were introduced to extensively hydrolyzed formula within the first 7 days of life were often switched to another formula at a later time, we adjusted our analyses additionally for a switch in formula (yes/no) until age 3 months.

The significance level for all analyses was set to 0.05. All calculations were done with SAS 9.3 (SAS Institute Inc., Cary, NC) and R 3.0.3 (<http://cran.r-project.org>) software.

## RESULTS

Median follow-up of the children analyzed was 8.0 (interquartile range 6.5–

9.0) years. Further characteristics of the study population are given in Table 1. During their first 3 months of age, 2,198 children did not receive any formula or cow's milk. Up to the age of 7 days, 2,737 children received nonhydrolyzed formula as a first formula, 116 received partially hydrolyzed formula, and 210 received extensively hydrolyzed formula. Infants who were introduced to extensively hydrolyzed formula until age 3 months as a first infant formula were predominantly from Finland (80.5%) (Table 2), were introduced to this formula at a median age of 0 days, and were often switched to another formula type during the first 3 months of life (63.5%), mainly to nonhydrolyzed cow's milk formula (in 160 of 169 infants). Duration of the first formula intake was missing in 1,611 subjects. When duration was known, more than 60% (156 of 246) of children introduced to extensively hydrolyzed infant formula as the first formula were fed this formula only for less than 7 days.

Infants who received extensively hydrolyzed formula as the first formula during the first 3 months had an increased risk for development of any islet autoantibodies in unadjusted analyses

**Table 1—Characteristics of children included in the analysis**

Variable	N (%)
	N = 8,506
Developed any islet autoantibodies	686 (8.1)
Developed multiple islet autoantibodies	410 (4.8)
Female child	4,193 (49.3)
HLA genotype	
DR3/4	3,319 (39.0)
DR4/4	1,664 (19.6)
DR3/3	1,782 (21.0)
Other	1,741 (20.5)
Having a first-degree relative with type 1 diabetes	922 (10.8)
Having a mother with type 1 diabetes	337 (4.0)
Delivery by C-section	2,205 (25.9)
Country of residence	
U.S.	3,632 (42.7)
Finland	1,805 (21.2)
Germany	572 (6.7)
Sweden	2,497 (29.4)
Type of first formula introduced during the first 3 months	
Nonhydrolyzed cow's milk-based formula	5,523 (64.9)
Extensively hydrolyzed cow's milk-based formula	266 (3.1)
Partially hydrolyzed cow's milk-based formula	274 (3.2)
Other formula†	214 (2.5)
No formula, no cow's milk	2,198 (25.8)
No formula, regular cow's milk	31 (0.4)

†Including soy protein-based and elemental formula.

**Table 2—Subject and feeding characteristics according to type of first formula introduced during the first 3 months of age**

