Preventive effect of hydrolyzed infant formulas persists until age 6 years: Long-term results from the German Infant Nutritional Intervention Study (GINI)

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Background: The long-term effect of nutritional intervention with hydrolyzed infant formulas on allergy development has not been sufficiently evaluated.
Objective: We performed a follow–up of the German Infant Nutritional Intervention study until 6 years of life to investigate the long-term allergy-preventive effect of 3 hydrolyzed infant formulas compared with cow's milk formula (CMF) in a randomized, double-blind trial.
Methods: Between 1995 and 1998, 2252 newborns with atopic heredity were randomly assigned at birth to receive one of 4 blinded formulas: partially or extensively hydrolyzed whey formula, extensively hydrolyzed casein formula, or CMF as milk substitute for the first 4 months when breast-feeding was insufficient. The cohort was followed from birth until 6 years of age with yearly questionnaires. Outcomes were physician-diagnosed allergic diseases (atopic dermatitis, food allergy, allergic urticaria, asthma, and hay fever/allergic rhinitis). Log-binomial regression modeled with generalized estimation equations was used for the statistical analysis.
Results: In the intent-to-treat analysis the relative risk of a physician's diagnosis of allergic manifestation (AM) compared with CMF was 0.82 (95% CI, 0.70-0.96) for partially hydrolyzed whey formula, 0.90 (95% CI, 0.78-1.04) for extensively hydrolyzed whey formula, and 0.80 (95% CI, 0.69-0.93) for extensively hydrolyzed casein formula. The corresponding figures for atopic eczema were 0.79 (95% CI, 0.64-0.97), 0.92 (95% CI, 0.76-1.11), and 0.71 (95% CI, 0.58-0.88), respectively. In the per-protocol analysis all effects were stronger and significant. No significant effect on other AMs was found.
Conclusion: The data confirm a long-term allergy-preventive effect of hydrolyzed infant formulas on AM and atopic eczema until 6 years of age. (J Allergy Clin Immunol 2008;121:1442-7.)

Key words: *Birth cohort, long-term allergy prevention, hydrolysates, double-blind randomized trial*

The rationale for the prevention of allergic diseases in infants with a positive family history for atopic diseases by allergen avoidance is based on the hypothesis that allergen exposure in the first months of life plays an important role for later development of allergic diseases.[1-4] Consequently, primary preventive approaches include the avoidance or at least reduction of food allergens during the vulnerable period when the innate immune system adapts to maturity.[5-7] Thus to reduce the allergenicity of cow's milk–based substitutes for breast milk, hydrolyzed infant formulas were introduced for primary prevention.[7-10] Dependent on the degree of hydrolysis, they are differentiated in partial hydrolysate formulas and extensive hydrolysate formulas and with respect to the protein source in whey and casein hydrolysates. Several studies have been conducted with either partial hydrolysate formulas, extensive hydrolysate formulas, or both that demonstrated a preventive effect on allergic manifestation (AM) in infancy (first 2 years) and early childhood.[11-16] Although there are some multifaceted intervention studies showing a persistent preventive effect on allergic diseases, mainly atopic eczema and asthma up to school age,[17,18] studies showing a long-term effect of early dietary prevention as monointervention are scarce,[9,19] and their results as a basis for recommendations have been questioned.[20]

Recently, we reported the results of the German Infant Nutritional Intervention (GINI) study, confirming the concept that

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Abbreviations used
AM: Allergic manifestation
AD: Atopic dermatitis used synonymously to eczema according to the new nomenclature for reasons of continuity with previous articles by the GINI
CMF: Cow's milk formula
eHF-C: Extensively hydrolyzed casein formula
eHF-W: Extensively hydrolyzed whey formula
GINI: German Infant Nutritional Intervention
ITT: Intent-to-treat
pHF-W: Partially hydrolyzed whey formula
PP: Per-protocol

feeding certain hydrolyzed infant formulas reduces the risk for allergic diseases in infants at high risk up to the age of 3 years.[21] Children who were supplemented with either extensively hydrolyzed casein formula (eHF-C) or partially hydrolyzed whey formula (pHF-W) instead of regular cow's milk formula (CMF) significantly less often had atopic dermatitis (AD).

