

The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: The German Infant Nutritional Intervention Study, a randomized double-blind trial

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Background: The potential of extensively or partially hydrolyzed formulas to reduce the risks for allergies is controversial.

Objective: We sought to assess the preventive effect of differently hydrolyzed formulas compared with cow's milk formula (CMF) in high-risk infants.

Methods: Between 1995 and 1998, 2252 infants with a hereditary risk for atopy were enrolled in the German Infant Nutritional Intervention Study and randomly assigned at birth to one of 4 blinded formulas: CMF, partially hydrolyzed whey formula, extensively hydrolyzed whey formula, and extensively hydrolyzed casein formula (eHF-C). The primary end point at 1 year of age was the presence of allergic manifestation, which was defined as atopic dermatitis (AD), gastrointestinal manifestation of food allergy, allergic urticaria, or a combination of these factors.

Results: At 12 months per protocol, analysis was performed on 945 infants exposed to study formula: 304 (13.5%) infants had left the study, 138 (6.1%) infants were excluded because of noncompliance, and 865 infants were exclusively breast-fed the first 4 months of life. The incidence of allergic manifestation was significantly reduced by using eHF-C compared with CMF (9% vs 16%; adjusted OR, 0.51; 95% CI, 0.28-0.92), and the incidence of AD was significantly reduced by using eHF-C (OR, 0.42; 95% CI, 0.22-0.79) and partially hydrolyzed whey formula (OR, 0.56; 95% CI, 0.32-0.99). Family history of AD

was a significant risk factor and modified the preventive effect of the hydrolysates.

Conclusions: Prevention of allergic diseases in the first year of life is feasible by means of dietary intervention but influenced by family history of AD. The preventive effect of each hydrolyzed formula needs to be clinically evaluated. (*J Allergy Clin Immunol* 2003;111:533-40.)

Key words: *Hydrolyzed formula, prevention, atopic dermatitis, food allergy, infants*

The prevalence of allergic diseases has increased in children and young adults over the last decades.^{1,2} A number of experimental and clinical data indicate that early exposure to dietary allergens might be crucial for the development of allergies, particularly food allergies and atopic dermatitis (AD).^{3,4} Therefore if breast-feeding is insufficient, partially hydrolyzed formula (pHF) and extensively hydrolyzed formula (eHF) have been recommended as substitutes for the first 4 to 6 months of life in infants at risk for allergic diseases to reduce early allergen exposure.⁵ The use of both eHF^{6,7} and pHF^{8,9} was reported to reduce early allergic manifestation (AM), mainly AD and food allergy. However, these studies neither allow a direct comparison because of substantial variations in study design, inclusion criteria, cointerventions, definitions of clinical end points, and diagnostic measures nor allow any final conclusions.¹⁰ Thus it was recently stated again that more studies comparing the preventive effects of formula with moderately and highly reduced allergenicity are needed.⁵

The German Infant Nutritional Intervention (GINI) Study was initiated to prospectively investigate, in a randomized and double-blind design, the allergy-preventive effect of 3 differently hydrolyzed infant formulas compared with a conventional cow's milk formula (CMF) in a cohort of infants with at least one first-degree relative (parent or sibling) with an allergic disease.

METHODS

This study is a prospective, randomized, double-blind intervention trial to assess the allergy-preventive effect of differently hydrolyzed formulas compared with CMF in infants at risk for atopy. Infants were recruited between September 1995 and June

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Abbreviations used

AD:	Atopic dermatitis
AM:	Allergic manifestation
CMF:	Cow's milk formula
eHF-C:	Extensively hydrolyzed casein formula
eHF-W:	Extensively hydrolyzed whey formula
FA-GIT:	Food allergy with manifestation in the gastrointestinal tract
GINI Study:	German Infant Nutritional Intervention Study
OR:	Odds ratio
pHF-W:	Partially hydrolyzed whey formula

1998 in obstetric units throughout 2 areas of Germany (Wesel, North Rhine Westphalia, and Munich, Bavaria).