Subject characteristic	Cow's milk-based, nonhydrolyzed n = 5,523	Cow's milk-based, extensively hydrolyzed n = 266	Cow's milk-based, partially hydrolyzed n = 274	Other formula n = 14	No formula, no cow's milk n = 2,198	No formula, regular cow's milk n = 31
Female, n (%)	2,702 (48.9)	127 (47.7)	149 (54.4)	110 (51.4)	1,088 (49.5)	17 (54.8)
HLA DR3/4, n (%)	2,162 (39.2)	87 (32.7)	112 (40.9)	100 (46.7)	843 (38.4)	15 (48.4)
HLA DR4/4, n (%)	1,079 (19.5)	49 (18.4)	45 (16.4)	28 (13.1)	452 (20.6)	11 (35.5)
HLA DR3/3, n (%)	1,150 (20.8)	39 (14.7)	63 (23.0)	48 (22.4)	479 (21.8)	3 (9.7)
First-degree relative with type 1 diabetes, n (%)	542 (9.8)	33 (12.4)	64 (23.4)	40 (18.7)	241 (11.0)	2 (6.5)
Mother with type 1 diabetes, n (%)	221 (4.0)	11 (4.1)	33 (12.0)	18 (8.4)	53 (2.4)	1 (3.2)
C-section, n (%)	1,593 (28.9)	52 (19.6)	113 (41.2)	85 (39.7)	359 (16.4)	3 (10.3)
Country, n (%)						
U.S.	2,580 (46.7)	24 (9.0)	115 (42.0)	166 (77.6)	731 (33.3)	16 (51.6)
Finland	1,052 (19.1)	214 (80.5)	4 (1.5)	27 (12.6)	506 (23.0)	2 (6.5)
Germany	203 (3.7)	2 (0.8)	151 (55.1)	15 (7.0)	198 (9.0)	3 (9.7)
Sweden	1,688 (30.6)	26 (9.8)	4 (1.5)	6 (2.8)	763 (34.7)	10 (32.3)
Birth seasonality, n (%)						
Spring	1,260 (22.8)	71 (26.7)	62 (22.6)	59 (27.6)	540 (24.6)	7 (22.6)
Summer	1,369 (24.8)	66 (24.8)	67 (24.5)	52 (24.3)	577 (26.3)	8 (25.8)
Fall	1,431 (25.9)	53 (19.9)	74 (27.0)	55 (25.7)	541 (24.6)	7 (22.6)
Winter	1,463 (26.5)	76 (28.6)	71 (25.9)	48 (22.4)	540 (24.6)	9 (29.0)
Feeding characteristics						
Age at introduction of first formula, median (IQR), days	7 (0–28)	0 (0–0)	7 (0–28)	0 (0–14)	—	—
Switching to another formula type during the first 3 months, n (%)	961 (17.4)	169 (63.5)	102 (37.2)	95 (44.4)	—	—
Duration of first formula intake (days), n (%)						
1–6	1,308 (32.3)	156 (63.4)	36 (18.2)	87 (49.7)	—	—
7–29	1,663 (41.1)	81 (32.9)	112 (56.6)	70 (40.0)	—	—
≥30	1,076 (26.6)	9 (3.7)	50 (25.3)	18 (10.3)	—	—
Any breast-feeding ≥3 months, n (%)	3,310 (60.5)	206 (77.4)	140 (54.5)	152 (71.4)	2,177 (99.1)	23 (74.2)

The percentages refer to the number of available subjects in the respective formula group. IQR, interquartile range.

(unadjusted HR 1.63 [95% CI 1.15; 2.33]), which did not remain significant after adjustment for potential confounders (adjusted HR 1.38 [0.95; 2.01]), compared with children who received nonhydrolyzed cow's milk formula (Table 3 and Fig. 1A). However, this association was statistically significant also in adjusted analyses when the first formula introduced during the first 7 days was examined (adjusted HR 1.57 [1.04; 2.38]) (Table 3 and Fig. 1B). No significant associations were observed in infants receiving partially hydrolyzed formula, other formula, or no formula during the first 3 months compared with nonhydrolyzed cow's milk formula. No significant interactions between type of first formula and HLA genotype or country were observed.

We observed almost identical HRs, although with slightly wider CIs, for multiple islet autoantibody development by the introduction of extensively hydrolyzed formula during the first 7 days (adjusted HR 1.58 [95% CI 0.94; 2.64]) and by introduction during the first 3 months (adjusted HR 1.39 [0.88; 2.20]). Exposure to extensively hydrolyzed formula as the first formula was significantly associated with the development of IAA autoantibodies ( $n = 268$ ; adjusted HR 1.75 [1.04; 2.93] for exposure in the first 3 months, and adjusted HR 1.88 [1.06; 3.34] for exposure in the first 7 days), but not with the development of GADA autoantibodies ( $n = 285$ ; adjusted HR 1.10 [0.56; 2.13] for exposure in the first 3 months and 1.29 [0.62; 2.69] for exposure in the first 7 days). The observed

associations did not change considerably when we additionally adjusted for a switch in formula type in the first 3 months (e.g., adjusted HR 1.63 [1.05, 2.52]) for development of any islet autoantibodies by exposure to extensively hydrolyzed formula during the first 7 days, whereas the switch itself was not associated with increased risk (e.g., adjusted HR 0.94 [0.73, 1.20] for development of any islet autoantibodies).