We report now on the follow-up of the GINI study until 6 years of life, when we examined whether study formula feeding in the first 4 months of life is continuously preventive in the development of AMs, including AD, food allergy/intolerance, allergic urticaria, asthma, and, for the first time, hay fever/allergic rhinitis.

METHODS

Subjects

Between September 1995 and July 1998, healthy term newborns were recruited at birth in 2 regions of Germany (rural Wesel and urban Munich) for the GINI study birth cohort. The infants were enrolled before any formula supplementation had occurred and at the latest at 14 days of age. Detailed description of screening and recruitment has been provided elsewhere.[22,23] High-risk infants, defined as having at least 1 parent or sibling with a history of allergic diseases, were selected for the GINI study (N = 2252). If the parents agreed to participate in the prospective, double-blind intervention trial, newborns were randomized at birth by a computer-generated list to one of 3 hydrolyzed study formulas (pHF-W, extensively hydrolyzed whey formula [eHF-W], or eHF-C) or a conventional CMF and stratified for single or biparental atopic heredity and study region.[22] Mothers were advised to feed the randomized formula as the only substitute to breast milk in case of insufficient breast-feeding during the strict intervention period of 4 months. Further intervention measures included written and verbal dietary recommendations and a tight schedule of visits to the study centers up to the age of 3 years.[21] Noncompliance was defined as either feeding a formula other than randomized or lacking information on the milk feeding from weekly diaries during the first 4 months.[22,24]

The study protocol was approved by the local ethics committees, and written informed consent was obtained from all participating families.

Questionnaires

At birth, information on parental history of allergic diseases, parental education level, maternal smoking during pregnancy, siblings, and pet ownership was collected from parents of all children by using self-administered questionnaires.

Children were followed with identical International Study of Asthma and Allergies in Childhood–modified questionnaires[25] at the ages of 1, 2, 3, 4, and 6 years to collect information on health outcomes, allergic symptoms, physician's diagnosis of allergic diseases, and covariates, such as children's nutrition, environmental tobacco smoke exposure, and pet ownership. The parents were asked to complete these questionnaires in addition to their regular intervention program during the first 3 years.[21,22]

Determination of outcomes and covariates by questionnaires

Parents were asked whether a physician had diagnosed an atopic disease since the last follow-up. At the last follow-up (6 years), the prevalence of the diagnosis was asked separately for years 5 and 6. The question was this: "Did a physician diagnose any of the following diseases during the 1st/2nd/3rd/4th/5th/6th year of life: … asthma, hay fever or allergic rhinitis, allergic or atopic eczema/dermatitis, urticaria or quincke edema, food allergy or intolerance? … ."

For reasons of continuity with previous articles on the GINI study,[21,22] we used "AD" synonymously with "eczema," according to the new nomenclature.[26] The definition of AM included physician's diagnosis of AD, food allergy/intolerance, and allergic urticaria. Asthma was added to the definition of AM from 3 years and hay fever/allergic rhinitis from 4 years onward because asthma and hay fever cannot be diagnosed with any degree of certainty before the third and fourth year of life, respectively.

The following covariates were reported at birth and considered as potential confounders: sex; study region (Munich or Wesel); family history of asthma, hay fever, and eczema; heredity of family allergy; parental education (3 groups: schooling <10 years, 10 years, and >10 years); and number of older siblings. Passive tobacco smoke exposure was asked for in the annual questionnaires on and after the second year, and mothers smoking before pregnancy were identified from the questions asked at recruitment. Information on furry pets in the home was gathered annually.

