Effect of Early Feeding on Intestinal Permeability and Inflammation Markers in Infants with Genetic Susceptibility to Type 1 Diabetes: A Randomized Clinical Trial

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Objectives To assess whether weaning to an extensively hydrolyzed formula (EHF) decreases gut permeability and/or markers of intestinal inflammation in infants with HLA-conferred diabetes susceptibility, when compared with conventional formula.

Study design By analyzing 1468 expecting biological parent pairs for HLA-conferred susceptibility for type 1 diabetes, 465 couples (32 %) potentially eligible for the study were identified. After further parental consent, 332 babies to be born were randomized at 35th gestational week. HLA genotyping was performed at birth in 309 infants. Out of 87 eligible children, 73 infants participated in the intervention study: 33 in the EHF group and 40 in the control group. Clinical visits took place at 3, 6, 9, and 12 months of age. The infants were provided either EHF or conventional formula whenever breastfeeding was not available or additional feeding was required over the first 9 months of life. The main outcome was the lactulose to mannitol ratio (L/M ratio) at 9 months. The secondary outcomes were L/M ratio at 3, 6, and 12 months of age, and fecal calprotectin and human beta-defensin 2 (HBD-2) levels at each visit.

Results Compared with controls, the median L/M ratio was lower in the EHF group at 9 months (.006 vs .028; $P = .005$). Otherwise, the levels of intestinal permeability, fecal calprotectin, and HBD-2 were comparable between the two groups, although slight differences in the age-related dynamics of these markers were observed.

Conclusions It is possible to decrease intestinal permeability in infancy through weaning to an extensively hydrolyzed formula. This may reduce the early exposure to dietary antigens. *(J Pediatr 2021;238:305-11)*.

Trial registration Clinicaltrials.gov: NCT01735123.

Studies on the pathogenesis of the type 1 diabetes (T1D)[1] have implied that impaired gut permeability may play a role in the disease process.[2] Increased intestinal permeability has been reported both before and after the diagnosis of T1D, although findings are controversial.[3-5] Intestinal permeability matures with age: permeability is high in preterm newborn infants and decreases during the first week of life to levels observed in term babies.[6-8]

In rodent models, an extensively hydrolyzed casein diet improved the intestinal gut barrier function and decreased the incidence of future autoimmune diabetes.[9-11] The effects of the hydrolyzed casein diet on the infants' gut are still largely unknown, but assuming they resemble those observed in animal models, weaning to an extensively hydrolyzed formula (EHF) might decrease the leakage of the intestinal mucosa, and the incidence of T1D in genetically susceptible children.[12] Indeed, preliminary findings from the Trial to Reduce IDDM in the Genetically at Risk (TRIGR) pilot study implied that such a weaning strategy might decrease by half the cumulative appearance of diabetes-associated autoantibodies by 10 years of age.[13] However, in the full-scale TRIGR study, the cumulative incidence of positivity for multiple (≥2) autoantibodies[12] and the incidence of clinical T1D[14] were comparable between children weaned to EFH vs a conventional formula. As no clear explanations to this discrepancy were identified in previous analyses, we set out to study further the effects of this weaning strategy on gut permeability and markers of intestinal inflammation in infants with

EHF	Extensively hydrolyzed formula
IDDM	Insulin dependent diabetes mellitus
HBD-2	Human beta-defensin 2
L/M	Lactulose/mannitol
T1D	Type 1 diabetes

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HLA-conferred susceptibility to T1D. We hypothesized that early EHF diet might decrease intestinal permeability and reduce the levels of fecal calprotectin and human beta-defensin 2 (HBD-2). The latter 2 have been used as biomarkers for intestinal inflammation, even though increased calprotectin more clearly reflects mucosal inflammatory processes, whereas elevated levels of the antimicrobial HBD-2 associate with perturbations in the intestinal microbial communities.[15,16] In addition to these main questions, our study provides novel information on the natural history of gut permeability and the degree of intestinal inflammation during the first year of life in healthy full-term infants.

Methods

We conducted a randomized, double blind clinical trial in Tampere, Finland. Whenever breast milk was not available and/or additional feeding was required, infants with HLA-conferred susceptibility to T1D were provided either extensively hydrolyzed casein-based formula (EHF group) or conventional cows' milk-based formula (control group, **Figure 1**; available at www.jpeds.com). To reach the estimated statistical power of >80 % for detecting a 50 % reduction in the intestinal permeability, 82 infants were to be randomized 1:1 in the 2 groups (**Appendix**; available at www.jpeds.com). The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki. The local ethics committee approved the study, and written informed consents were acquired from the parents as described below.