## CONCLUSIONS

Our study indicated that islet autoimmunity risk is not reduced and might even be increased in children who received extensively hydrolyzed cow's milk-based formula compared with nonhydrolyzed cow's milk-based formula as a first formula. Islet autoimmunity risk was not associated with the first introduction of partially hydrolyzed or other formulas and of regular cow's milk or no cow's milk (compared with nonhydrolyzed cow's milk).

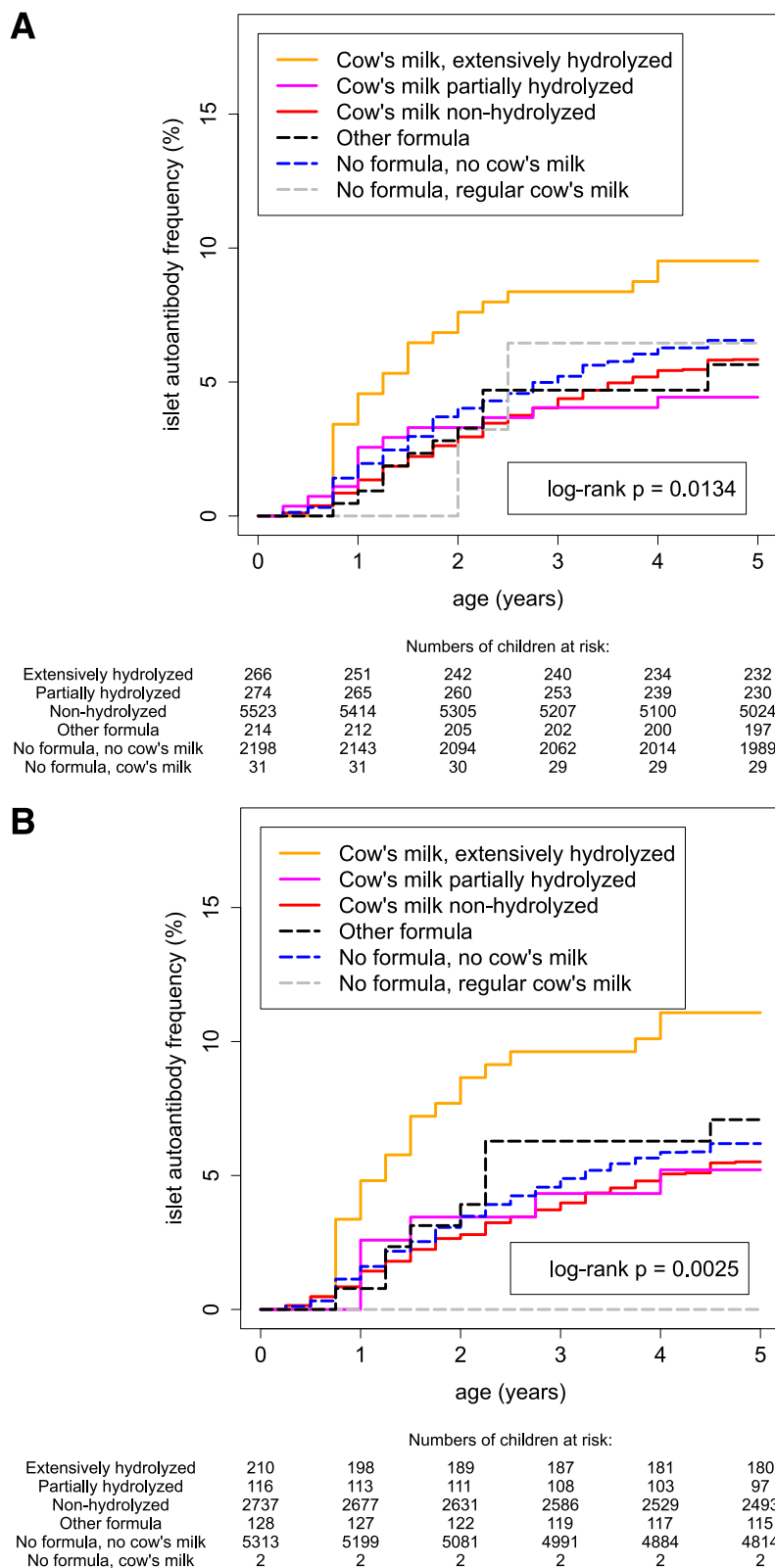
Consistent with findings from the TRIGR trial (11), our results provide evidence that introducing an extensively hydrolyzed formula as the first infant formula does not protect children with an HLA-conferred increased risk for type 1 diabetes from the development of islet autoimmunity and, specifically, of IAA autoantibodies. Rather, our findings indicate that early weaning to an extensively hydrolyzed cow's milk-based formula is associated with an increased risk for islet autoimmunity. It is noteworthy that 80.5% of infants receiving extensively hydrolyzed formula as