Patient selection criteria and randomization

All candidate mothers in these regions were contacted before or shortly after delivery and were asked to fill in a questionnaire with 18 items on past or present asthma, allergic rhinitis, AD, allergic urticaria, or food allergy (symptoms, physician diagnosis, or relevant treatment), according to the International Study of Asthma and Allergies in Childhood and the German Multicentric Allergy Study.^{11,12}

Parents with a healthy newborn and at least one family member (mother, father, or biologic sibling of the newborn) with an allergic disease according to the questionnaire were invited to participate, and written informed consent was obtained from both subjects before enrollment. Exclusion criteria were as follows: severe acquired or congenital diseases, gestational age of less than 37 weeks, birth weight of less than 2500 g, age of greater than 14 days, intake of any cow's milk-based formula before inclusion, or incapability of the parents to comply with the study protocol. The study protocol was approved by the ethic committees of each of the study centers.

At inclusion, infants were randomized by means of a computer-generated list to one of the study formula stratified by an independent person for single or double (biparental only) heredity of atopy and study region. Blinding of parents and the study team was guaranteed by using identically labeled tins for the study formula coded with 4 different letters for each of the 4 formulas.

Study formula

The following 3 hydrolyzed formulas were compared with standard CMF (Nutrilon Premium; Nutricia/Numico, Zoetermeer, Netherlands): (1) a partially hydrolyzed whey formula (pHF-W; Beba HA; Nestlé, Vevey, Switzerland); (2) an extensively hydrolyzed whey formula (eHF-W; Hipp HA/ Hipp, Pfaffenhofen, Germany, until 1999 on the German market and identical to Nutrilon Pepti [Nutricia/Numico, Zoetermeer, Netherlands]); and (3) a lactose-free, extensively hydrolyzed casein formula (eHF-C; Nutramigen; Mead Johnson, Diezenbach, Germany). The formulas differ in terms of the nitrogen source and the molecular weight profile. The ratios of casein to whey are 0 to 100 in pHF-W and eHF-W, 100 to 0 in eHF-C, and 40 to 60 in CMF. The percentage of peptides of less than 1500 d is 54%, 84%, and 95.5%, and the percentage of peptides of greater than 6000 d is 18%, 2%, and 0.5% in pHF-W, eHF-W, and eHF-C, respectively.

Study protocol

All mothers received written recommendations for feeding of the infants. Mothers were encouraged to exclusively breast-feed for at least 4 months (strict intervention period) and preferably 6 months. No dietary restrictions during lactation were recommended. The timing of weaning and introduction of study formula was left exclusive-

ly to the mother. Study formula was provided until the child was 6 months of age. Mothers were asked not to feed solid food during the strict intervention period and thereafter to add not more than one new food per week but to avoid milk and dairy products, hen's egg, soy products, fish, nuts, tomatoes, and citrus fruits during the first year.

During the first 6 months, mothers documented in weekly diaries the kind of milk the infant was fed (breast milk, study formula, or a brand of nonstudy formula if fed), time of first introduction, kind of new solid foods, and any health problems. Parental compliance with the feeding recommendations was assessed on the basis of these diary entries in addition to structured interviews.¹³ Children were excluded from the adherence to protocol analysis if the diaries during the intervention period showed noncompliance with the milk-feeding recommendations or if one of the weekly diaries was missing.

Children were examined on visits at the clinical centers at 1, 4, 8, and 12 months of age, and a structured interview on health problems, including symptoms related to AD, allergic urticaria, and food allergy with manifestation in the gastrointestinal tract (FA-GIT), was carried out by a study physician. Information on sociodemographic factors, family and living conditions, and smoking habits were documented. Unscheduled visits were made when possible allergic symptoms, particularly skin lesions, appeared.

Definition of end points

A case with an AM was assumed when at least one of the following allergic diseases was documented during the first 12 months: AD, allergic urticaria, or FA-GIT.

