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Removal of Bovine Insulin From Cow's Milk Formula and Early Initiation of Beta-Cell Autoimmunity in the FINDIA Pilot Study

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Objective: To test whether weaning to a bovine insulin–free cow's milk formula (CMF) reduces type 1 diabetes mellitus–associated autoantibodies in children at genetic risk.

Design: Randomized, double-blind pilot trial (Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes [FINDIA]).

Setting: Three pediatric hospitals in Finland from May 15, 2002, to November 22, 2005.

Participants: A total of 1113 infants with HLA-conferred susceptibility to type 1 diabetes were randomly assigned to receive study infant formulas; 908 children provided at least 1 follow-up blood sample (last follow-up, June 2009).

Intervention: The CMF (n=389), whey-based hydrolyzed formula (WHF) (n=350), or whey-based FINDIA formula essentially free of bovine insulin (n=365) during the first 6 months of life whenever breast milk was not available.

Main Outcome Measures: Primary outcome was beta-cell autoimmunity monitored at ages 3, 6, and 12 months and then annually until age 3 years. Autoantibodies to insulin, the 65-kDa isoform of glutamic acid decarboxylase, and the tyrosine phosphatase–related IA-2 molecule were screened, and islet cell autoantibodies and autoantibodies to zinc transporter 8 were analyzed in infants whose primary screening test results were positive.

Results: In the intention-to-treat analysis, 6.3% of children in the CMF group, 4.9% of those in the WHF group, and 2.6% of children in the FINDIA group were positive for at least 1 autoantibody by age 3 years. The odds ratios were 0.75 (95% CI, 0.37-1.54) in the WHF group and 0.39 (0.17-0.91) in the FINDIA group when compared with the CMF group. In the treatment-received analysis, the corresponding odds ratios were 0.81 (95% CI, 0.37-1.76) and 0.23 (0.08-0.69).

Conclusion: In comparison with ordinary CMF, weaning to an insulin-free CMF reduced the cumulative incidence of autoantibodies by age 3 years in children at genetic risk of type 1 diabetes mellitus.

Trial Registration: clinicaltrials.gov Identifier: NCT01055080

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TYPE 1 DIABETES MELLITUS (T1DM) is perceived as an immune-mediated disease resulting from T cell–mediated destruction of the insulin-producing beta cells in the pancreatic islets. Although controversial, epidemiologic studies have identified dietary wheat and cow's milk (CM)–derived antigens as candidate triggers of T1DM, but identification of the diabetogenic antigen has remained challenging.[1] A recent report[2] showed that the risk of beta-cell autoimmunity was reduced by the age of 10 years when infants at genetic risk of T1DM were weaned to an extensively hydrolyzed casein-based formula instead of a regular CM formula (CMF), but the mechanisms of protection were not studied. Earlier studies[3,4] have shown that exposure to bovine insulin in CMF induced