the first formula were from Finland. The incidence of type 1 diabetes has been reported to be higher in Finnish children compared with the other TEDDY countries (16). Further, the association between extensively hydrolyzed infant formula and islet autoimmunity risk was attenuated after adjusting for country, HLA genotype, having a first-degree relative with type 1 diabetes, sex, delivery mode, breast-feeding duration, and seasonality of birth. However, interaction analyses did not indicate that this association was modified by genotype or country. Thus, it appears unlikely that country-specific differences are the major factor that would explain these associations. Furthermore, children receiving extensively hydrolyzed formula were introduced to this formula type very early, and more than 60% consumed it only for a short duration and were frequently switched to a nonhydrolyzed cow's milk formula during the first 3 months of life. When we restricted our analyses to formulas introduced during the first 7 days of life, the association between extensively hydrolyzed infant formula and risk for islet autoimmunity became stronger, suggesting that very early decisions about infant milk feeding may be important in islet autoimmunity risk. It appears unlikely, though, that an early switch in formula type is an important confounder in this context, because such a switch during the first 3 months of life did not mitigate the observed associations and was also not associated with increased autoimmunity risk itself.

**Table 3—Risk for any islet autoantibody by type of first formula introduced during the first 3 months of age and first 7 days of age**

	Events/exposed (%)	Unadjusted HR (95% CI)	P value	Adjusted* HR (95% CI)	P value
First formula introduced during first 3 months					
Cow's milk-based, nonhydrolyzed	421/5,523 (7.6)	Reference	—	Reference	—
Cow's milk-based, extensively hydrolyzed	33/266 (12.4)	1.63 (1.15; 2.33)	0.007	1.38 (0.95; 2.01)	0.09
Cow's milk-based, partially hydrolyzed	17/274 (6.2)	0.86 (0.53; 1.40)	0.55	0.83 (0.49; 1.41)	0.49
Other formula	15/214 (7.0)	0.93 (0.55; 1.56)	0.78	0.91 (0.54; 1.54)	0.73
No formula, no cow's milk	196/2,198 (8.9)	1.17 (0.99; 1.39)	0.07	1.00 (0.84; 1.21)	0.97
No formula, regular cow's milk	4/31 (12.9)	1.67 (0.62; 4.47)	0.31	1.72 (0.64; 4.61)	0.28
First formula introduced during first 7 days					
Cow's milk-based, nonhydrolyzed	200/2,737 (7.3)	Reference	—	Reference	—
Cow's milk-based, extensively hydrolyzed	30/210 (14.3)	1.96 (1.34; 2.88)	<0.001	1.57 (1.04; 2.38)	0.03
Cow's milk-based, partially hydrolyzed	9/116 (7.8)	1.10 (0.56; 2.14)	0.78	0.99 (0.48; 2.06)	0.99
Other formula	11/128 (8.6)	1.21 (0.66; 2.21)	0.55	1.12 (0.61; 2.07)	0.71
No formula, no cow's milk	436/5,313 (8.2)	1.11 (0.94; 1.31)	0.22	0.97 (0.82; 1.16)	0.76
No formula, regular cow's milk	0/2 (0.0)	—	—	—	—

\*Adjusted for HLA genotype, first-degree relative with type 1 diabetes, mother with type 1 diabetes, sex, country, mode of delivery, any breast-feeding  $\geq 3$  months, and seasonality of birth.