AD was diagnosed on the basis of the modified diagnostic criteria¹⁴: (1) typical morphology and distribution of skin lesions (face, neck and scalp, flexural folds, hands, and extensor sides of the extremities); (2) pruritus (signs of scratching); and (3) tendency toward chronicity (duration ≥ 14 days, chronically relapsing, or both). The morphologic diagnosis of AD had to be confirmed on skin examination by a second physician who was a specially trained allergologist of the local GINI Study team.

The severity of AD was rated by using the SCORAD method.¹⁵ Final diagnosis was made by using an algorithm, and AD was defined only if all 3 diagnostic criteria were fulfilled. Only patients with definite diagnoses were included as cases in the statistical analysis.

Allergic urticaria was diagnosed if at least 2 episodes of itching eruptions or swelling with typical appearance, observed by the parents or a physician, were caused by the same allergen. In case of a single episode, immunologic evidence (a skin prick test with the accused undiluted native allergen causing a wheal reaction of ≥ 3 mm or an allergen-specific IgE level of ≥ 0.35 KU/L; CAP, Pharmacia, Uppsala, Sweden) or a positive provocation response with the suspected allergen was needed for definite diagnosis.

For the diagnosis of FA-GIT, both IgE and non-IgE-mediated immunologic reactions were considered.¹⁶ FA-GIT was suspected in the presence of at least one of the following: bloodstained stools, diarrhea, vomiting or frequent regurgitation, colicky behavior, or failure to thrive not explained by any other condition. Symptoms had to disappear during elimination of the suspected formula or food and be reproduced during challenge. In symptomatic breast-fed infants the mothers were advised not to eat cow's milk products, eggs, fish, nuts, and peanuts. If symptoms continued and were severe, discontinuation of breast-feeding and supplementation with an amino acid-derived infant formula (Neocate; SHS, Heilbronn, Germany) was recommended. FA-GIT was defined as definite if a standardized elimination-challenge procedure was performed and had positive results under supervision of the GINI Study team in the clinic (for early reactions) or at home (for late reactions). Double-blind, placebo-controlled food challenge was performed in cases of uncertain reactions.

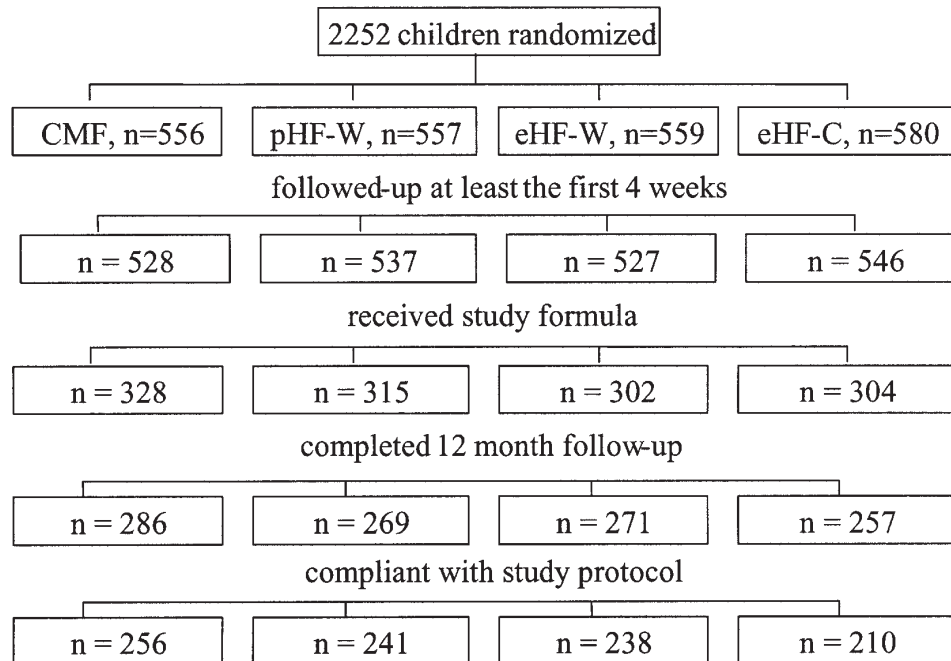


FIG 1. Trial profile.