Participants

Pregnant women were recruited from January 2013 to February 2015. Families were contacted at the fetal ultrasonography visit around gestational week 20. After informed consent, the expecting parents were analyzed for T1D-associated HLA genotypes. Pairs who proved to be expecting an offspring potentially carrying T1D-associated risk genotypes were contacted by the third trimester. Informed consents from the parents were obtained for analyzing the newborn infants' genotypes at birth and for the eligible infants' participation in the intervention study. Eligible genotypes, analyzed as previously described,[17] were the high-risk genotype (*DRB1*03-DQA1*05-DQB1*02* with *DRB1*0401/2/4/5-DQA1*03-DQB1*03:02*), and the moderate-risk genotypes (homozygosity for either of the above; *DRB1*04:01/2/4/5-DQA1*03-DQB1*03:02* with a neutral haplotype, or the *DRB1*03-DQA1*05-DQB1*02/DRB1*09-DQA1*03-DQB1*03:03* genotype). Neutral haplotypes comprised *DRB1*01-DQB1*05:01, DRB1*08-DQB1*04, DRB1*07-DQA1*02:01-DQB1*02,* and *DRB1*09-DQA1*03-DQB1*03:03*.

Exclusion criteria were: (1) an older sibling participating in the study; (2) multiple gestation; (3) the parents unwilling to feed the infant any cows' milk based products; (4) prematurity (gestational age at birth <35 weeks); (5) technical challenges hindering the participation; (6) severe neonatal illnesses and/or abnormalities; (7) infant fed with other type of formula than the study formula in the delivery hospital; (8) no HLA sample available from the newborn before the age of 8 days.

Study Protocol

Eligible pregnant women came to their first clinical study visit at the beginning of the last trimester. Randomization for the study formula was performed during the 35th gestational week, and families received the first batch of the study formula before the delivery. Cord blood samples were used for the HLA genotyping in the newborn infants, and the families were informed about the genotyping results within 10 days after the delivery. Eligible infants visited the study center at the age of 3, 6, 9, and 12 months. During the visits, blood samples were taken and the lactulose-mannitol (L/M) tests were performed in order to assess the intestinal permeability. Stool samples were collected at the age of 2 weeks and monthly thereafter.

The recruited mothers were encouraged to breastfeed. Study formulas were used as a part of the diet until the age of 9 months, whenever infants needed additional feeding. During the intervention period, the infants were on a diet free of cow-based proteins, while their mothers' diet remained unaltered. Study formula use and the compliance to the intervention diet were monitored with frequent dietary questionnaires and interviews (at the age of 2 weeks, monthly between the age of 1 and 9 months, and at 12 months of age). Follow-up ended when the infant reached the age of 12 months, but participants were offered a possibility to continue in the Finnish Type 1 Diabetes Prediction and Prevention study follow-up.[18]

The primary outcome of this study was the L/M ratio at 9 months of age. By the protocol, at that age the intended exposure to study formula could have reached 90 days, even if the infants were exclusively breastfed for the first 6 months. The secondary outcomes were the L/M ratios at 3, 6, and 12 months, and the levels of fecal calprotectin and HBD-2 at the age of 3, 6, 9, and 12 months. Details regarding these tests, other analyses, nutritional contents of the formulas, and adverse events during the intervention are reported in the **Appendix**.

Randomization

The infants were randomized to 1 of the 4 color-coded, blinded formulas, 2 of which contained the EHF and 2 the control formula. A data handling software (BC CLIN version 3.6, Biocomputing Platforms Ltd) was used for the randomization (1:1 block permutation). The manufacturer of the formulas (Mead Johnson Nutrition, Glenview, IN) kindly provided packing and labeling of the study formulas, as well as guarded the randomization codes during the intervention period. Participating families and all study personnel remained blinded until the last participant's 12-month visit had been completed.

Table I. Maternal and infant characteristics

	Study formula group	
	Control (n = 40)	EHF (n = 33)
Mothers		
Age, years (range; SD)	32.7 (21.0-44.3; 4.8)	32.4 (25.2-45.4; 4.8)
Smoking, n (%)[a]	3 (7.5)	4 (12.1)
Parity prior to the study participant, n (SD)	1.0 (.97)	1.0 (.68)
Infants		
Birth weight, grams (range; SD)	3630 (2960-4410; 381)	3570 (2650-4720; 419)
Birth length, cm (range; SD)	50.0 (47.0-54.0; 1.5)	50.0 (46.0-55.0; 1.9)
Gestational age, weeks (range; SD)	40.3 (37.0-42.4; 1.2)	39.7 (36.6-42.1; 1.3)
Males, n (%)[b]	24 (60.0)	11 (33.3)
Route of delivery: Cesarean, n (%)	4 (10)	3 (9.1)
High-risk HLA genotype, n (%)	13 (32.5)	7 (21.2)
Positivity for one or more T1D-associated autoantibody by 12 months of age, n (%)	2 (5.0)	3 (9.1)

EHF, extensively hydrolyzed casein-based formula.
[a]Continuous variables are medians, if not otherwise indicated.
[b]Due to prenatal randomization Chi^2 $P = .023$; other distributions comparable between the 2 groups.

