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Effect of Hydrolyzed Infant Formula vs Conventional Formula on Risk of Type 1 Diabetes
The TRIGR Randomized Clinical Trial

Writing Group for the TRIGR Study Group

IMPORTANCE Early exposure to complex dietary proteins may increase the risk of type 1 diabetes in children with genetic disease susceptibility. There are no intact proteins in extensively hydrolyzed formulas.

OBJECTIVE To test the hypothesis that weaning to an extensively hydrolyzed formula decreases the cumulative incidence of type 1 diabetes in young children.

DESIGN, SETTING, AND PARTICIPANTS An international double-blind randomized clinical trial of 2159 infants with human leukocyte antigen–conferred disease susceptibility and a first-degree relative with type 1 diabetes recruited from May 2002 to January 2007 in 78 study centers in 15 countries; 1081 were randomized to be weaned to the extensively hydrolyzed casein formula and 1078 to a conventional formula. The follow-up of the participants ended on February 28, 2017.

INTERVENTIONS The participants received either a casein hydrolysate or a conventional adapted cow's milk formula supplemented with 20% of the casein hydrolysate. The minimum duration of study formula exposure was 60 days by 6 to 8 months of age.

MAIN OUTCOMES AND MEASURES Primary outcome was type 1 diabetes diagnosed according to World Health Organization criteria. Secondary outcomes included age at diabetes diagnosis and safety (adverse events).

RESULTS Among 2159 newborn infants (1021 female [47.3%]) who were randomized, 1744 (80.8%) completed the trial. The participants were observed for a median of 11.5 years (quartile [Q] 1-Q3, 10.2-12.8). The absolute risk of type 1 diabetes was 8.4% among those randomized to the casein hydrolysate (n = 91) vs 7.6% among those randomized to the conventional formula (n = 82) (difference, 0.8% [95% CI, −1.6% to 3.2%]). The hazard ratio for type 1 diabetes adjusted for human leukocyte antigen risk group, duration of breastfeeding, duration of study formula consumption, sex, and region while treating study center as a random effect was 1.1 (95% CI, 0.8 to 1.5; *P* = .46). The median age at diagnosis of type 1 diabetes was similar in the 2 groups (6.0 years [Q1-Q3, 3.1-8.9] vs 5.8 years [Q1-Q3, 2.6-9.1]; difference, 0.2 years [95% CI, −0.9 to 1.2]). Upper respiratory infections were the most common adverse event reported (frequency, 0.48 events/year in the hydrolysate group and 0.50 events/year in the control group).

CONCLUSIONS AND RELEVANCE Among infants at risk for type 1 diabetes, weaning to a hydrolyzed formula compared with a conventional formula did not reduce the cumulative incidence of type 1 diabetes after median follow-up for 11.5 years. These findings do not support a need to revise the dietary recommendations for infants at risk for type 1 diabetes.

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Group Information: The writing group and members of the TRIGR Study Group are listed at the end of this article.

Corresponding Author: Mikael Knip, MD, DMSc, Children's Hospital, University of Helsinki, PO Box 22 (Stenbäckinkatu 11), FI-00014 Helsinki, Finland (mikael.knip @helsinki.fi).

Type 1 diabetes is considered to be a chronic immune-mediated disease characterized by selective loss of insulin-producing β cells in the pancreatic islets in genetically susceptible individuals. Overt clinical disease is preceded by an asymptomatic period of highly variable duration during which diabetes-associated autoantibodies appear in the peripheral circulation as markers of emerging β-cell autoimmunity.[1,2] Several disease-related autoantibodies predict clinical type 1 diabetes, including classic islet cell antibodies (ICAs), insulin autoantibodies (IAAs), and autoantibodies to glutamic acid decarboxylase (GAD); the tyrosine phosphatase-related insulinoma-associated 2 molecule (IA-2); and zinc transporter 8.[2] In natural history studies from infancy, positivity for 2 or more autoantibodies signals a risk of approximately 70% for the development of clinical diabetes over the subsequent 10 years.[3]

The incidence of type 1 diabetes is increasing at an accelerating rate among children in North America and in most European countries.[4,5] Accumulating evidence suggests that β-cell autoimmunity emerges early in life.[6,7] Accordingly, any measure aimed at primary prevention of type 1 diabetes (ie, prevention of the initiation of the diabetic disease process) has to be initiated in infancy. In addition, there is a growing body of data suggesting that factors affecting the emergence of autoimmunity may be different from those associated with progression from autoimmunity to diabetes.[8,9]

Some epidemiological and immunological studies suggest that exposure to complex foreign proteins in early infancy may increase the risk of β-cell autoimmunity and type 1 diabetes in genetically susceptible individuals,[10-12] although others do not.[13,14] In our previous study, weaning to an extensively hydrolyzed casein formula did not decrease the cumulative incidence of diabetes-associated autoantibodies by 7 years of age in at-risk children.[15] This article reports on the intervention effect on diabetes incidence by 11.5 years of age in the TRIGR (Trial to Reduce Insulin-Dependent Diabetes Mellitus in the Genetically at Risk) Study.

Methods

Study Design

A randomized, double-blind study was conducted in 78 study centers from 15 countries as previously described.[16] The study protocol is available in Supplement 1. Newborn infants who had a first-degree relative with type 1 diabetes and defined human leukocyte antigen (HLA) genotypes were recruited between May 2002 and January 2007 and followed up until the youngest participant reached 10 years of age in February 2017. Randomization of the infants who met the inclusion criteria took place before birth or immediately after birth (Figure 1). Randomization was stratified by study center, with a block size of 4. Written informed consent was obtained from the family before enrollment. The study was approved by the ethics committees of all participating centers.

Key Points

Question Does weaning to an extensively hydrolyzed formula decrease the cumulative incidence of type 1 diabetes in children at risk?

Findings In this randomized clinical trial that included 2159 children with human leukocyte antigen–conferred susceptibility to type 1 diabetes and at least 1 affected family member, weaning to a hydrolyzed formula compared with a conventional formula did not significantly decrease the cumulative incidence of type 1 diabetes after a median of 11.5 years (8.4% vs 7.6%).