Statistical methods

The following assumptions were made to estimate the sample size: (1) a loss caused by drop out and exclusively breast-feeding of approximately 50% was expected^{7,17}; (2) the prevalence of allergic diseases in the cow's milk group was estimated to be 30%^{8,9}; and (3) the expected reduction in prevalence with the hydrolyzed formula was 30% (which corresponds to an expected incidence of 20%).⁶ We calculated with a significance error of 5% (2-tailed) and power of 80% a sample size of at least 313 infants for each formula.¹⁸ Assuming the loss, we aimed to enroll 600 infants per formula, adding to a total of 2400 included children.

Frequencies in baseline characteristics (inclusive exclusion criteria, such as noncompliant or drop out) were compared with the Pearson χ^2 test, ANOVA for unbalanced data were used for the comparison of means, and in all cases the exact levels of significance are given. The incidence was calculated as the number of cases during the first year of life per group size. The effects on incidence were analyzed first by means of simple logistic regression models and odds ratios (ORs) with 95% CIs, and the exact level of significance on the basis of Wald χ^2 statistics are given. Multiple logistic regression models were used to adjust for potential risk factors and confounders, which took into account information on family history of atopy (single: one parent, sibling, or both allergic; double: biparental allergy) and AD (number of family members with AD), sex, level of parental education, parental nationality, maternal smoking after birth, pet ownership, and study center. Factors were dropped out in a backward stepping procedure if they had influence on neither the dependent variable nor on the effect of the feeding regimen. For the final model, adjusted ORs with 95% CIs are reported.

If hints for nonhomogeneity of the ORs are given, stratified analyses were performed as a hypothesis-generating exercise. In addition to the adherence to protocol analysis, the final multiple regression model was repeated in the intention-to-treat population

with and without the exclusively breast-fed infants. All calculations were performed by using the statistical Analysis System (SAS, release 6.12).

RESULTS

Baseline characteristics

A total of 2252 infants were included in the study and randomized to the study formula (Fig 1). Within the first 4 weeks, 114 (5%) of 2252 children left the study without physical examination and information on feeding modalities. A total of 889 (42%) of 2138 mothers exclusively breast-fed their infants during the first 4 months; all but 24 (3%) of 889 could be followed-up during the entire study period. Study formula was given to 1249 infants, but 166 (13%) of 1249 dropped out before the 12-month follow-up. A further 138 children were excluded from the analyses because of noncompliance (138/1083 [13%]). A total of 945 infants were therefore included in the adherence to protocol analysis for clinical end points at 12 months of age. In the step-by-step analyses for imbalances between the groups, we found a significant bias in the eHF-C group because disproportionately more children had to be excluded as a result of noncompliance with the study formula (18% [47/257] in the eHF-C group vs 10%-12% in the other study formula groups, $P = .02$). Major reasons for drop outs were as follows: personal problems within families (124/304 [41%]), nutritional problems of the infant (96/304 [32%]), change of permanent residence (31/304 [10%]),

TABLE I. Baseline characteristics of children included in the adherence-to-protocol analysis (n = 945) by study formula

	CMF (N* = 256)	pHF-W (N* = 241)	eHF-W (N* = 238)	eHF-C (N* = 210)	P value†
Male sex	139 (54)	129 (54)	128 (54)	103 (49)	.669
Birth weight (g), mean ± SD	3469 ± 515	3465 ± 473	3511 ± 479	3441 ± 454‡	.502§
Length (cm), mean ± SD	52.4 ± 2.6	52.3 ± 2.6	52.2 ± 2.4	52.1 ± 2.4‡	.552§
SF during first 4 weeks	168 (66)	160 (66)	165 (69)	149 (71)	.576
13-16 wk of SF feeding	126 (49)	113 (47)	123 (52)	96 (46)	.589
Exclusive SF	45 (18)	32 (13)	32 (13)	30 (14)	.493
FH of allergy					.694
Single	188 (73)	168 (70)	164 (69)	147 (70)	
Double	68 (27)	73 (30)	74 (31)	63 (30)	
AD in FH	91/252 (36)	79/241 (33)	96/237 (41)	76/208 (37)	.377
Parental education					.636
<10 y	32 (13)	20 (8)	25 (11)	18/209 (9)	
10 y	83 (32)	85 (36)	72 (30)	73/209 (35)	
≥12 y	241 (55)	136 (56)	141 (59)	118/209 (56)	
Parental nationality, German	218/255 (85)	204/240 (85)	207 (87)	185/209 (89)	.695
Siblings, yes	111/252 (44)	82/240 (34)	96/236 (41)	80/210 (38)	.148
Study region, North Rhine Westphalia	133 (52)	130 (54)	120 (50)	120 (57)	.520