**Figure 1**—Cumulative risk of any islet autoimmunity with respect to type of first formula introduced during the first 3 months (A) and 7 days (B). The  $P$  values refer to log-rank tests. The numbers below the graphs indicate the number of subjects in each formula group at each follow-up.

The molecular weights of the proteins contained in nonhydrolyzed cow's milk formula range from 14 to 67 kD. In

contrast, peptides have a molecular weight of <3 kD in extensively hydrolyzed formulas and of 3–10 kD in partially

hydrolyzed infant formulas (9). Thus, compared with nonhydrolyzed cow's milk formula, extensively hydrolyzed infant formula does not contain intact bovine insulin, which has been hypothesized to be associated with increased type 1 diabetes risk in previous studies (19,20). Our results do not support the role of bovine insulin in the development of islet autoimmunity because cumulative islet autoimmunity risk and, specifically, risk for IAA autoantibodies at seroconversion was rather increased in infants receiving extensively hydrolyzed cow's milk-based formula. Interestingly, consistent with our results, hydrolyzed formula intake was also associated with increased islet autoimmunity risk, although not significantly, in the TRIGR study (11).

In fact, the potential increase of islet autoimmunity risk in infants fed an extensively hydrolyzed infant formula during the first 3 months needs further investigation. Recent research activities focusing on proteins included in human and bovine milk, as well as in colostrum, indicated that milk from both species contains proteins and peptides that are involved in the modulation of the immune system and maturation of the gastrointestinal tract of the newborn infant, although the protein type and quantity in milk differs between species (21–23). A recent study investigated the composition of human milk and observed changes in the human milk serum proteome during the first 2 weeks of lactation, which coincide with the gradual maturation of the digestive and immune system (24). These findings further strengthen the relevance of protein composition during the first 2 weeks in child development. Although proteins included in infant formula made from nonhydrolyzed cow's milk differ quantitatively and qualitatively from those included in human breast milk, proteins may lose their functionality dependent on the degree of hydrolyzation. Further targeted investigations are needed to identify which bovine proteins may be of importance here.

The strengths of this study include the prospective collection of detailed information on the use of different formula types, minimizing recall bias. In addition, the multinational large sample size gave us the possibility to investigate associations between various types of infant formulas and islet autoimmunity risk and potential country-specific differences.



A current limitation of our study is the follow-up time. Although this follow-up covers early islet autoimmunity, studying whether infant formula feeding is associated with the progression to clinical type 1 diabetes will require further follow-up of the TEDDY cohort. Furthermore, most of the infants fed the extensively hydrolyzed formula were from Finland. Unfortunately, the TEDDY data do not contain information about why parents introduced formula milk or chose a specific formula type. Although we tried to take into account specific characteristics of children receiving hydrolyzed formula, such as very early introduction and any breast-feeding duration, we cannot exclude that other unmeasured variables are associated with the use of extensively hydrolyzed infant formula and act as confounders in this analysis. In addition, the study population was selected based on an HLA genotype conferring risk for type 1 diabetes and may therefore not be generalizable to the general population.

In conclusion, our study provides further evidence that there is no benefit for infants at increased genetic risk for type 1 diabetes to be fed extensively or partially hydrolyzed infant formula as a first formula if breast-feeding is not possible. For infants who cannot be breast-fed, hydrolyzed infant formula should therefore be considered in the context of atopy prevention, according to recommendations published by pediatric authorities.

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