Results

HLA-genotyping was performed in 1468 expecting mothers and fathers (Figure 1). Based on the parental genotypes, 462 of their future offspring (31.5 %) were potentially eligible for the intervention trial. Out of the 462 pregnant women, 332 (71.9 %) consented to the follow-up and HLA genotyping of their offspring. At the 35th gestational week, 332 future infants were randomized, 161 (48.5 %) to the EHF group and 171 (51.5 %) to the control group. After delivery, 309 newborn infants were HLA-genotyped and 87 of them (28.2 %) were eligible for the intervention trial. Thirteen infants never participated in the intervention due to neonatal health problems, exposure to a formula other than the study formula, or due to declining further participation.

Seventy-three infants, 33 (45.2 %) participants in the EHF group and 40 (54.8 %) participants in the control group completed the trial. As the prenatal randomization included no sex adjustments, males were over-represented in the control group (Table I). Otherwise, the 2 groups were comparable regarding their basic characteristics and growth-related measures. Neither were there any significant differences in health-related and nutritional characteristics between the 2 groups (Table II). Seven participants received only breast milk throughout the intervention period and had no exposure to study formula by the age of 9 months (group of breastfed infants with no other milk exposure). All participants were introduced to other foods according to normal feeding recommendations. The compliance was relatively poor considering per protocol analyses (daily use of study formula at least 90 days by the age of 9 months; n = 16 in the EHF and n = 15 in the control group), and no further per protocol analyses were performed.

Intestinal Permeability

Intestinal permeability (L/M ratio) declined with advancing age and maturation of the gut, and the decrease was mostly obvious between the age of 6 to 9 months (Figure 2 and Table III). According to the intention-to-treat analysis, intestinal permeability was lower in the EHF group infants at the age of 9 months (Table III), even if breastfed infants (with no other milk exposure) were analyzed as a separate group (Figure 3 and Table IV; available at www.jpeds.com). No other significant differences between the intervention groups (intention-to-treat analyses) or nutritional groups

Table II. Health- and nutrition-related characteristics in the two groups

	Study formula group		
	Control (n = 40)	EHF (n = 32)	*P* value
Episodes of otitis media during the first year of life, (range; SD)	0 (0-6; 1.0)	0 (0-6; 1.7)	.11
Episodes of gastroenteritis during the first year of life (range; SD)	0 (0-2; 0.7)	0 (0-3: 0.7)	.14
Number of antibiotic courses during the first year of life, (range; SD)	0 (0-7; 1.2)	0 (0-7; 2.1)	.19
Use of probiotics during the first 3 months of life; n (%)	30 (75)	25 (78)	1.00
Exclusively breastfed, n (%)	3 (7.5)	4 (12.1)	.69
Age when study formula was introduced, days (range; SD)	120 (0-260; 89.5)	8 (1-252; 89.9)	.29
Age at the end of breastfeeding, days (range; SD)	359 (53-735; 168)	344 (11-487; 144)	.87
Age when other foods were introduced, days (range; SD)	133 (71-202; 3)	131 (80-188; 26)	.80
Duration of the daily study formula use, days (range; SD)	77 (0-329; 105)	89 (0-365; 108)	.59
Amount of study formula per day, g (range; SD)	16 (0-168; 45)	15 (0-141; 42)	.64

Continuous variables are medians, if not otherwise indicated.