Meaning Weaning to a hydrolyzed formula did not reduce the risk of type 1 diabetes in children with an increased disease risk.
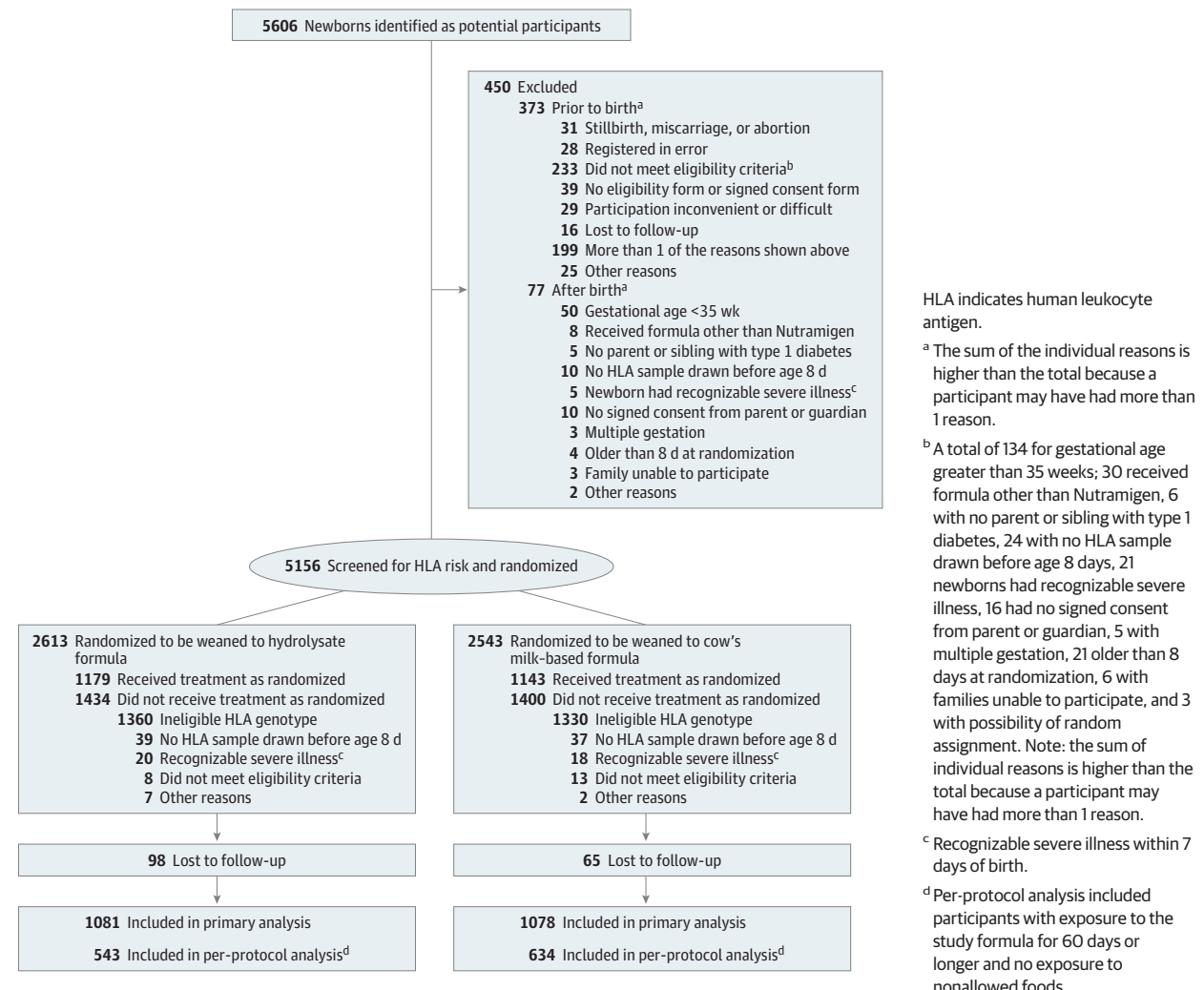
Dietary Intervention

Infants were randomly assigned weaning to either the intervention or control formulas, which were produced specifically for this study. Randomization was carried out in each strata within 4 blocks. The intervention formula was an extensively hydrolyzed casein-based formula, while the control formula was composed of 80% intact cow's milk protein and 20% hydrolyzed milk protein and formulated so that the taste and smell would be indistinguishable from the intervention formula. Study formulas were prepared and coded with the use of 4 colors by Mead Johnson Nutritional and were blinded to all investigators except the data management unit. Newborn infants requiring supplemental feeding before randomization (eg, infants born at night or on weekends) received banked breast milk or Nutramigen, an extensively hydrolyzed casein-based formula.

Breastfeeding was practiced at the discretion of the participating mothers, and maternal diets were unmodified. Breastfeeding was encouraged and exceeded national averages in both groups.[17] The dietary intervention period lasted until the infant was at least 6 months of age and, if by that time the child had not received the study formula for at least 60 days, study formula feeding was continued until 60 days of study formula exposure was reached, but not beyond 8 months of age. Parents were asked not to feed the children any commercial or other baby foods containing bovine protein during the intervention period. Adherence to the protocol was monitored by means of regular family nutrition interviews (at the age of 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 months) and by the analysis of cow's milk antibodies in serum samples.

HLA Genotyping

Cord blood or a heel stick blood sample collected on filter paper shortly after birth was immediately sent to the Turku (Europe and Australia) or Pittsburgh (North America) laboratories for HLA genotyping. HLA genotyping for the selected DQB1 and DQA1 alleles was performed using sequence-specific oligonucleotide hybridization, with quality control between the 2 laboratories carefully maintained. The following genotypes were regarded as eligible: (1) *HLA DQB1*02/DQB1*03:02* (high risk); (2) *HLA DQB1*03:*

Figure 1. Screening, Randomization, and Follow-up

HLA indicates human leukocyte antigen.

[a] The sum of the individual reasons is higher than the total because a participant may have had more than 1 reason.

[b] A total of 134 for gestational age greater than 35 weeks; 30 received formula other than Nutramigen, 6 with no parent or sibling with type 1 diabetes, 24 with no HLA sample drawn before age 8 days, 21 newborns had recognizable severe illness, 16 had no signed consent from parent or guardian, 5 with multiple gestation, 21 older than 8 days at randomization, 6 with families unable to participate, and 3 with possibility of random assignment. Note: the sum of individual reasons is higher than the total because a participant may have had more than 1 reason.