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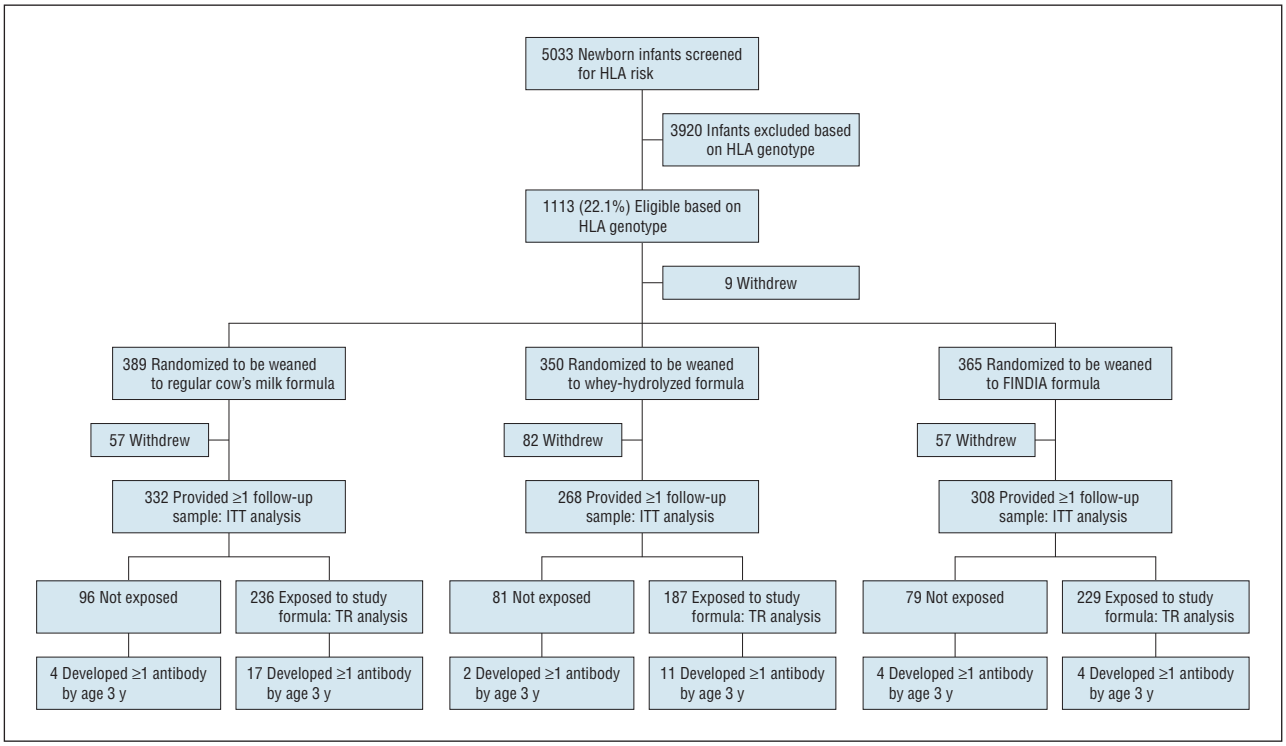

Figure 1. Participant flow diagram. FINDIA indicates Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes; ITT, intention-to-treat; and TR, treatment-received.

insulin-binding antibodies and T-cell responses to insulin in formula-fed infants. There is a difference in 3 amino acids, 2 in the A chain and 1 in the B chain, between human and bovine insulin. In humans, the immunogenicity of bovine insulin was recognized when it was used in therapy for patients with overt T1DM.[5] In animal studies, the immunogenic and diabetogenic nature of insulin have been shown[6,7] to depend on the replacement of a single amino acid residue.

According to our hypothesis, immunization to bovine insulin during early infancy at the time when maturation of the gut is incomplete could explain the findings implicating CM as a risk factor for T1DM. The induction of oral tolerance to dietary antigens is affected by age-induced maturation of the gut, which is also supported by breastfeeding.[8] During the first months of life, gut permeability decreases[9,10] and the number of intestinal IgA-positive plasma cells increases remarkably.[11] In animal studies, tolerance is classically induced only after infancy[12] and autoantigen administration in the neonatal period may exacerbate disease.[13] In the Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes (FINDIA) pilot study, we set out specifically to investigate whether bovine insulin is a diabetogenic factor in CMF. We tested whether weaning to a nonhydrolyzed whey-based bovine insulin–free formula during the first 6 months of life decreases the cumulative incidence of diabetes-associated autoantibodies by age 3 years. The results indicate that weaning to the essentially bovine insulin–free formula reduced or postponed the early initiation of beta-cell autoimmunity, which supports the role of bovine insulin as an early trigger of beta-cell autoimmunity and encourages further studies aimed at dietary prevention of T1DM on the basis of weaning to an insulin-free infant formula.

METHODS

STUDY DESIGN

The study was a multisite, randomized, controlled, double-blind clinical pilot trial in which newborn infants were randomized to receive standard CMF (Tutteli; Valio Ltd), a whey-based hydrolyzed formula (WHF) (Peptidi-Tutteli; Valio Ltd), or the whey-based FINDIA formula from which bovine insulin was removed according to the method described in patent EP1124436.[14] Study formulas were prepared and coded by Valio Ltd, which maintained the codes. Infants were allocated to the 3 study groups according to a computer-generated randomization list. Breastfeeding was encouraged; thus, a group of infants not exposed to any study formula became available during the study. Newborn infants requiring supplemental feeding in the delivery hospital received frozen collected and pooled breast milk or WHF. The codes were opened when statistical analysis of the emergence of diabetes-predictive autoantibodies was performed after all children in the follow-up groups had reached the age of 3 years. The study was approved by the ethics committees of the participating hospitals, and the families provided written informed consent.