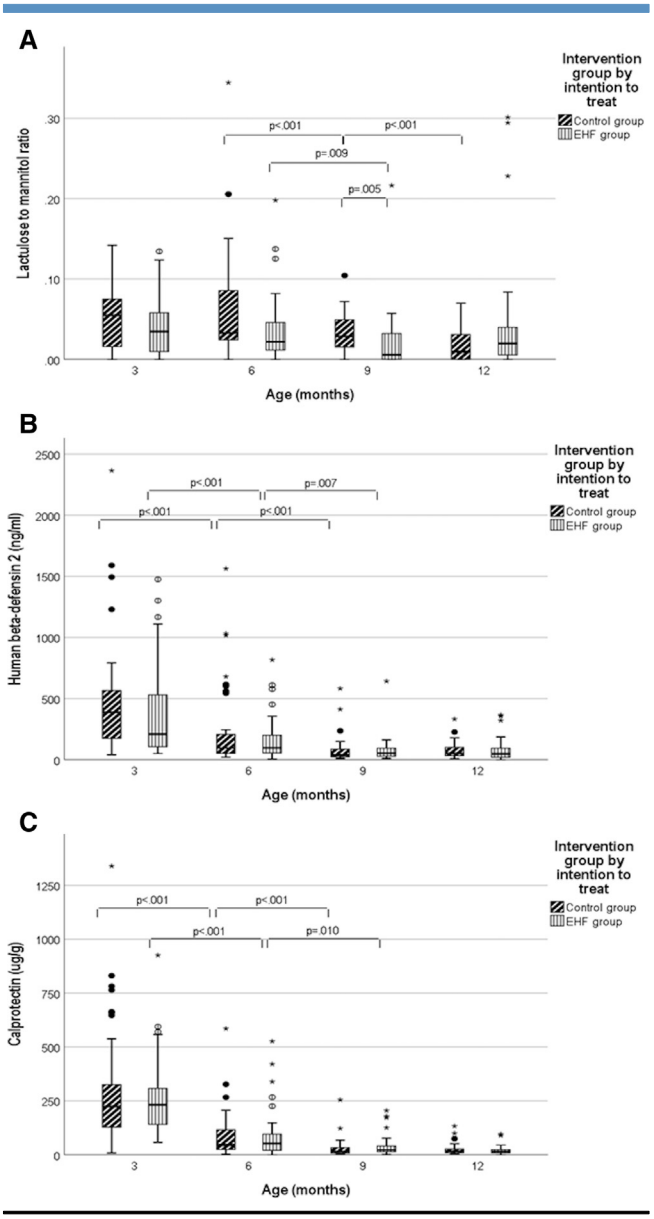

Figure 2. **A**, L/M ratio, **B**, fecal HBD-2, and **C**, calprotectin levels in the 2 intervention groups according to intention-to-treat analysis. Upper outlines of the boxes represent the 75th percentiles and the lower outlines the 25th percentiles of the values, and the whiskers indicate the minimum and maximum values (statistical outliers marked with dots and extreme values with asterisks). *P* values indicate statistical differences between the groups (Mann Whitney U Test) and between different time points (Wilcoxon Signed Rank Test). The main findings indicated in the figures with *P* values; detailed analyses available in **Table II**. *EHF*, extensively hydrolyzed casein-based formula.

(comparisons between infants in the EHF, control and breastfed group) were observed regarding the intestinal permeability at the visits. The decrease in the median permeability occurred slightly differently in the three nutritional groups (**Figure 3** and **Table III**). No interaction between sex and infant formula group was observed at any age. The decrease continued through 3 to 12 months (from 0.053 to 0.008; $P < .001$) in the control group, and it occurred between 3 to 9 months (from 0.031 to 0.005; $P = .016$) in the EHF group. In the breastfed infants the longitudinal permeability changes were statistically non-significant.

Intestinal permeability levels at various ages showed strong correlations with each other (Spearman rho correlation coefficients [r_S] between .405 and .575; **Table IV**), but not with calprotectin levels. Only at the 3-month visit, permeability correlated with HBD-2 levels at 3 and 6 months (r_S .239 and .340, respectively). Regarding categorical factors, higher permeability levels were observed at 6 months for infants with preceding acute gastroenteritis (.093 vs .030 in unaffected infants; $P = .040$), and at 9 months in males (.031 vs .006 in females; $P = .005$) and in participants with the high-risk HLA genotype (.030 vs .014 in participants with moderate risk genotypes; $P = .048$).

Intestinal Inflammation Markers

No significant differences in the levels of fecal calprotectin or HBD-2 were observed between the intervention groups or nutritional groups at any given time points (**Figure 2**, **Figure 3**, **Tables III** and **IV**). In the case of fecal calprotectin, we observed an interaction between sex and infant formula group at the age of 9 month ($P = .020$), so that the intervention was associated with higher fecal calprotectin levels in girls only. Levels of fecal calprotectin and HBD-2 had significant intercorrelations (r_S between .250 and .564), and both showed normal physiological declines mainly by the age of 9 months.