[c] Recognizable severe illness within 7 days of birth.

[d] Per-protocol analysis included participants with exposure to the study formula for 60 days or longer and no exposure to nonallowed foods.

02/x (x not DQB1*02, DQB1*03:01, or DQB1*06:02) (moderate risk); (3) HLA DQA1*05-DQB1*02/y (y not DQA1*02:01-DQB1*02, DQB1*03:01, DQB1*06:02, or DQB1*06:03) (mild risk); and (4) HLA DQA1*03-DQB1*02/y (y not DQA1*02:01-DQB1*02, DQB1*03:01, DQB1*06:02, or DQB1*06:03) (rare mild risk).

β-Cell Autoimmunity

ICAs were detected using indirect immunofluorescence. The other 3 autoantibodies were quantified with the use of specific radiobinding assays in the Scientific Laboratory, Children's Hospital, University of Helsinki, Helsinki, Finland, with cutoff limits for positivity of 2.5 JDF units for ICAs, 2.80 relative units (RU) for IAA, 5.36 RU for GAD autoantibodies, and 0.77 RU for IA-2 autoantibodies.[18] The disease sensitivity and specificity of the ICA assay were 100% and 98%, respectively, in the fourth round of the international workshops on standardization of the ICA assay. According to the Diabetes Autoantibody Standardization Program and the International Autoantibody Standardization Program workshop results in 2002-2016, the disease sensitivities of the IAA, GAD autoantibody, and IA-2 autoantibody radiobinding assays were 42% to 62%, 70% to 92%, and 62% to 80%, respectively. The corresponding disease specificities were 93% to 99%, 90% to 98%, and 93% to 100%, respectively.

Outcomes

The primary end point was the diagnosis of diabetes according to World Health Organization criteria.[19] According to those criteria, the diagnosis is based on (1) symptoms + a single random plasma glucose level of 200 mg/dL or greater (to convert to mmol/L, multiply by 0.0555) or (2) if no symptoms, the diagnosis requires a raised random plasma glucose reading of 200 mg/dL or greater on 2 occasions, a raised fasting plasma glucose reading of 126 mg/dL or greater, or a diabetic oral glucose tolerance test (OGTT, fasting venous plasma glucose ≥126 mg/dL and/or a 2-hour venous plasma glucose ≥200 mg/dL) on 2 occasions. OGTTs were performed by protocol on all study participants who had not been previously diagnosed at 6 and 10 years of age and at study end. Additional OGTTs were performed as clinically indicated. All diagnosed cases were centrally reviewed.

Adverse Events

Undesirable experiences occurring to a child during the trial, whether or not considered related to the investigational product, were reported as adverse events. Serious adverse events were reviewed centrally by the safety monitoring group for this study and were reported annually in tabular form to an external data safety and monitoring board, which reviewed each serious adverse event individually.

Statistical Analyses

The cumulative incidence of diabetes onset from the time of randomization within each group was estimated using a modified Kaplan-Meier diabetes-free survival function. The difference between groups in the cumulative incidence functions, and the associated hazard functions, was tested using the Mantel–log rank test on discrete time to type 1 diabetes (6-month intervals). The relative risk of diabetes onset between groups was estimated from the discrete Cox proportional hazard model.[20] The proportionality assumption of the Cox proportional hazard model was tested. First, the Schoenfeld residuals were examined to determine whether there was an association with time. Second, the interaction of parameters of interest and time were included in the models and tested for significance. For treatment and the variables used in the adjusted models, the null hypothesis of proportionality failed to be rejected. The analyses were adjusted for HLA risk, duration of breastfeeding, duration of study formula consumption, sex, and region, while treating study center as a random effect. The critical value for the test statistic ($P = .047$) and confidence intervals in this primary analysis were adjusted for multiple looks, which took place during the trial and were based on the Lan and DeMets[21] spending function. When comparing data between the 2 study groups, the t test was applied for normally distributed variables and the nonparametric Mann-Whitney U test for skewed variables.

The effects of weaning to the casein hydrolysate vs conventional formula were tested using the intention-to-treat principle including all HLA-eligible participants who were randomized to a treatment group. Tests of significance reported herein were 2-tailed. Statistical analyses were performed using SAS version 9.4 (SAS Institute). No imputation for missing values was performed; rather, observations with relevant missing values were excluded from respective analyses. The analysis of diabetes risk was also performed according to treatment received (per-protocol analysis). Participants were included in this analysis if they had exposure to the study formula for 60 days or longer and were not exposed to nonallowed foods. This study was designed such that given a confidence level of 95%, an estimated cumulative incidence of diabetes of 7.6% by the age of 10 years in the control group and an expected dropout rate of 20% by 10 years and a frequency of 10% of exclusive breastfeeding (up to age of 6 months), the study would have 80% power to detect a 40% change in the end point. As a post hoc analysis, the hazard ratio of the treatment groups was also calculated after adjusting for the age at which multiple autoantibodies appeared as an exploratory analysis.

Results

Altogether, 2159 newborn infants (1021 female [47.3%]) with an eligible HLA genotype (41.9% of the genotyped infants) were randomized to the intervention study. Five hundred sixteen infants (23.9%) carried the high-risk HLA genotype; 953 (44.1%), moderate-risk genotypes; 668 (31.0%), mild-risk genotypes; and 22 (1.0%), the rare mild-risk genotype. The first-degree relative with type 1 diabetes was the mother in 1052 infants (48.8%), the father in 722 (33.4%), and a sibling in 308 (14.3%), and 77 participants (3.5%) had multiple affected relatives. The median follow-up time for the diagnosis of diabetes was 11.5 years (Q1-Q3, 10.2-12.8 years; mean, 11.0 years). Randomization resulted in 1081 infants in the casein hydrolysate group and 1078 in the control group. There were no differences in the demographics or the distribution of HLA genotypes between the 2 groups (**Table 1**).