Data are expressed as number (percentage) unless otherwise indicated.

SF, Study formula; FH, family history.

*Number of children unless indicated otherwise because of missing values.

‡ χ^2 Test of homogeneity unless indicated otherwise.

‡n = 1 missing value.

§ANOVA F test.

refusal to use blinded study formula (17/304 [6%]), death unrelated to study formula or design (4/304 [1%]), and others (32/304 [11%]); there were no significant differences between the groups (data not shown).

In infants included in the adherence to protocol analysis, no significant differences among the 4 study formula groups were observed with reference to feeding and baseline characteristics, family history of allergies, and sociodemographic data (Table I).

Incidence of AMs

Of the infants exposed to study formula, 119 (13%) of 945 had AMs during the first year of life; AD had been diagnosed in 106 (11%), allergic urticaria in 5 (0.5%), and FA-GIT in 12 (1.3%) infants (Table II). Allergic diseases other than AD were distributed homogeneously among groups, and more than one allergic disorder was diagnosed in 4 children. The incidence of AM was significantly lower in infants fed eHF-C compared with that seen in infants fed CMF ($P = .036$), whereas the reduction in incidence in both groups fed whey hydrolysates did not reach significance ($P = .114$ in the pHF-W group and $P = .677$ in the eHF-W group, Table II).

Potential risk factors for AM

We observed a significant crude association between individual potential risk factors and the development of AM, such as male sex (15% in boys compared with 10% in girls, $P = .018$) and AD in parents, siblings, or both (18% in children with AD in the family compared with

10% in children without AD in the family, $P < .001$). Although there was a trend toward a higher incidence of AM in children with a double (16%) compared with a single family history of any allergic disease (11%), this was not significant ($P = .087$). Neither parental education, nationality and siblings in the family, nor study center influenced the incidence of AM (Table III).

Multivariable analyses

Multiple logistic regression models were used to adjust for potential risk factors and confounders. The final model included sex ($P = .037$), AD in the family, ($P < .001$), and maternal smoking after birth ($P = .015$).

After adjusting the effects of the feeding regimens on the incidence of AM, eHF-C remained the only formula with a significant protective effect (Table IV). However, when the data were reanalyzed with AD as the only outcome parameter, both eHF-C and pHF-W reduced the incidence of AD significantly by greater than 50% (Table IV). Hints for nonhomogeneity of the ORs are given across the presence of AD in the core family, and after stratification with respect to this factor, we observed different effects of the formulas on the incidence of AM and AD (Table IV). In infants without AD in the core family ($n = 603$), feeding the 3 hydrolysates reduced the incidence of AM by 37% to 49% compared with that seen in the CMF-fed group (Table IV). When we combined the group of infants fed pHF-W, eHF-W, or eHF-C, the reduction in comparison with CMF was almost significant ($P = .054$). In contrast, in infants with a positive family history of AD ($n = 342$), the eHF-C reduced the risk for AM by 47%,

whereas pHF-W led to a reduction of 28% only, and eHF-W had no preventive effect (Table IV). Consistent results were observed for the incidence of AD with a more preventive effect in all subgroups (Table IV).

Intention-to-treat analysis

An intention-to-treat analysis was performed with all children who at least had a 4-week follow-up ($n = 2138$, level 2 in Fig 1). As expected, effects were less prominent because up to 44% of the children in each group were exclusively breast-fed (Fig 1). The intention-to-treat analysis of data, including all infants exposed to study formula ($n = 1249$, level 3 in Fig 1), confirmed the results of the adherence-to-protocol analysis (not shown).