PARTICIPANTS

Newborn infants were enrolled into the FINDIA pilot trial between May 2002 and November 2005 in 3 pediatric Finnish hospitals. Maternal T1DM, gestational diabetes treated with insulin, and preterm birth (<35 weeks of gestation) were exclusion criteria. Of 5033 children screened, 1104 infants (21.9%) were identified as having an eligible HLA risk genotype (**Figure 1**). The

Table 1. Duration of Study Formula Feeding and Follow-up Time in the Intervention Groups

Variable	CMF	WHF	FINDIA	Total	*P* Value
Duration of formula feeding, mo					
ITT					
No.	332	268	308	908	.01[a]
Median	2.71	1.04	2.83	2.37	
IQR	0.00-5.86	0.00-5.03	0.00-5.76	0.00-5.72	
Range	0.00-18.13	0.00-12.24	0.00-17.50	0.00-18.13	
TR					
No.	236	187	229	652	.002[a]
Median	5.10	3.62	5.13	4.74	
IQR	1.99-6.02	0.79-5.79	2.07-5.99	1.55-5.95	
Range	0.03-18.13	0.03-12.24	0.03-17.50	0.03-18.13	
Follow-up time, No. (%)					
ITT, y					
≥2	277 (83.4)	218 (81.3)	262 (85.1)	757 (83.4)	.45[b]
≥3	186 (56.0)	140 (52.2)	158 (51.3)	484 (53.3)	
TR, y					
≥2	198 (83.9)	153 (81.8)	190 (83.0)	541 (83.0)	.88[b]
≥3	124 (52.5)	96 (51.3)	115 (50.2)	335 (51.4)	

Abbreviations: CMF, cow's milk formula; IQR, interquartile range; ITT, intention to treat; FINDIA, Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes (insulin-free whey-based formula); TR, treatment received; WHF, whey-based hydrolyzed formula.
[a] Kruskal-Wallis test.
[b] χ^2 Test.

HLA-DQB1 genotyping was performed from cord blood to define selected alleles (*DQB1*02*, **0301*, **0302*, **0602*, and **0603*) associated with either susceptibility to or protection against T1DM.[15]

Infants carrying the *DQB1*02* allele were also evaluated for the possible presence of the *DQA1*05* allele. The children carrying the following genotypes were considered eligible for the trial: the high-risk *HLA-DQB1*02/DQB1*0302* genotype (13.4%), the moderate-risk *DQB1*0302/x* genotype (*x* ≠ *DQB1*0301* or **0602*; 54.2%), and the *HLA-DQA1*05-DQB1*02 (DR3)/y* genotype (*y* ≠ *DQB1*0301*, **0602*, or **0603*; 32.5%). The distribution of different HLA-associated risk genotypes did not differ significantly in the intervention groups.

DIETARY INTERVENTION

Breastfeeding was encouraged, and study formulas were given only when breast milk was not available during the dietary intervention period until the age of 6 months. The percentage of children who did not receive study formula during the first 6 months of life did not differ significantly between the intervention groups: 28.2% in the whole study, 28.9% in the CMF group, 30.2% in the WHF group, and 25.6% in the FINDIA group. All infant food products containing CM or beef were excluded during the intervention period. Adherence was monitored by regular family interviews. The duration of the use of the study formula differed in the intervention groups; the exposure time was shorter in the WHF group than in the FINDIA or CMF groups (**Table 1**).

MEASUREMENTS

At the follow-up visits, blood was drawn at 3, 6, and 12 months of age and annually thereafter up to the age of 6 years. Screening for autoantibody positivity included the analysis of insulin autoantibodies (IAA) and antibodies to the 65-kDa isoform of glutamic acid decarboxylase (GADA) and the tyrosine phosphatase–related IA-2 molecule (IA-2A) with specific radiobinding assays in the Scientific Laboratory, Children's Hospital, University of Helsinki, Helsinki, Finland.[16] The cutoff limits for positivity for the different autoantibody tests were determined as the levels corresponding to the 99th percentile of antibody levels in 354 nondiabetic Finnish children. We used cutoff limits for positivity of 2.80 relative units (RU) for IAA, 5.36 RU for GADA, and 0.78 RU for IA-2A. The disease sensitivity of the IAA assay was 44% and the specificity was 100% in the 2005 Diabetes Autoantibody Standardization Program Workshop.[17] The same characteristics of the GADA and IA-2A assays were 82%, 72% and 96%, 100%, respectively. Samples with IAA, GADA, or IA-2A levels between the 97th and 99.5th percentiles were reanalyzed for confirmation. Transplacentally transferred maternal antibodies were disregarded in the analysis. Islet cell autoantibodies (ICA) and autoantibodies to zinc transporter 8 (ZnT8A) were analyzed in individuals positive for at least 1 of the 3 initial autoantibodies. The cutoff levels for ICA and ZnT8A were 2.5 Juvenile Diabetes Foundation units and 0.61 RU, respectively.