Study Intervention

Eighty percent of infants in the casein hydrolysate group and 80.9% in the control group were exposed to the study formula during the intervention period. The mean (SD) ages of the infants at the time of study formula introduction were 2.0 (2.3) months in the hydrolysate group and 1.8 (2.2) months in the control group (difference, 0.2 months [95% CI, 0-0.42]). The mean (SD) duration of study-formula feeding was 10.2 (9.3) weeks in the casein hydrolysate group and 11.7 (9.7) weeks in the control group (difference, 1.5 weeks [95% CI, 0.7-2.3]; $P < .001$). As previously reported, the analysis of cow's milk antibodies confirmed that the families adhered well to the dietary intervention, resulting in conspicuous differences in the antibody levels between treatment groups.[13]

Progression to Diabetes

The median age at initial seroconversion was 1.6 years (Q1-Q3, 1.0-3.0 years) in the casein hydrolysate group among those who progressed to clinical diabetes, whereas it was 1.5 years (Q1-Q3, 1.0-3.0 years; $P = .38$) among the progressors in the control group. The mean duration from seroconversion to clinical diabetes was 4.1 years (median, 3.5 years [Q1-Q3, 1.4-6.6]) in the casein hydrolysate group and 3.9 years (median, 3.1 years [Q1-Q3, 1.1-6.2]) in the control group (difference, 0.2 years [95% CI, −0.8 to 1.1]; $P = .76$). The number of participants who were positive for each specific autoantibody during the preclinical period is shown in Table 1. Five children (5.5%) in the casein hydrolysate group and 6 (7.3%) in the control group had no detectable autoantibodies before the diagnosis of diabetes ($P = .62$). At diagnosis, the number of autoantibody-negative participants had dropped to 4 (4.4%) and 5 (6.1%), respectively (difference, 1.7% [95% CI, −6.4% to 10.4%]).

Diabetes

During follow-up, diabetes developed in 91 children in the casein hydrolysate group (8.4%) and in 82 in the control

Table 1. Demographic Characteristics, Dietary Exposure, and Autoantibody Status of the Trial Participants

Characteristic	Casein Hydrolysate (n = 1081[a])	Control Formula (n = 1078[a])
Baseline Characteristics		
HLA risk category, No. (%)		
HLA-DQB1*0302/DQB1*02 [high risk]	260 (24.1)	256 (23.7)
HLA-DQB1*0302/x (x not DQB1*02, DQB1*0301, or DQB1*0602) [moderate risk]	478 (44.2)	475 (44.1)
HLA-DQA1*05-DQB1*02/y (y not DQA1*0201-DQB1*02, DQB1*0301, DQB1*0302, DQB1*0602, or DQB1*0603) [mild risk]	332 (30.7)	336 (31.2)
HLA-DQA1*03-DQB1*02/y (y not DQA1*0201-DQB1*02, DQB1*0301, DQB1*0302, DQB1*0602, or DQB1*0603) [rare mild risk]	11 (1.0)	11 (1.0)
Region, No. (%)		
Finland	212 (19.6)	212 (19.7)
Canada	265 (24.5)	263 (24.4)
United States	199 (18.4)	196 (18.2)
Other	405 (37.5)	407 (37.8)
Maternal age, mean (SD), y	30.7 (5.1)	30.9 (4.9)
Female infants, No. (%)	505 (46.7)	516 (47.9)
Characteristics Obtained After Randomization		
Relative with type 1 diabetes, No. (%)		
Mother only	530 (49.0)	522 (48.4)
Father only	355 (32.8)	367 (34.0)
1 Sibling only	151 (14.0)	157 (14.6)
>1 Family member	45 (4.2)	32 (3.0)
Breastfeeding duration, median (Q1-Q3), mo	7.8 (2.1-9.0)	7.1 (2.1-9.0)
No. of infants	1071	1066
Exclusive breastfeeding duration, median (Q1-Q3), wk	0.29 (0.14-10.0)	0.29 (0.14-7.0)
No. of infants	1071	1065
Age at first study formula intake, mean (SD), mo	2.0 (2.3)	1.8 (2.2)
No. of infants	865	872
Study formula duration, median (Q1-Q3), wk	9.0 (0.4-18)	10.0 (1.0-22)
No. of infants	1071	1065
Islet autoantibodies, No. (%)[b]		
ICA+	394 (36.5)	373 (34.8)
IAA+	183 (17.0)	162 (15.1)
GADA+	207 (19.2)	186 (17.3)
IA-2A+	115 (10.7)	102 (9.5)
Duration of follow-up, mean (SD), y	10.9 (2.8)	11.0 (2.7)
Duration of follow-up, median (Q1-Q3), y	11.5 (10.1-12.8)	11.4 (10.2-12.8)

Abbreviations: GADA, glutamic acid decarboxylase autoantibody; HLA, human leukocyte antigen; IAA, insulin autoantibody; IA-2A: tyrosine phosphatase–related insulinoma-associated 2 molecule autoantibody; ICA, islet cell antibody; Q, quartile.

[a] Sample sizes are reported when they differ from the overall sample sizes.

[b] Participants were considered positive for a specific autoantibody if they had 1 or more measurements greater than the specified threshold during follow-up (ICA >2.5 JDF units, IAA >2.80 RU, GADA >5.36 RU, and IA-2A >0.77 RU). Islet autoantibodies were measured at birth, 3 months, 6 months, 9 months, 12 months, and then annually to age 14 years.

group (7.6%) (difference, 0.8% [95% CI, −1.6% to 3.2%]; P = .47; **Figure 2**). The hazard ratio for type 1 diabetes adjusted for HLA risk group, duration of breastfeeding, duration of study formula consumption, sex, and region, while treating study center as a random effect, was 1.1 (95% CI, 0.8-1.5; P = .46). There was no significant difference in the median age at diagnosis between the 2 groups (6.0 years [Q1-Q3, 3.1-8.9] vs 5.8 years [Q1-Q3, 2.6-9.1]; P = .75; difference, 0.2 years [95% CI, −0.9 to 1.2]). About one-fourth of the cases in each group were diagnosed without clinical symptoms (**Table 2**). Five children (5.5%) in the casein hydrolysate

group and 3 (3.7%) in the control group presented with diabetic ketoacidosis (difference, 1.8% [95% CI, −6.3% to 9.8%]; P = .57). Comparisons between the treatment groups within HLA risk groups, according to the relationship to the affected family member (father, mother, or sibling with diabetes), geographic region associated with the clinical site of enrollment, or sex were not statistically significant (**Table 3**).