Three month's HBD-2 correlated with the infant's birth length. HBD-2 levels were lower at 6 months in infants with preceding acute gastroenteritis (38.0 vs 97.0 ng/ml in unaffected infants). At 12 months, lower HBD-2 values were observed in females (36.0 vs 67.0 ng/ml in males) and in infants receiving daily probiotics (35.0 vs 58.0 ng/ml in infants without probiotics). Calprotectin levels were lower at 3 months in infants developing T1D-associated autoantibodies during their first year of life (123.3 vs 231.4 ug/g in seronegative participants; $P = .047$). They were also lower at 6 and 9 months in infants with acute gastroenteritis between the age of 3 and 6 months (11.7 and 5.4 vs 48.6 and 16.6 ug/g in unaffected infants; $P = .002$ and $P = .046$, respectively). Interestingly, lower calprotectin level at 6 months associated also with upcoming acute gastroenteritis (1.7 vs 48.6 ug/g in unaffected participants; $P = .008$).

Discussion

This study tested the effect of weaning to different formulas on the intestinal permeability and two intestinal inflammation markers. The outcome showed that weaning to EHF resulted in lower permeability at the age of 9 months when compared with a conventional formula. At the age of 9 months a higher proportion of the infants were consuming

Table III. L/M ratio and fecal HBD-2 and calprotectin levels in the 2 intervention groups according to intention-to-treat analysis

	Control group			EHF group			
	N	Median (IQR)	Between visits, P^a	N	Median (IQR)	Between visits, P^a	Between study groups, P^b
L/M ratio							
At 3 months	38	.055 (.016-.075)	3 to 6 months: .802	32	.035 (.008-.059)	3 to 6 months: 0.76	.117
At 6 months	37	.033 (.024-.086)	6 to 9 months: .001	32	.022 (.011-.047)	6 to 9 months: .009	.134
At 9 months	35	.028 (.015-.050)	9 to 12 months: .001	30	.006 (.000-.032)	9 to 12 months: .259	.005
At 12 months	33	.009 (.000-.031)	3 to 9 months: .009	31	.020 (.005-.043)	3 to 9 months: .010	.169
			3 to 12 months: <.001			3 to 12 months: .074	
			6 to 12 months: <.001			6 to 12 months: .510	
HBD-2 (ng/ml)							
At 3 months	40	388.0 (170.0-584.0)	3 to 6 months: <.001	33	209.2 (100.0-535.0)	3 to 6 months: <.001	.061
At 6 months	40	95.5 (50.0-232.0)	6 to 9 months: <.001	33	96.0 (54.5-251.0)	6 to 9 months: .007	.764
At 9 months	39	38.0 (22.5-92.0)	9 to 12 months: .524	31	52.0 (26.0-109.0)	9 to 12 months: .990	.444
At 12 months	37	51.0 (26.0-94.0)	3 to 9 months: <.001	30	54.0 (23.0-112.5)	3 to 9 months: <.001	.923
			3 to 12 months: <.001			3 to 12 months: <.001	
			6 to 12 months: .002			6 to 12 months: .030	
Calprotectin (ug/g)							
At 3 months	40	224.2 (136.1-331.1)	3 to 6 months: <.001	33	231.4 (134.0-309.7)	3 to 6 months: <.001	.914
At 6 months	40	45.2 (24.6-119.2)	6 to 9 months: <.001	33	52.5 (22.6-114.3)	6 to 9 months: .010	.656
At 9 months	39	15.1 (8.0-34.0)	9 to 12 months: .902	31	22.7 (13.1-45.1)	9 to 12 months: .007	.157
At 12 months	37	16.4 (8.4-27.4)	3 to 9 months: <.001	30	15.2 (8.5-24.2)	3 to 9 months: <.001	.595
			3 to 12 months: <.001			3 to 12 months: <.001	
			6 to 12 months: <.001			6 to 12 months: <.001	

P values indicate statistical differences between the groups[b] and between different time points[a].

the study formula than at earlier time points, and from that point of view our observation is not surprising. The lower gut permeability in the EHF group leads to a reduced exposure to foreign antigens through the intestinal wall, which will likely affect the training of the host immune system. No differences were observed in fecal calprotectin and HBD-2 levels between the two groups over the first year of life.