Bovine insulin–binding IgG antibodies were analyzed in a subgroup of 463 infants at the age of 3 months and in 291 infants at the age of 6 months using an enzyme-linked solid-phase immunoassay method described elsewhere.[3]

Although the primary aim of this study was not to test the effect of intervention on the development of T1DM, we registered progression to clinical disease. The information on the diagnosis of T1DM was primarily derived from data obtained from pediatric clinics and information obtained during scheduled study follow-up visits.

STATISTICAL ANALYSIS

The difference in the duration of exposure to study formula feeding between the groups was compared by Kruskal-Wallis analysis, and the χ^2 test was used to compare the follow-up time. The primary variable was seroconversion to autoantibody positivity (IAA, GADA, or IA-2A) by the age of 3 years. The primary analysis was binary logistic regression analysis in the intention-to-treat population. It was appropriate to perform the treatment-received analysis because 28% of children did not receive the study formula. The preplanned pairwise comparisons were performed between the FINDIA and CMF groups and between the WHF and

Table 2. Positivity for at Least 1 or 2 Beta-Cell Autoantibodies by the Age of 3 Years[a]

	Intention-to-Treat Analysis				Treatment-Received Analysis[b]			
Variable	Formula	Positivity (%)	OR (95% CI)	*P* Value	Formula	Positivity (%)	OR (95% CI)	*P* Value
Positivity for ≥1 autoantibody[c]	CMF	6.3	1 [Reference]		CMF	7.2	1 [Reference]	
	WHF	4.9	0.75 (0.37-1.54)	.44	WHF	5.9	0.81 (0.37-1.76)	.59
	FINDIA	2.6	0.39 (0.17-0.91)	.03	FINDIA	1.7	0.23 (0.08-0.69)	.01
Positivity for ≥2 autoantibodies in the same sample[c]	CMF	3.0	1 [Reference]		CMF	3.4	1 [Reference]	
	WHF	2.2	0.74 (0.26-2.06)	.56	WHF		0.78 (0.25-2.43)	.67
	FINDIA	1.3	0.42 (0.13-1.37)	.15	FINDIA	0.9	0.25 (0.05-1.20)	.08
Positivity for ≥2 autoantibodies in the same sample or repeated positivity, ≥2 samples[c]	CMF	3.9	1 [Reference]		CMF	4.7	1 [Reference]	
	WHF	2.6	0.66 (0.26-1.67)	.38	WHF	3.2	0.68 (0.25-1.87)	.45
	FINDIA	1.9	0.49 (0.18-1.30)	.15	FINDIA	1.3	0.27 (0.07-0.99)	.048

Abbreviations: CMF, cow's milk formula; FINDIA, Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes (insulin-free whey-based formula);
OR, odds ratio; WHF, whey-based hydrolyzed formula.

[a] The ORs associated with the use of FINDIA or WHF vs CMF. Numbers of children in the intention-to-treat groups were CMF, 332; WHF, 268; and FINDIA, 308. Numbers of children in the treatment-received group were CMF, 236; WHF, 187; and FINDIA, 229

[b] Only the infants who received the study formulas during the first 6 months of life were included in the analysis. Breastfeeding was encouraged, and study formulas were given only when breast milk was not available during the dietary intervention period until the age of 6 months.

[c] All samples were screened for positivity for insulin autoantibodies and antibodies to the 65-kDa isoform of glutamic acid decarboxylase and the tyrosine phosphatase–related IA-2 molecule, and all follow-up samples from an individual testing initially positive for at least 1 autoantibody were analyzed for islet cell autoantibodies and autoantibodies to zinc transporter 8.

CMF groups according to the study hypothesis. The *P* values from the primary analysis were not adjusted.