The prespecified per-protocol analysis was defined to include those who were not exposed to any nonallowed foods containing cow's milk and had exposure to study formula for at least 60 days. The hazard ratio for type 1 diabetes

Figure 2. Cumulative Survival Without Type 1 Diabetes
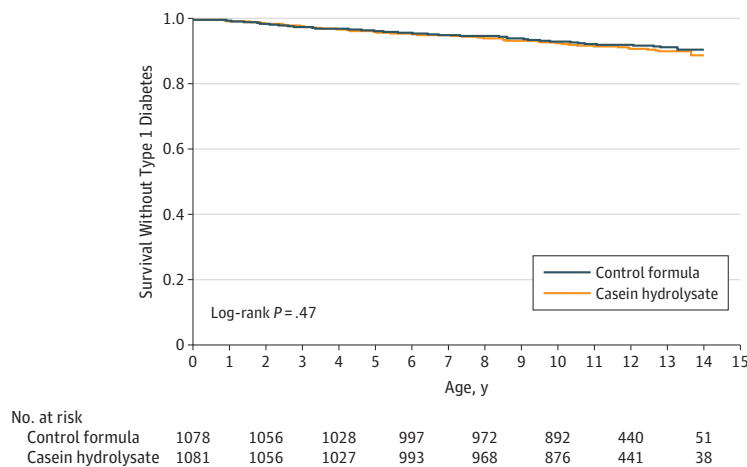
The median follow-up time was 11.5 years (quartile [Q] 1-Q3, 10.1-12.8 years) in the casein hydrolysate group and 11.4 years (Q1-Q3, 10.2-12.8 years) in the control group.

No. at risk							
Control formula	1078	1056	1028	997	972	892	440	51
Casein hydrolysate	1081	1056	1027	993	968	876	441	38

Table 2. Characteristics of the Participants Who Progressed to Type 1 Diabetes

Characteristic	Casein Hydrolysate (n = 91[a])	Control Formula (n = 82[a])	Between-Group Difference (95% CI)	P Value
Male, No. (%)	49 (53.8)	33 (40.2)	13.6 (−2.1 to 28.4)	.07
Age at diagnosis, median (Q1-Q3), y	6.0 (3.1-8.9)	5.8 (2.6-9.1)	0.2 (−0.9 to 1.2)	.75
Maximum No. of autoantibodies before diagnosis, median (Q1-Q3)	4 (3-4)	4 (3-4)	0 (−0.3 to 0.3)	.55
Participants with detectable autoantibodies before diagnosis, No. (%)				
1 Autoantibody	1 (1.1)	2 (2.4)	1.3 (−4.7 to 8.3)	
2 Autoantibodies	7 (7.7)	4 (4.9)	2.8 (−6.1 to 11.5)	.56
3 Autoantibodies	29 (31.9)	20 (24.4)	7.5 (−6.8 to 21.2)	
4 Autoantibodies	52 (57.1)	52 (63.4)	6.3 (−9.0 to 21.1)	
Clinical symptoms at diagnosis, No. (%)	70 (76.9)	61 (74.4)	2.5 (−10.9 to 16.1)	
Diabetic ketoacidosis at diagnosis, No. (%)	5 (5.5)	3 (3.7)	1.8 (−6.3 to 9.8)	.57
Hemoglobin A_{1c} at diagnosis, median (Q1-Q3)				
% (DCCT unit)	7.9 (6.5-9.4)	8.1 (7.0-9.3)	0.2 (−0.5 to 0.7)	.77
No. of infants	80	72		
mmol/mol	62.4 (47.8-79.1)	65.0 (53.0-78.1)	2.6 (−5.3 to 7.7)	.80
No. of infants	80	72		
Family history of type 1 diabetes, No. (%)				
Mother	30 (33.0)	29 (35.4)	2.4 (−12.3 to 17.1)	
Father	32 (35.2)	28 (34.2)	1.0 (−13.8 to 15.6)	.54
Sibling	18 (19.8)	20 (24.4)	4.6 (−8.5 to 17.8)	
>1 Sibling	11 (12.1)	5 (6.1)	6.0 (−3.9 to 15.7)	
Breastfeeding >6 mo, No. (%)	60 (65.9)	48 (58.5)	7.4 (−7.7 to 22.2)	.32
No breastfeeding, No. (%)	4 (4.4)	4 (4.9)	0.5 (−7.4 to 8.9)	.88

Abbreviation: Q, quartile.

[a] Sample sizes are reported when they differ from the overall sample sizes.

in this subpopulation (n = 1177), adjusted for HLA risk group, duration of breastfeeding, duration of study formula consumption, sex, and region, while treating study center as a random effect, was 1.1 (95% CI, 0.7-1.7; P = .63).

As noted previously, the 2 treatment groups did not differ according to the characteristics of participants who developed diabetes (Table 2) or when analyzed within prespecified subgroups (Table 3). As a post-hoc analysis, the hazard ratio of the treatment groups was estimated after adjusting for the age at which multiple autoantibodies appeared (median, 3.2 years [Q1-Q3, 1.6-6.3] in the casein hydrolysate group and 3.0 years [Q1-Q3, 1.5-6.1] in the control group; P = .42) with little effect on the overall results (hazard ratio, 0.95 [95% CI, 0.70-1.28]; P = .95).