Statistics

The outcomes were based on parent-reported physician's diagnosis during the first, second, third, fourth, fifth, and sixth years. The risk of 1 or more atopic diseases in a period (birth to 3 or 6 years) was estimated by using the life-table method for the 4 study formulas.[27] Intent–to-treat (ITT) and per-protocol (PP) analyses were performed. Generalized estimating equations were used to model longitudinal data with missing outcomes because of loss to follow-up,[28] and the results of the log-binomial models were presented as relative risks with 95% CIs. The PP analysis included all infants fed (fully or partially) with study formula within the first 4 months and who were compliant with the milk-feeding recommendations.[24] Statistical analyses were done with the statistical software SAS for Windows, Release 9.1 (SAS Institute, Cary, NC).

RESULTS

Study population and participation

The present analysis is based on the 2252 children primarily randomized to one of the 4 study formulas. The pattern concerning the beginning and duration of feeding with the study formula was equally distributed between the study formula groups. Only 139 (6%) infants were fed exclusively with study formula, whereas 889 (39.4%) of 2252 were exclusively breast-fed. During the strict intervention period (birth to 4 months of age), 988 children were compliant with the milk-feeding recommendation, 184 were noncompliant, and 191 children dropped out with unknown exposure (also noncompliant). Participation in the annual questionnaires is depicted in Fig 1. The proportion of loss to follow-up was at most 10%. At least 1 questionnaire was answered by 90.4% of the 2252 randomized children and 95.9% of the PP population. The 6-year questionnaire was answered by 74.6% of the total cohort and 80.4% of the PP population. A lower level of parental education, the presence of more than 1 siblings, and residency in Wesel were significantly associated with discontinuation of the study. However, these factors remained equally distributed over the study formula groups. Participation did not depend on allergies in the family. Baseline characteristics of the cohort, including data on feeding with the study formulas in the first 4 months, are described elsewhere.[22]

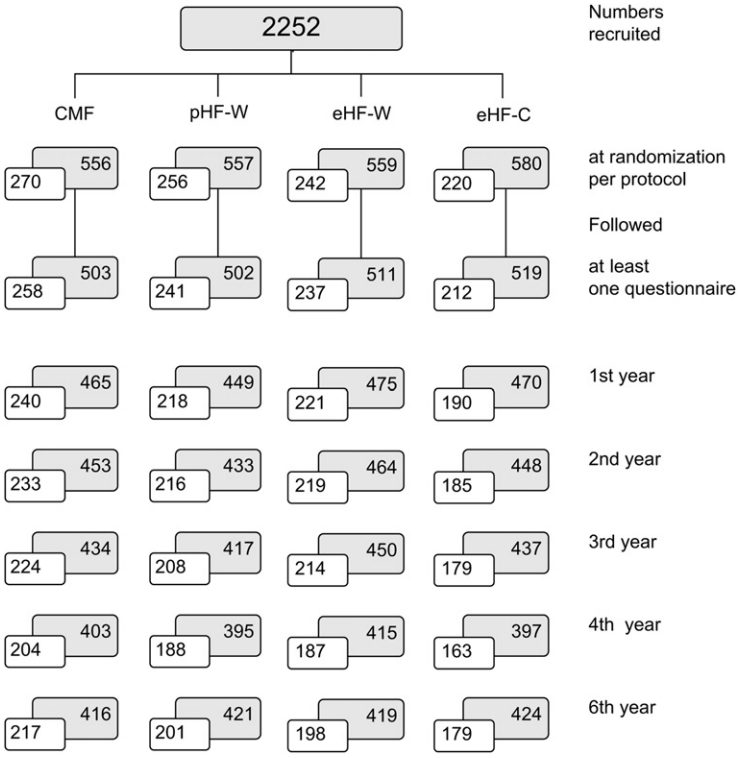

FIG 1. GINI study profile from birth to 6 years. Number of followed children in the ITT population (all randomized, *shaded boxes*) and the PP population *(open boxes)* in which fully breast-fed (n = 889) and non-compliant (n = 375) children were excluded.