Intestinal permeability has previously been reported to be comparable in breastfed and formula-fed infants, although the physiological decline has been postulated to be faster in breastfed infants.[19] In the current study, intestinal permeability decreased with age during the first year of life in all study groups. However, the dynamics of the permeability changes differed between the intervention groups: compared with control infants, the main decline was observed 3 months earlier (between the age of 6 and 9 months) in infants weaned to EHF. Considering the three nutritional groups, between 9 to 12 months of age both breastfed and control infants had still decreasing permeability, whereas participants in the EHF group had somewhat increasing values. This increase was likely due to the recent introduction of novel dietary proteins into the expanding repertoire of nutrients in the infants in the EHF group. It appears that early nutrition with EHF, containing small peptides instead of whole proteins, leads to lower intestinal permeability at an earlier age, and the effects of breast milk and conventional formula on the dynamics of the intestinal permeability are comparable, even if these 2 types of nourishment are immunologically remarkably different.[20]

As previously reported,[6,8,21,22] inverse correlations between age and markers of intestinal inflammation and permeability were notable also in the current analyses (r_S between −.255 and −634; $P < .001$ for all comparisons). Fecal calprotectin and HDB-2 levels decreased between the age of 3 and 9 months in both intervention groups and in all nutritional groups. Age explained associations between intestinal permeability and HBD-2 levels, but correlations between HBD-2 and calprotectin values remained significant even after taking age into account (partial r = .343; $P < .001$). The high HDB-2 levels (**Figure 3**) observed at 3 months may be explained by the fact that at that age, the majority of the infants were receiving breast milk. In addition to various microbial components and immunomodulatory molecules, breast milk contains hyaluronan, which is an inducer of HBD-2.[20,23]

Although the 2 intervention groups had dissimilar permeability at 9 months, their fecal calprotectin and HBD-2 levels were comparable, suggesting that the higher permeability observed in controls did not associate with intestinal inflammation. That levels of the 2 inflammation markers were comparable between the groups contradicts a previously reported observation[21] that breastfed infants have higher fecal calprotectin levels than their formula-fed peers. Assuming that fecal calprotectin is a biomarker for intestinal inflammation, as previously described,[2,15] low calprotectin levels might indicate reduced extra-intestinal exposure to foreign antigens and abated inflammatory responses.

Previous reports have suggested that compared with healthy controls, both L/M ratio and lactulose excretion are higher in subjects with preclinical T1D and in patients with established T1D.[3,4] Compared with controls, patients

with T1D carrying the HLA-DQB1*02 allele have been reported to have an increased permeability for mannitol and lactulose, but comparable intestinal permeability.[5] In the current analyses, no such differences were observed. The only T1D-autoimmunity related finding was that infants with early islet autoimmunity had lower calprotectin levels at 3 months than their seronegative peers. Mannitol, a small sugar molecule, uses transcellular pathways to permeate the intestinal epithelium, whereas the larger lactulose molecules pass the mucosa through paracellular routes.[4] These sugars have different peak excretion times, and several factors, such as pro-inflammatory stimuli, affect their excretion dynamics.[24] To minimize the effects of the variable gastric emptying times and mucosal surface areas,[8] we used a 5-hour collection time to cover the peak excretion times of both sugars.

Some limitations have to be taken into consideration when assessing the outcome of the current trial. The study protocol was demanding and time-consuming for the families. Thus, the number of the consenting families was limited, leading to a smaller participation rate than expected. Performing a successful lactulose/mannitol test is challenging in infants: not all babies are eager to drink the test solution, and the plastic bags used for the urine collection leak easily, if not carefully followed. These circumstances produced limitations to the measurements, and 2 unreliable results had to be excluded from the final analyses. The uneven sex distribution between the 2 groups may induce a bias. Therefore we carried out a sensitivity analysis by including sex, infant formula group, and their interaction term in the analysis of the intestinal permeability. No interaction was observed between sex and the intervention. Regarding potential generalization of our results, one should keep in mind that our participants, children carrying HLA-conferred susceptible to T1D, may have currently unknown inherited and/or acquired differences in their absorptive system and their intestinal immunology, when compared with their non-susceptible peers.

The current results show that it is possible to affect the intestinal permeability with a dietary intervention in infancy. The decreased gut permeability at the age of 9 months in the infants fed extensively hydrolyzed formula implies that exposure to dietary antigens may be reduced in these infants. Whether this has beneficial or adverse consequences in relation to the education of the immune system and the establishment of oral tolerance in infants, and to the development of immune-mediated diseases later in childhood, remains an open question that should be addressed in future studies. ■