Table 3. Development of Type 1 Diabetes: Unadjusted Treatment Effect in Subgroups

Subgroup	Casein Hydrolysate			Control Formula			Hazard Ratio (95% CI)
	Total No.	No. With Diabetes	No. Without Diabetes at End of Study[a]	Total No.	No. With Diabetes	No. Without Diabetes at End of Study[a]	
Overall	1081	91	960	1078	82	968	1.116 (0.828-1.504)
HLA risk group							
1	260	37	212	256	36	213	1.019 (0.644-1.612)
2	478	39	433	475	25	438	1.549 (0.938-2.560)
3	332	15	305	336	19	309	0.797 (0.405-1.570)
4	11	0	10	11	2	8	0
Proband							
Sibling only	151	18	130	157	20	135	0.934 (0.493-1.769)
Parent only	901	66	808	902	59	817	1.134 (0.798-1.611)
Sibling and parent	27	6	21	17	3	14	1.297 (0.324-5.187)
Other	2	1	1	2	0	2	
Region							
Finland	212	20	191	212	14	198	1.421 (0.718-2.814)
United States	199	14	176	196	18	168	0.720 (0.358-1.449)
Canada	265	20	233	263	26	229	0.763 (0.426-1.367)
Other	405	37	360	407	24	373	1.610 (0.963-2.691)
Male	576	42	520	562	49	502	0.820 (0.543-1.238)
Female	505	49	440	516	33	466	1.575 (1.013-2.448)

Abbreviation: HLA, human leukocyte antigen.

[a] Does not include 58 participants (30 in the casein hydrolysate group and 28 in the control group) who died or became lost to follow-up prior to the end of the study.

Adverse Events

The frequency of any infection was 0.90 events/year in the hydrolysate group and 0.93 events/year in the control group. The corresponding frequencies of upper respiratory infections were 0.48 and 0.50, respectively. The rate of other adverse events was of the same magnitude in the 2 groups (eTable in Supplement 2). Similar linear growth and weight gain were observed in both groups.

Discussion

In this international randomized trial in children with an HLA genotype conferring increased risk for type 1 diabetes and an affected first-degree relative, weaning to a highly hydrolyzed formula during infancy did not reduce the incidence of type 1 diabetes compared with cow's milk–based formula. This outcome is consistent with the report of this trial that showed no difference between the study groups in the appearance of islet autoantibodies,[15] but is not consistent with data from the pilot study,[22] which reported that weaning to an extensively hydrolyzed formula in infancy was associated with a decrease in the frequency of disease-associated autoantibodies by the age of 7.5 years. That study was conducted in 230 Finnish children, while the current trial included 2159 high-risk children from 15 different countries, most participants being from Canada, Finland, and the United States. The larger number of participants in this study provides substantially greater statistical power in a more heterogeneous study population compared with

the pilot study and, therefore, provides a more definitive answer to whether weaning to an extensively hydrolyzed formula is protective of diabetes.

Overall, 173 participants (8.0%) progressed to type 1 diabetes during the follow-up for 11.5 years. This is close to an expected rate of 7.5% by the age of 10 years in the control group, on which the sample size estimate was based. For unknown reasons, the rate of diabetes was higher, although not significantly so, among females compared with males in the casein hydrolysate group. About 49% of the participants had a mother affected by type 1 diabetes, while only around 35% of those who presented with clinical disease had an affected mother. This reflects the well-known fact that offspring of mothers with type 1 diabetes have a reduced disease risk compared with offspring of affected fathers.[23,24]

Additional strengths of the current trial include a very high retention rate of participants and dietary adherence. The fact that the study was performed in 15 countries on 3 continents also supports the generalizability of the results. This study was planned to have 2 end points, namely (1) positivity for 2 autoantibodies by the age of 6 years and (2) clinical diabetes by the age of 10 years. While the previous report of this study showed no benefit in terms of a reduction in seroconversion to autoantibody positivity,[15] the follow-up of the trial participants to 10 to 14 years of age enabled the study to evaluate the possible effect of the treatment on progression from autoimmunity to diabetes.

The study was not designed to test the effect of breastfeeding because random assignment of infants to breastfeeding or formula feeding was not considered ethical. However,

no effect of exclusive breastfeeding was seen on progression to seroconversion or diabetes. Some prospective studies assessing the associations between infant feeding patterns and the development of β-cell autoimmunity in children who are at genetic risk for type 1 diabetes have not observed any associations between the duration of either exclusive or total breastfeeding and β-cell autoimmunity.[11,12] However, a recently published study involving children from the general population showed that no breastfeeding was related to an increased risk of diabetes compared with infants with history of any breastfeeding.[25]

The casein-based formula used as the intervention modality in this study was highly hydrolyzed and did not contain intact proteins. Less than 0.3% of the peptides had a molecular weight exceeding 2000 Da. Accordingly, the formula should be free of intact bovine insulin, which is present in cow's milk.[26] Vaarala et al[26] showed that infants fed a conventional cow's milk–based formula before the age of 3 months developed a strong immune response to bovine insulin, which differs from human insulin by 3 amino acids. Infants developing early signs of β-cell autoimmunity lacked the capacity to mount oral tolerance to bovine insulin. It has been speculated that sustained bovine insulin immunity might contribute to prediabetes progression, as weaning to an insulin-free formula reduced the cumulative incidence of autoantibodies by more than half in young children at genetic risk for type 1 diabetes.[27] The current data do not, however, support the bovine insulin hypothesis.

To our knowledge, this is the first trial to test with adequate power whether eliminating exposure to foreign intact protein in the infant diet could prevent type 1 diabetes in a genetically high-risk population. This trial suggests that cow's milk does not play a critical role in the development of type 1 diabetes.

Limitations

The results of this study are not directly generalizable to the background population because participants were selected based on a positive family history for type 1 diabetes and an HLA genotype conferring risk for type 1 diabetes. In addition, the outcome is not necessarily applicable to children with other HLA genotypes.

Conclusions

Among infants at risk for type 1 diabetes, weaning to a hydrolyzed formula compared with a conventional formula did not reduce the cumulative incidence of type 1 diabetes after a median follow-up for 11.5 years. These findings do not support a need to revise the current dietary recommendations for infants at increased risk for type 1 diabetes.