The secondary variables were multiple positivity (≥2 of IAA, GADA, IA-2A, ICA, or ZnT8) or repeated positivity (≥2 samples with autoantibody positivity) by the age of 3 years and survival time without autoantibody positivity by the age of 3 and 6 years, separately. The analysis of secondary variables was supportive because it was conducted to confirm the results from the primary analysis. The *P* values from analysis of the secondary variables were not adjusted.

Binary logistic regression analysis was used to compare the cumulative incidence of beta-cell autoantibodies by the age of 3 years, and the results are given as odds ratios (ORs) with 95% CIs. Cox regression analysis was applied to compare the time without autoantibody positivity during the follow-up until the age of 3 and 6 years. In the Cox regression analysis, the 4 groups were compared: CMF, WHF, and FINDIA formulas as well as the group without exposure to study formula. The children who were lost during follow-up without a positive autoantibody test result were treated as censored cases. The proportionality of the hazards was checked using the cumulative hazard plot and the log-minus-log plot. The results are given as hazard ratios with 95% CIs. Kaplan-Meier curves were drawn to describe the survival without positive autoantibodies. Logistic regression and Cox regression analyses were performed without covariates. In addition, an adjustment in the analysis was made for the duration of the exposure time to study formula by treating it as a binary variable with a cutoff point at 3 months (<3 and ≥3 months) and for HLA genotypes (high, moderate, and low risk). Missing values in the primary or secondary variables were not imputed regardless of the reason (eg, missing blood sample or lost to follow-up).

The distributions of IgG class antibodies to bovine insulin were skewed to the right at ages 3 and 6 months and were logarithmically transformed before the analysis. Analysis of variance was used as a global test to compare the 4 groups, and 3 pairwise tests (WHF vs CMF, FINDIA vs CMF, and no exposure vs CMF) were performed using the Fisher LSD test. Because of logarithmic transformation, the paired comparisons are given as ratios WHF:CMF, FINDIA:CMF, and no exposure:CMF.

All tests were 2-tailed, and the level of significance was set at 5%. The statistical analysis was performed with commercial software (SPSS, release 17.0; SPSS Inc).

RESULTS

BETA-CELL AUTOIMMUNITY

A total of 908 children provided at least 1 blood sample during the follow-up from age 3 months for analysis of diabetes-associated autoantibodies (Figure 1). By the age of 3 years, 42 children (4.6%) had seroconverted to positivity for at least 1 autoantibody: 21 children (6.3%) in the CMF control group, 8 (2.6%) in the FINDIA group, and 13 (4.9%) in the WHF group. The ORs for autoantibody positivity by the age of 3 years are reported in **Table 2**. The use of bovine insulin–free FINDIA formula was associated with a reduced risk of at least 1 autoantibody (IAA, GADA, or IA-2A) and for repeated autoantibody positivity or positivity for multiple autoantibodies (≥2 of IAA, GADA, IA-2A, ICA, and ZnT8A) compared with the use of CMF. The number needed to treat was 19 in the FINDIA group compared with the CMF group for positivity for at least 1 autoantibody as well as for positivity for multiple autoantibodies or repeated positivity by age 3. The appearance of autoantibodies in the children is reported in eTable 1 (http://www.archpediatrics.com).

The timing for the first appearance of autoantibody positivity according to the treatment-received principle is presented up to age 6 years in the Kaplan-Meier curves shown in **Figure 2**. The number of children studied for beta-cell autoimmunity after the age of 4 years remains low.

The hazard ratios associated with the use of FINDIA formula, WHF, and for the group of children that was not exposed to study formula compared with the use of CMF are given by the Cox regression model in **Table 3**.

The outcome of the intervention was independent of the child's HLA genotype and of the duration of exposure to study formula feeding (data available on request).