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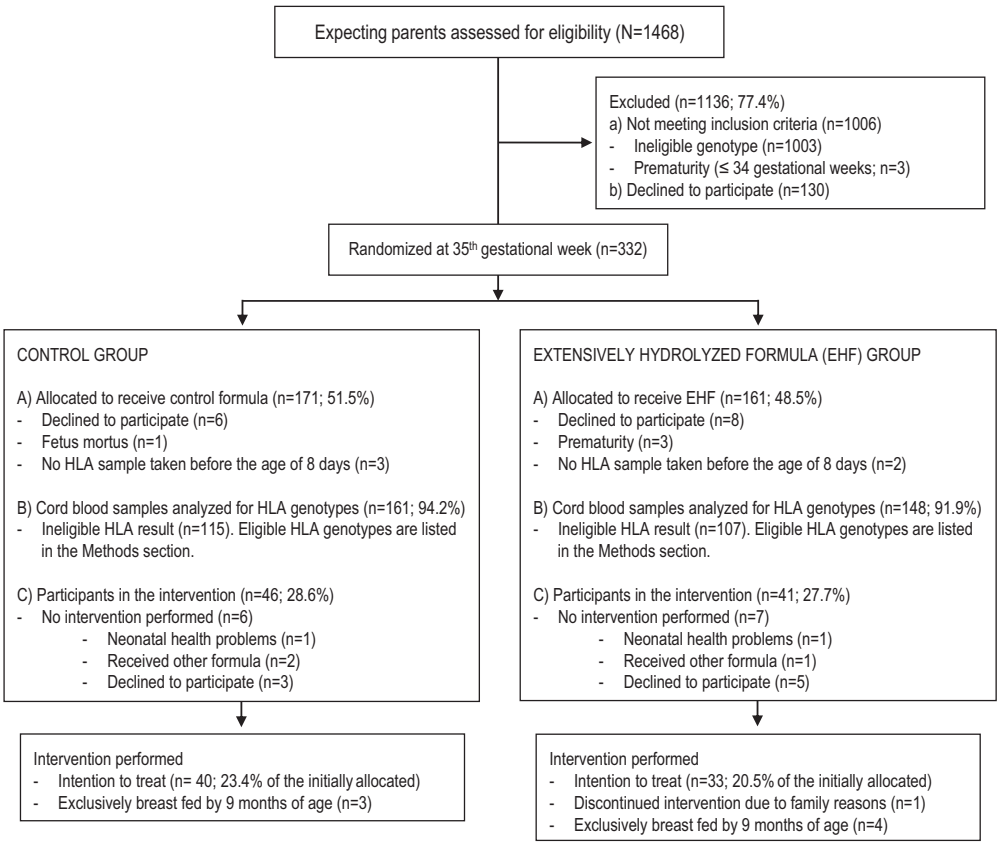

Figure 1. Recruitment for the intervention study.