TRIGR Study Group Members: *Data Safety and Monitoring Board:* Thomas Mandrup-Poulsen (chair), Elja Arjas, Åke Lernmark, Esa Läärä, Barbara Schmidt, and Jeffrey P. Krischer (observer). *International Coordinating Center (ICC), Helsinki, Finland:* Hans K. Åkerblom, Mila Hyytinen, Mikael Knip, Katriina Koski, Matti Koski, Kristiina Merentie, Eeva Pajakkala, Antti Reunanen, Marja Salonen, Tuija Terhonen, and Seija Virkkunen. *Data Management Unit (DMU), Tampa, Florida:* David Cuthbertson, Bruce Gainer, David Hadley, Jeffrey P. Krischer, Jamie Malloy, Lavanya Nallamshetty, and Linda Shanker. *Canadian Coordinating Center, London and Ottawa, Ontario, Canada:* Brenda Bradley, John Dupré, Jeffrey L. Mahon, Gigi Lough, William Fraser, Margaret L. Lawson, Mathew Sermer, and Shayne P. Taback. *USA Coordinating Center, Pittsburgh, Pennsylvania, and Seattle, Washington:* Dorothy Becker, Margaret Franciscus, Anita Nucci, and Jerry Palmer. *Nutritional Epidemiology Unit, National Institute for Health and Welfare, Helsinki, Finland:* Kirsi Alahuhta, Sonja Bärlund, Tuuli Korhonen, Lea Kovanen, Eveliina Lehtonen, Sari Niinistö, Minna Pekkala, Susa Sorkio, Liisa Toivanen, Liisa Vähätalo, Suvi M. Virtanen, Ulla Uusitalo, and Taina Öhman. *Australia:* Children's Hospital, Westmead: Ros Bongiorno, Jacki Catteau, Glenda Fraser, Neville Howard, and Margaret Lloyd. *John Hunter Children's Hospital, Newcastle:* Patricia Crock, Michelle Giles, Krystyna Siech, and Denise Wong See. *Sydney Children's Hospital, Sydney:* Christina Brown, Jacki Catteau, Maria Craig, and Amanda Johnston. *Canada:* St Joseph's Health Care Centre (University of Western Ontario), London: Lynda J. Bere, Cheril L. Clarson, Morris Jenner, Jeffrey L. Mahon, Ruth McManus, Natale Renato, and Marge Lovell. *Children's and Women's Health Centre of British Columbia (University of British Columbia), Vancouver:* Debbie Higo, Nancy Kent, Jennifer Kwan, Colleen Marshall, Daniel Metzger, Jean-Pierre Chanoine, Laura Stewart, and David Thompson. *Alberta Children's Hospital (University of Calgary), Calgary:* Alun Edwards, Ian Lange, Julia Mercer, Daniele Pacaud, Ho Josephine, Wendy Schwarz, and David K. Stephure. *Stollery Children's Hospital (University of Alberta), Edmonton:* Jeanne Boer, Tameeza Chatur, Connie Chick, Bob Couch, Nestor Demianczuk, Rose Girgis, Seth Marks, Edmond Ryan, and Marilyn Thompson. *Children's Hospital Research Institute of Manitoba (University of Manitoba), Winnipeg:* Heather J. Dean, Lorna Grant, Kathy Hamelin, Janine LaForte, Liam Murphy, Daniel Catte, Carol Schneider, Elizabeth A. C. Sellers, Shayne P. Taback, and Vincent Woo. *Children's Hospital of Eastern Ontario and The Ottawa Hospital (University of Ottawa), Ottawa:*

Alice Boland, Brenda Bradley, Heather D. Clark, Tammy Cooper, Andrée Gruslin, Alan Karovitch, Erin Keely, Margaret L. Lawson, Gigi Lough, Janine C. Malcolm, Victor Sauro, and George F. Tawagi. *Mount Sinai Hospital/Hospital for Sick Children (University of Toronto), Toronto:* Santina Andrighetti, George Arnold, Jon Barrett, Ian Blumer, Denis Daneman, Diane Donat, Robert Ehrlich, Denice Feig, Irving Gottesman, Mathias Gysler, Samuel Karkanis, Anne Kenshole, Byrton Knight, Elyse Lackie, Valerie Lewis, Mary Jean Martin, Cynthia Maxwell, Gillian Oliver, Paul Panchum, Mathew Sermer, Nicholas Shilletto, Angelo Simone, Martin Skidmore, Tania Turrini, and Suzanne Wong. *CHU de Quebec (Université Laval), Quebec:* Christine Allen, Lise Bélanger, Isabelle Bouchard, Suzanne Ferland, Line Frenette, Maria Garrido-Russo, Mylene Leblanc, Jérôme Imbeault, Valérie Morin, Guy Olivier, and John Weisnagell. *Saint John Regional Hospital–a health care facility within Horizon Health Network (Dalhousie University):* Garry Costain, John Dornan, Kathy Heath, Mary-Catherine MacSween, Angela McGibbon, Carolyn Ramsay, Frank Sanderson, and Susan Sanderson. *L' Hôpital Sainte-Justine (University of Montreal), Montreal:* Linda Benabdesselam, William Fraser, Monique Gonthier, Céline Huot, and Maryse Thibeault. *Montreal Children's Hospital–McGill University Health Centre/Centre hospitalier universitaire e Sherbrooke (McGill University), Montreal:* Diane Laforte, Laurent Legault, and Patrice Perron. *IWK Health Centre (Dalhousie University), Halifax:* Anthony Armson, Paula Canning, Elizabeth A. Cummings, Vanda Ivanko, Lynne McLeod, Arati Mokashi, and Karen Scott. *Janeway Child Health Center (Memorial University), St John's:* Tracey Bridger, Joan Crane, Cheryl Crummell, Joseph C. Curtis, Colette Dawson, Carol Joyce, Leigh Anne Newhook, Sharon Newman, Eileen Druken, and Jahanara Begum-Hasan. *Kingston General Hospital (Queen's University), Kingston:* Adriana Breen, Robyn Houlden, and Marie Woods. *Regina General Hospital–Regina Qu'Appelle Health Region (University of Saskatchewan), Regina:* George Carrson and Sheila Kelly. *Royal University Hospital (University of Saskatchewan), Saskatoon:* Marie Jocelyn Martel, Marie Penner, and Koravangattu Sankaran. *Peterborough Regional Health Centre (University of Ottawa), Peterborough:* Karolyn Hardy-Brown, Nancy King, and Richard A. White. *Vancouver Island Health Research Centre (University of British Columbia), Victoria:* Marilyn Park, James Popkin, Laurie Robson, and Karen Coles. *Czech Republic:* Faculty Hospital Kralovske Vinohrady, Prague: Eva Al Taji, Marcela Cerna, Milos Cerny, Helena Francova, Irena Hainerova, Hana Kothankova, Renata Koukalova, Vladimira Krakorova, Pavla Mendlova, Radka Sitova, Katerina Stechova, Jan Vavrinec, Jan Vosahlo, and Blanka Zlatohlavkova. *Hospital Milosrdnych Bratri, Brno:* Ludmila Brazdova. *Faculty Hospital Olomouc, Olomouc:* Petra Faksova, Dana Gregorova, Lumir Kantor, Kamila Malkova, Jitrenka Venhacova, and Petra Venhacova. *Hospital of Masaryk, Usti nad Labem:* Adam Cipra and Jaroslav Skvor. *Hospital Ceske Budejovice, Ceske Budejovice:* Zdenka Tomsikova. *Faculty Hospital Plzen, Plzen:* Hana Botkova-Krauseova, Alice Mockova, and Petra Paterová. *Hospital of Bata, Zlin:* Pavla Gogelova and Jitka Kandrnalova. *Estonia:* Tallinn Children's Hospital, Tallinn: Ülle Einberg, Ülle Jakovlev, Svetlana Posiadlo, Eve Rannaste, Reet Raukas,