ITT analysis

The ITT analysis at 6 years included all 2252 children (Fig 1). The proportion of missing outcome information causes by drop-outs at all examination times was equal in the 4 randomized groups (statistically tested, data not shown), and participation did not depend on the answers to the diagnoses on previous questionnaires. In the first 6 years, a physician's diagnosis of any allergy was given in 885 children (AM), and two thirds of the patients were given diagnoses by a physician as having atopic eczema (AD; n = 575; Table I). The diagnosis of atopic eczema alone was given by 35%, food intolerance by 11%, urticaria by 9%, hay fever by 6.2%, and asthma by 2.5%, and the residual cases (36.5%) with any physician's diagnosis had more than 1 disease. Children in the group fed with pHF-W and eHF-C less often had AMs during the first 6 years (significant reduction up to 20%; $P = .008$ and .004, respectively) compared with children fed CMF, whereas in the eHF-W group a nonsignificant effect of 10% could be observed. The reduction of AD is in a similar range as for AM in the pHF-W ($P = .021$) and eHF-W (not significant) groups; in the eHF-C group, however, the effect is stronger ($P = .002$).

The effect of formula on any AM is primarily driven by eczema. No significant effect could be shown when we calculated the effect of nutritional intervention on any AM in the first 6 years of life after having excluded AD from the definition of AM (data no shown).

PP analysis

The results for the PP analysis, which includes all children who were compliant with the milk-feeding recommendations (N = 988), are summarized in Table II. Of 384 children with a physician's diagnosis of any allergy, 253 had been given diagnoses of AD and only 43 had been given diagnoses of asthma between 3 and 6 years (not shown). The relative risk of any allergy up to 6 years and also of AD is significantly reduced by 25% to 49% at all time points (at 1 year and until 3 and 6 years) in the pHF-W and eHF-C groups. Additionally, in contrast to the ITT analysis, the effect of eHF-W on the cumulative incidence of AM and AD until 6 years also reached significance.

Prevalence of atopic manifestation in the fourth to sixth year

The data confirm a significant long-term effect of all 3 hydrolysates on AM and AD in the PP analysis. Certain, although not statistically significant, effects on allergic rhinitis could be observed, especially in the eHF-W group. The results in the ITT analysis are less strong (Table III). The prevalence of asthma is not affected by any of the formulas (Table III).

DISCUSSION

The results of the GINI study with a follow-up until 6 years of age revealed the important finding that nutritional intervention with hydrolyzed infant formulas in the first 4 months of life has a long-lasting preventive effect on atopic eczema in high-risk children. The preventive effect of pHF-W and eHF-C on AM and AD found in the 2 previous analyses at age 1 year and until 3 years[21,22] could now be confirmed up to age 6 years in both the ITT and the PP analyses. Although we previously analyzed the 3-year data in 2 ITT analyses (in or excluding fully breast-fed

TABLE I. ITT analyses: relative risk from marginal log-binomial models with generalized estimating equations by study formula in comparison with cow's milk feeding