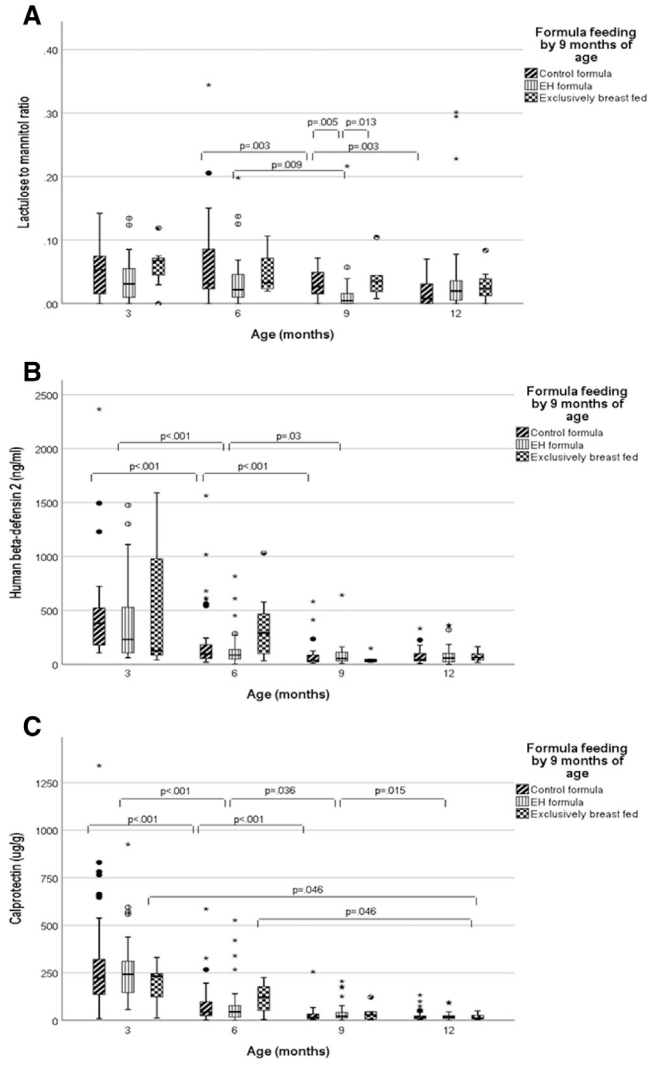

Figure 3. **A**, L/M ratio **B**, fecal HBD-2, and **C**, calprotectin levels in relation to formula feeding by 9 months of age. Upper outlines of the boxes represent the 75th percentiles and the lower outlines the 25th percentiles of the values, and the whiskers indicate the minimum and maximum values (statistical outliers marked with dots and extreme values with asterisks). *P* values indicate statistical differences between the groups (Mann Whitney U Test) and between different time points (Wilcoxon Signed Rank Test). The main findings indicated in the figures with *P* values; detailed analyses available in **Table IV**. *EHF*, extensively hydrolyzed casein-based formula.

Table IV. L/M ratio and fecal HBD-2 and calprotectin levels in relation to formula feeding by 9 months of age

L/M ratio

	Control group			EHF group			BF group			
	N	Median (IQR)	Between visits, P^a	N	Median (IQR)	Between visits, P^a	N	Median (IQR)	Between visits, P^a	Between study groups, P^b
At 3 months	35	.053 (.015-.077)	3 to 6 months: .688	28	.037 (.008-.055)	3 to 6 months: .755	7	.068 (.003-.075)	3 to 6 months: .237	.144
At 6 months	34	.031 (.023-.086)	6 to 9 months: .003	28	.022 (.010-.047)	6 to 9 months: .009	7	.033 (.021-.082)	6 to 9 months: .173	.358
At 9 months	32	.027 (.014-.050)	9 to 12 months: .003	27	.005 (.000-.017)	9 to 12 months: .130	6	.035 (.016-.060)	9 to 12 months: .116	.005
At 12 months	30	.008 (.000-.032)	3 to 9 months: .005	27	.020 (.005-.037)	3 to 9 months: .016	7	.024 (.009-.047)	3 to 9 months: .463	.259
			3 to 12 months: <.001			3 to 12 months: .074			3 to 12 months: .075	
			6 to 12 months: <.001			6 to 12 months: 0.619			6 to 12 months: .176	
HBD-2 (ng/ml)										
At 3 months	37	380.0 (172.0-542.0)	3 to 6 months: <.001	29	231.2 (100.0-535.0)	3 to 6 months: <.001	7	126.0 (50.0-1167.1)	3 to 6 months: .398	.291
At 6 months	37	96.0 (51.0-219.5)	6 to 9 months: <.001	29	86.0 (50.8-184.5)	6 to 9 months: .030	7	290.0 (84.0-578.0)	6 to 9 months: .116	.208
At 9 months	36	37.0 (23.0-92.0)	9 to 12 months: .812	28	54.0 (30.5-114.3)	9 to 12 months: .988	6	34.0 (23.0-73.1)	9 to 12 months: .988	.401
At 12 months	34	44.0 (24.0-84.0)	3 to 9 months: <.001	27	58.0 (24.0-120.0)	3 to 9 months: <.001	6	63.0 (38.5-123.0)	3 to 9 months: .225	.823
			3 to 12 months: <.001			3 to 12 months: <.001			3 to 12 months: .043	
			6 to 12 months: .002			6 to 12 months: .315			6 to 12 months: .043	
Calprotectin (ug/g)										
At 3 months	37	223.6 (140.6-456.9)	3 to 6 months: <.001	29	241.9 (136.8-344.6)	3 to 6 months: <.001	7	231.1 (105.9-255.5)	3 to 6 months: .310	.642
At 6 months	37	43.6 (25.0-108.9)	6 to 9 months: <.001	29	44.7 (18.2-91.4)	6 to 9 months: .036	7	121.5 (38.3-206.9)	6 to 9 months: .116	.428
At 9 months	36	15.1 (8.2-33.3)	9 to 12 months: .922	28	22.4 (13.4-43.0)	9 to 12 months: .015	6	28.6 (6.5-64.7)	9 to 12 months: .249	.297
At 12 months	34	16.0 (7.9-23.2)	3 to 9 months: <.001	27	16.8 (9.0-25.1)	3 to 9 months: <.001	6	12.4 (6.8-32.5)	3 to 9 months: .075	.900
			3 to 12 months: <.001			3 to 12 months: <.001			3 to 12 months: .046	
			6 to 12 months: <.001			6 to 12 months: <.001			6 to 12 months: .046	

BF, breastfeeding without any other milk intake.

P values indicate statistical differences between the groups[b] and between different time points[a].