DISCUSSION

Our results confirm that allergy prevention in the first year of life in infants with a familial risk for atopy is possible by feeding hydrolyzed formulas instead of CMF as a supplement or substitute for breast milk. AM was significantly prevented by eHF-C, whereas AD as the predominant allergic disease during infancy was prevented by eHF-C and pHF-W but not by eHF-W. For the first time, we could demonstrate that the preventive potential of the different formulas depends on the family history of AD as the main risk factor for AM in the first year. The beneficial effect of the nutritional intervention was generally more pronounced and differently influenced in children without this genetic background.

From previous cohort studies with hydrolysates in atopy-prone infants, it remains inconclusive whether eHF or pHF are preferable for allergy prevention. The published studies are not comparable for many reasons, such as different inclusion criteria, insufficient randomization, blinding, monitoring and reporting of compliance and drop outs, and, most important, often poorly defined diagnostic criteria of AMs as outcome parameters and small numbers of infants.¹⁰ With our study design, we tried to overcome most of the flaws of other studies.

Although the study protocol was rather demanding for the mothers, the total drop-out rate was low, without any differences among the 4 formula groups (Fig 1). However, in the group of children receiving eHF-C, significantly ($P = .02$) more mothers were noncompliant with the milk-feeding recommendations. Because of the processing, eHF-C has probably the worst taste and smell of all study formula, and more infants might have refused the formula, more mothers might have considered this nutrition inappropriate, or both. However, a strong bias is unlikely because the intention-to-treat analysis revealed a similar protective effect of eHF-C (data not shown).

The results of the exclusively breast-fed infants were not included in the analysis because a direct comparison with the infants exposed to study formula has severe limitation: randomization of breast-feeding is not possible for ethical reasons, and mothers who decided not to give formula differed significantly from the mothers of the 945 infants exposed to study formula with respect to the

TABLE II. First-year incidence of AD, allergic urticaria, FA-GIT, and AM with crude ORs from logistic regression dependent on the feeding regimen

	CMF	pHF-W	eHF-W	eHF-C
No. of patients	256	241	238	210
AD				
n	38	22	31	15
%	14.8	9.1	13.0	7.1
Urticaria				
n	1	0	1	3
%	0.4	0.0	0.4	1.4
FA-GIT				
n	1	5	2	4
%	0.4	2.1	0.8	1.9
AM				
n	40	26	34	19
%	15.6	10.8	14.3	9.1
Crude OR	1	0.65	0.90	0.54
95% CI		(0.39-1.1)	(0.55-1.5)	(0.30-0.96)
P value		.114	.677	.036

TABLE III. Incidence of AM in groups of potential risk factors and crude ORs by simple logistic regression models

	n/N* (%)	OR (95% CI)	P value
Sex			
Female	44/446 (10)	1	
Male	75/499 (15)	1.6 (1.1-2.4)	.018
FH of allergy			
Single	76/667 (11)	1	
Double	43/278 (16)	1.4 (0.95-2.1)	.087
AD in FH			
No	57/596 (10)	1	
Yes	62/342 (18)	2.1 (1.4-3.1)	<.001
Parental education			
<10 y	13/95 (14)	1.0 (0.51-1.9)	.665
10 y	43/313 (14)	1	
≥12 y	63/536 (12)	0.84 (0.55-1.3)	.399
Parental nationality			
German	103/814 (13)	1	
Non-German	15/128 (12)	0.92 (0.52-1.6)	.767
Siblings			
No	70/569 (12)	1	
Yes	48/369 (13)	1.1 (0.72-1.6)	.750
Study center			
North Rhine Westphalia	64/503 (13)	1	
Bavaria	55/442 (12)	0.98 (0.66-1.4)	.897

FH, Family history.