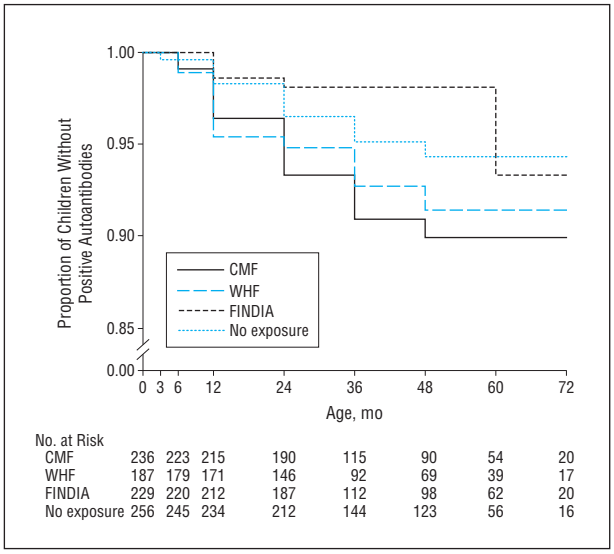

Figure 2. Kaplan-Meier curves for survival without positive autoantibodies according to the treatment-received analysis. Curves represent survival in infants who received cow's milk formula (CMF) (n=236), whey-based hydrolyzed formula (WHF) (n=187), or insulin-free whey-based formula (Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes [FINDIA]) (n=229) and in the group not exposed to any study formula (no exposure) (n=256). The CMF group was included as a reference group.

Table 3. Survival Without Positive Autoantibodies During the Follow-up Time 0 to 3 Years and 0 to 6 Years[a]

Follow-up Time	Formula	HR (95% CI)	*P* Value
0-3 y	CMF	1 [Reference]	
	WHF	0.82 (0.38-1.74)	.60
	FINDIA	0.24 (0.08-0.72)	.01
	No exposure	0.54 (0.25-1.18)	.13
0-6 y	CMF	1 [Reference]	
	WHF	0.85 (0.41-1.76)	.66
	FINDIA	0.39 (0.16-0.93)	.03
	No exposure	0.59 (0.29-1.23)	.16

Abbreviations: CMF, cow's milk formula; FINDIA, Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes (insulin-free whey-based formula); HR, hazard ratio; WHF, whey-based hydrolyzed formula.

[a] Numbers of children who were CMF, 236; FINDIA, 229; WHF, 187; and group that was not exposed to any study formula, 256. The results are given by the Cox regression model.

PROGRESSION TO MANIFEST DIABETES

According to our knowledge, 10 children had developed T1DM in the whole study group by December 2010. Three affected children were withdrawn from the study soon after birth without any follow-up data. In the follow-up group, 4 children were randomized to the WHF group and 3 children to the FINDIA group. One child in the FINDIA group did not receive study formula during the intervention period. According to the treatment-received principle, the number of progressors was 4 in the WHF group, 2 in the FINDIA group, and none in the CMF group. In the treatment-received analysis, 13 children (7.0%) developed T1DM and/or beta-cell autoimmunity in the WHF group, as well as 7 children (3.1%) in the FINDIA group and 18 (7.6%) in the CMF group during the 6-year follow-up period. Twelve children (4.7%) who were not exposed to study formulas developed T1DM and/or beta-cell autoimmunity.

INDUCTION OF BOVINE INSULIN–BINDING ANTIBODIES

The antibody levels binding to bovine insulin were higher in the CMF group than in the other groups; at 3 months, the ratio of antibody levels in comparison with the CMF group (n=108) was 0.62 (95% CI, 0.48-0.81; $P<.001$) in the WHF group (n=70), 0.79 (0.62-1.00; $P=.05$) in the FINDIA group (n=95), and 0.56 (0.45-0.68; $P<.001$) in the group not exposed to study formula (n=190). At 6 months, the corresponding ratios in comparison with the CMF group (n=67) were 0.43 (95% CI, 0.30-0.62; $P<.001$) in the WHF group (n=61), 0.70 (0.51-0.97; $P=.03$) in the FINDIA group (n=63), and 0.51 (0.36-0.72; $P<.001$) in the group not exposed to study formula (n=70).

COMMENT

Weaning to a whey-based cow's milk formula essentially free of bovine insulin reduced the early induction of beta-cell autoimmunity during the first 3 years of life compared with the use of conventional CMF. The reduced OR for autoantibody positivity was more pronounced when only children who received study formulas were included in the treatment-received analysis, which emphasizes the effect of the intervention. Because of the short follow-up time, we cannot conclude whether weaning to bovine insulin–free formula during the first 6 months of life protects against or postpones early induction of beta-cell autoimmunity. The importance of early induction of beta-cell autoimmunity has been strengthened by recent studies[16] in which autoantibody positivity during the first 3 years of life was associated with high risk of progression to T1DM when compared with seroconversion later in life. In addition, weaning to the insulin-free FINDIA formula was associated with a reduction in the frequency of positivity for multiple autoantibodies, which is highly predictive for T1DM among individuals carrying increased HLA-conferred disease susceptibility.[16] The development of clinical T1DM is a rare phenomenon before the age of 3 years, and the number of progressors was thus low in our study. The assessment of a potential preventive effect in terms of T1DM requires a longer follow-up time as seen in the TRIGR (Trial to Reduce IDDM [Insulin-Dependent Diabetes Mellitus] in the Genetically at Risk) pilot study.[2] Our results support the hypothesis of a critical role of dietary bovine insulin as an early trigger of beta-cell autoimmunity in infancy.