Mall-Anne Riikjärv, and Kairit Valla. *Tartu University Children's Hospital, Tartu:* Valve Astover, Anne Kirss, Anne Ormisson, Jana Retpap, Ene Täht, Vallo Tillmann, and Sille Vahtra. *Finland:* Children's Hospital, University of Helsinki, Helsinki: Hans K. Åkerblom, Maija Heikkilä, Minna Hirvasniemi, Kristiina Luopajärvi, Susanne Johansson, Päivi Kleemola, Mikael Knip, Elina Laukkanen, Anna Parkkola, Hanna-Mari Pigg, Hilkka Puttonen, Martin Renlund, Kirsi Salonen, Heli Suomalainen, Tarja Tenkula, and Kari Teramo. *Department of Obstetrics and Gynecology, University of Helsinki, Helsinki:* Anna-Liisa Järvenpää. *Jorvi Hospital, Espoo:* Anu-Maaria Hämäläinen, Rea Jussila, and Sanne Kiiveri. *Kymenlaakso Central Hospital, Kotka:* Hannu Haavisto, Seija Holopainen, Harriet Kupiainen, Tuula Leeve, Kari Lumme, Tuula Nironen, Maria Salonen, Sirpa Tenhola, and Teea Tiilikainen. *Paijat-Hame Central Hospital, Lahti:* Hilkka Keinonen, Pentti Lautala, Pia Salonen, and Maarit Vesanto. *Department of Pediatrics, Tampere University Hospital, Tampere:* Anna Susanna Aspholm, Paula Asunta, Heidi Ikävalko, Eeva Jason, Sinikka Jäminki, Päivi Kekki, Merja Koskinen, Susanna Lehtimäki, Jyrki Lähde, Mariariita Mäkelä, Sari Peltoniemi, Laura Poutiainen, Kari Ranta, Tiina Salonsaari, Sanna-Leena Sarviharju-Tujula, Jenni Selvenius, and Heli Siljander. *Satakunta Central Hospital, Pori:* Pirkko-Liisa Haanpää, Carita Holm, Annikki Juutilainen, Virpi Järveläinen, Anna-Mari Kangaskolkka-Keskilohko, Eila Laino, Liisa Marjamäki, Eila Suominen, and Samuli Ylitalo. *Central Finland Cental Hospital, Jyväskylä:* Marika Hokkanen, Raisa Lounamaa, Minna Matikainen, Anja Nuuja, Ilkka Paalanen, Anna Riikka Puupponen, and Heli Salo-Edwards. *South Ostrobotnia Central Hospital, Seinäjoki:* Soili Alanne, Tiina Kultti, Hilpi Linjama, Katja Muhonen, Maija Vääräniemi, and Timo Talvitie. *Hyvinkää Hospital, Hyvinkää:* Mirja Backman, Raija Hanhijärvi, Pirjo Koivula, Kaija Lindström, Anni Martikainen, and Pirjo Nurmi. *Department of Pediatrics, Kuopio University Hospital, Kuopio:* Anne Björk, Hanna Huopio, Jorma Komulainen, Soili Lehtomäki, Eeva Muikku, Jouni Pesola, and Ulla Sankilampi. *Department of Pediatrics, Oulu University Hospital, Oulu:* Tuula Arkkola, Anne Hekkala, Sanna Jurvakainen, Minna-Liisa Koivikko, Miia Kähönen, Erja Leinonen, Teija Mykkänen, Hilkka Pohjola, Kaisu Riikonen, Aune Niittyvuopio, Aino Stenius, Päivi Tapanainen, and Riitta Veijola. *Kanta-Hame Central Hospital, Hämeenlinna:* Abram Alar, Senja Jovio, Paavo Korpela, and Enja Mäkinen. *Vaasa Central Hospital, Vaasa:* Liisa Hietanen, Johanna Kivistö, Marja-Liisa Käär, Päivi Lehtimäki, Taina Mustila, Erik Popov, Sirkku Säätelä, and Leena Taittonen. *South Carelian Central Hospital, Lappeenranta:* Krista Ahtiainen, Nina Laaksonen, Minna Luoto, Juha Viitala, and Ritva Virransalo. *Mikkeli Central Hospital, Mikkeli:* Päivi Nykänen, Satu Paajanen, Satu Parkkinen, Heli Pyrhönen, and Terttu Särkkä. *Germany:* Kinder-und Jugendkrankenhaus–Auf der Bult, Hannover: Bärbel Aschemeier, Sevim Bektas, Torben Biester, Thomas Danne, Nicolin Datz, Dorothee Deiss, Maryam Fath, Olga Kordonouri, Kerstin Lüpke, Babette Müller, Claudia Nestoris, Silke Rothes, Evelin Sadeghian, and Kerstin Semler. *Hungary:* Semmelweis Medical University, Budapest: András Arató, Dóra Krikovszky, László Madácsy, András Nobilis, and József Szénási. *Italy:* University Campus Bio-Medico of Rome, Rome: Danila Benevento, Giuseppina Beretta Anguissola, Martina Biagioni, Carla Bizzarri, Valentino Cherubini, Lucia Ferrito, Carla Giordano,

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