*Sum partially not equal to 945 because of missing values.

following variables: higher prevalence of AD in the family, higher rate of Cesarean section, higher parental education, higher rate of siblings ($P = .01$) and mothers older than 30 years of age, less smoking mothers, less homes with pets, and less from the study region in North-Rhine-Westphalia (all $P < .001$).¹⁹ A similar observation has been reported recently by Halken et al.¹⁷ In addition, the occurrence of allergic symptoms, such as AD, during the first weeks of life might influence the mother's decision to continue exclusive breast-feeding.

TABLE IV. Results of the multivariable models: Adjusted OR for AM and AD dependent on the feeding regimen and stratified by AD in family history

			CMF	pHF-W	eHF-W	eHF-C
AM	All	Incidence, n/N (%)	40/256 (16)	26/241 (11)	34/238 (14)	19/210 (9)
		Adjusted OR* (95% CI)	1	0.65 (0.38-1.1)	0.86 (0.52-1.4)	0.51 (0.28-0.92)
		P value		.109	.544	.025
	No AD in FH	Incidence, n/N (%)	22/165 (13)	14/162 (9)	11/142 (8)	10/134 (7)
		Adjusted OR† (95% CI)	1	0.63 (0.31-1.3)	0.55 (0.26-1.2)	0.51 (0.23-1.1)
		P value		.210	.131	.101
	AD in FH	Incidence, n/N (%)	18/91 (20)	12/79 (15)	23/96 (24)	9/76 (12)
		Adjusted OR† (95% CI)	1	0.72 (0.32-1.6)	1.3 (0.63-2.5)	0.53 (0.22-1.3)
		P value		.426	.515	.148
AD	All	Incidence, n/N (%)	38/256 (15)	22/241 (9)	31/238 (13)	15/210 (7)
		Adjusted OR* (95% CI)	1	0.56 (0.32-0.99)	0.81 (0.48-1.4)	0.42 (0.22-0.79)
		P value		.048	.44	.007
	No AD in FH	Incidence, n/N (%)	21/165 (13)	10/162 (6)	11/142 (8)	8/134 (6)
		Adjusted OR† (95% CI)	1	0.46 (0.21-1.02)	0.58 (0.27-1.3)	0.42 (0.18-1.00)
		P value		.055	.173	.050
	AD in FH	Incidence, n/N (%)	17/91 (19)	12/79 (15)	20/96 (21)	7/76 (9)
		Adjusted OR† (95% CI)	1	0.75 (0.33-1.7)	1.1 (0.54-2.3)	0.43 (0.17-1.1)
		P value		.494	.757	.077

FH, Family history.

*Adjusted for AD in family history, sex, and maternal smoking after birth.

†Adjusted for sex and maternal smoking after birth.

The incidence of AM in our study was lower, both in the formula-fed infants (hydrolysates mean, 11%; CMF, 16%) and in the exclusively breast-fed children (11% and 9.5% AD)¹⁹ compared with that seen in other studies, in which allergic diseases were reported from 12% to 58% in intervention groups and from 24% to 78% in CMF groups.^{8,9,17,20} Reasons for this difference might be that we excluded asthma and allergic rhinitis because these manifestations are not well defined at 1 year of age and because of our strict criteria for definition of allergic diseases. AD had to be confirmed by 2 independent physicians. The presence of eczema and all other criteria needed to be fulfilled. Urticaria was finally proved and diagnosed in only 5 of 67 infants initially reported to have had urticaria. Also, from 68 children suspected of having FA-GIT, only 12 could be confirmed as having the illness by means of elimination-challenge procedures. When we analyzed the incidence of AD in the subgroup of children with biparental allergy, the reported incidence rates of AD remained 2- to 4-fold lower compared with those in 2 previous studies with identical inclusion criteria and comparable feeding groups (CMF, eHF-C, and exclusive breastfeeding).^{17,20} The earlier time of introduction of study formula and the longer duration of exposure in our study might also have contributed to differences. Because the duration of exposure to study formula did not result in meaningful differences in the incidence rates between our study groups, this does not explain our lower incidence rates. However, genetic differences in the cohorts cannot be excluded. Although we might have underestimated the crude incidence of allergic diseases, particularly of FA-GIT, in our total population, the comparison between feeding groups is unaffected by this argument.