Bovine insulin may sensitize intestinal T lymphocytes early in life for later participation in the autoimmune destruction of the insulin-secreting beta cells based on the immunologic gut-pancreas axis.[1] Intestinal antigens are capable of stimulating lymphocytes infiltrating the pancreas, as shown when the activation of oral antigen-specific T cells in the pancreatic lymph nodes was dem-

onstrated experimentally.[18] Furthermore, lymphocytes may circulate between the gut and pancreas, since lymphocytes infiltrating the Langerhans islets express the gut-homing receptor α-4β-7-integrin, and the islet endothelium displays its ligand MadCam-1.[19-21]

In animal models, oral insulin has been studied as an inducer of oral tolerance in the strategy to prevent T1DM.[22] Mucosal autoantigen administration is, however, a double-edged sword, and in rodents it can lead not only to regulatory and protective immunity but also to pathogenic, tissue-destructive immunity and exacerbation of autoimmune disease.[22-24] The majority of children exposed to dietary antigens, such as bovine insulin, develop oral tolerance and not beta-cell autoimmunity; therefore, bovine insulin cannot be considered diabetogenic per se. In animal models, parenteral immunization with insulin or its peptides was able to induce immune-mediated T1DM only in the context of appropriate major histocompatibility complex molecules and with engineer-enhanced diabetes susceptibility, namely, poly I:C in immunization and transgenic expression of the costimulatory B7-1 molecule in the pancreatic beta cells.[25] In human T1DM, the dysregulation of the gut immune system might result in altered immune responses to dietary insulin (and other dietary antigens). Dysfunction of oral tolerance has been reported[3,26] in children who later develop T1DM or beta-cell autoimmunity, not only against dietary insulin but also against CM proteins in general. Wheat protein–induced intestinal inflammation and enteroviral infections have been proposed as triggers of intestinal inflammation in T1DM[27-29] and could serve as enhancers for the development of insulin-specific autoimmunity leading to beta-cell destruction. Furthermore, aberrancies in the gut immune system, such as increased permeability[30,31] and intestinal inflammation,[27,32] have been reported in T1DM.

We could not observe any reduction in signs of beta-cell autoimmunity among children who received a WHF, although the use of an extensively hydrolyzed casein-based formula reduced beta-cell autoimmunity by age 10 years in the TRIGR pilot study.[2] Both FINDIA and WHF are whey-based formulas, but the reduction of beta-cell autoimmunity was demonstrated only with the FINDIA formula, which questions the role of caseins in the development of T1DM.[33] In the WHF, insulin was hydrolyzed and we observed very low levels of antibodies binding to whole insulin by enzyme-linked solid-phase immunoassay. It is possible that the nature of peptides, insulin, and other derived whey proteins differs between the extensively hydrolyzed formulas resulting from differences in the hydrolysis process. The composition of the peptides in casein-based formulas has been shown to affect the development of intestinal microbiota and permeability, which are regulators of autoimmune diabetes.[34,35] In addition, the composition of insulin peptides may differ between hydrolyzed formulas. Insulin A chain peptide 1-13 contains a sequence that is resistant to enzymatic hydrolysis with gastric enzymes, trypsin, pepsin, or chymotrypsin[36] and includes the known difference of 2 amino acids between human and bovine insulin. This part of the A chain has also been recognized as an immunogenic T-cell epitope in T1DM.[37-39] Insulin peptides in WHF may be able to induce harmful T-cell responses similar to wheat gliadin peptides in celiac disease.[40] The role of altered peptides in the activation of autoantigen-specific T-cell reactivity and loss of tolerance to primary autoantigens has been implicated by studies on insulin peptides in nonobese diabetic mice.[6,7,25] Accordingly, bovine insulin peptides may act as environmental mimotopes of autoantigenic peptides and result in nontolerogenic T cells that activate autoreactivity against the primary autoantigen, ie, human insulin expressed in the islets.

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