We found a significant reduction in the incidence of AM, defined as the presence of AD, allergic urticaria, FA-GIT, or a combination of these illnesses, only with the eHF-C. When AD as major manifestation of allergy was considered as the only outcome parameter, pHF-W also led to a significant reduction compared with CMF, whereas eHF-W hardly showed any preventive effect. Our results are difficult to compare with the findings of the 2 Scandinavian studies that investigated the allergy-preventive effect of pHF and eHF.^{17,20} Halcken et al¹⁷ used the same eHF-C and pHF-W as we did, but a different brand of eHF-W and no CMF group for comparison. They did not find any differences regarding the incidence of AD between the groups. Oldaeus et al²⁰ compared the same eHF-C as we did with CMF but used a partially hydrolyzed whey-casein formula, which is not commercially available. In addition, they recommended a hypoallergenic diet to the pregnant and lactating mother as cointervention. The cumulative incidence of AD was significantly reduced at 9 months (17% in eHF-C vs 44% in pHF and 41% in CMF) but not at 12 and 18 months of age.²⁰ In our study the different preventive effects of the 2 extensively hydrolyzed formulas eHF-C and eHF-W were unexpected and difficult to explain. It can be speculated that the different processing of hydrolyzation with different enzymes rather than the degree of hydrolyzation or the protein source influences the remaining epitopes and the residual antigenicity of a hydrolysate.²¹ Our findings are supported by the *in vitro* results of Rugo et al,²² who found that eHF-C had the least residual allergenic potential in cow's milk-sensitive patients compared with different whey hydrolysates.

AD in the core family was found to be a stronger risk factor for the development of AM during infancy than double history of any allergic disease (Table III). This is in agreement with epidemiologic studies that showed a genetic influence for AD²³ and is further supported by the genetic linkage of AD to chromosome 3q21.²⁴ Because of the large number of children in our study, it was possible to stratify for different parental phenotypes. Our results suggested that the preventive effect of different feeding regimens might be influenced by the phenotype of AD in the core family. In the absence of AD in the family, preventive effects on AD were observed for the hydrolyzed formulas (Table IV) and also for exclusive breast-feeding (AD incidence in children without AD vs those with AD in the family, 5.8% vs 14.3%; data not shown). In contrast, in the presence of AD, only eHF-C reduced the risk by greater than 50% compared with CMF feeding. Our findings raise the question of whether nutritional intervention, including breast-feeding, is less effective in infants with a stronger genetic background for AD. This result could have far-reaching implications for future approaches to allergy prevention. Because these findings resulted from an additional subgroup analysis, they should be interpreted with caution until results of follow-up data or future studies confirm or contradict the consistency of these results.

In conclusion, our results clearly indicate that feeding a hydrolyzed formula instead of CMF as a supplement or substitute to breast milk during the first 4 months of life reduces the risk of AM during the first year of life. However, the different hydrolysates do not offer the same degree of prevention. Our data show that neither the degree of hydrolyzation nor the protein source is predictive of the preventive effect and that the genetic background might modify the preventive potential of a hydrolysate. Therefore the effect of each hydrolyzed formula aiming for prevention of AM needs to be clinically evaluated.

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APPENDIX

A. von Berg was study coordinator and responsible for protocol design and recruitment of patients and wrote the manuscript. S. Koletzko was involved in protocol design and recruitment of patients and wrote the manuscript with A. von Berg. A. Gruebl was responsible for protocol design, recruitment of patients, and biochemical analyses. B. Filipiak-Pittroff performed statistical analysis and assisted in writing the manuscript. H. E. Wichmann was responsible for data entry and data management, and he assisted in acquiring

ing funding and writing the manuscript. C. P. Bauer provided biochemical analyses and helped designing the study protocol. D. Reinhardt helped with the design of the study. D. Berdel was principle investigator of the study, developed the initial and final protocol, and assisted in acquiring, funding, and coordinating the study.

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