	No. of followed children (N = 2252)			
	CMF (N = 556)	**pHF-W (N = 557)**	**eHF-W (N = 559)**	**eHF-C (N = 580)**
AM*				
Cumulative incidence, birth to 1 y				
Cases (%)	83 (18.0)	66 (14.8)	88 (18.7)	70 (15.3)
RR (95% CI)	1	0.82 (0.61-1.10)	1.04 (0.79-1.36)	0.85 (0.63-1.14)
Cumulative incidence, birth to 3 y				
Cases (%)	183 (40.5)	136 (31.2)	168 (36.2)	137 (30.4)
RR (95% CI)	1	0.78 (0.64-0.95)	0.92 (0.77-1.10)	0.76 (0.63-0.93)
Cumulative incidence, birth to 6 y				
Cases (%)	249 (56.0)	206 (47.1)	226 (49.9)	204 (46.1)
RR (95% CI)	1	0.82 (0.70-0.96)	0.90 (0.78-1.04)	0.80 (0.69-0.93)
AD				
Cumulative incidence, birth to 1 y				
Cases (%)	65 (14.1)	53 (11.9)	71 (15.0)	52 (11.3)
RR (95% CI)	1	0.84 (0.60-1.18)	1.07 (0.78-1.46)	0.80 (0.57-1.13)
Cumulative incidence, birth to 3 y				
Cases (%)	139 (30.8)	99 (22.6)	124 (26.6)	91 (20.1)
RR (95% CI)	1	0.77 (0.61-0.98)	0.93 (0.75-1.16)	0.69 (0.54-0.88)
Cumulative incidence, birth to 6 y				
Cases (%)	169 (37.9)	135 (31.1)	151 (33.1)	120 (27.1)
RR (95% CI)	1	0.79 (0.64-0.97)	0.92 (0.76-1.11)	0.71 (0.58-0.88)

RR, Relative risk.
*Defined by any of physician-diagnosed AD, urticaria, and food allergy/intolerance for the first 6 years; asthma in the third to sixth year; and rhinitis in the fourth to sixth year.

TABLE II. PP analysis: adjusted relative risk from marginal log-binomial models with generalized estimating equations by study formula in comparison with cow's milk feeding

	No. of followed children (N = 988)			
	CMF (N = 270)	**pHF-W (N = 256)**	**eHF-W (N = 242)**	**eHF-C (N = 220)**
AM*†				
Cumulative incidence, birth to 1 y				
Cases (%)	50 (21.1)	26 (12.0)	35 (16.0)	24 (12.8)
aRR (95% CI)	1	0.56 (0.37-0.86)	0.73 (0.50-1.07)	0.60 (0.39-0.92)
Cumulative incidence, birth to 3 y				
Cases (%)	97 (41.7)	62 (29.0)	74 (33.7)	44 (23.9)
aRR (95% CI)	1	0.67 (0.51-0.89)	0.82 (0.64-1.07)	0.60 (0.44-0.83)
Cumulative incidence, birth to 6 y				
Cases (%)	128 (55.7)	96 (45.46)	89 (42.2)	71 (39.0)
aRR (95% CI)	1	0.75 (0.60-0.93)	0.78 (0.63-0.97)	0.67 (0.53-0.85)
AD‡				
Cumulative incidence, birth to 1 y				
Cases (%)	40 (16.8)	21 (9.7)	30 (13.6)	18 (9.6)
aRR (95% CI)	1	0.54 (0.34-0.87)	0.73 (0.47-1.12)	0.54 (0.33-0.89)
Cumulative incidence, birth to 3 y				
Cases (%)	78 (33.5)	42 (19.5)	56 (25.6)	30 (162)
aRR (95% CI)	1	0.58 (0.41-0.82)	0.76 (0.56-1.04)	0.51 (0.34-0.75)
Cumulative incidence, birth to 6 y				
Cases (%)	90 (39.1)	58 (27.4)	64 (29.9)	41 (22.5)
aRR (95% CI)	1	0.64 (0.48-0.86)	0.74 (0.56-0.98)	0.55 (0.39-0.76)

aRR, Adjusted relative risk.
*Defined by any of physician-diagnosed AD, urticaria, and food allergy/intolerance for the first 6 years; asthma in the third to sixth year; and rhinitis in the fourth to sixth year.
†Adjusted for family history of AD, hay fever, and asthma, heredity of family allergy, sex, education, exposure to tobacco smoke, pets in the household, siblings, and study region.
‡Adjusted for family history of AD, sex, education, exposure to tobacco smoke, pets in the household, siblings, and study region.

children: N = 2252 or N = 1363, respectively), in the present analysis we decided for the ITT analysis to be based on all randomized children (N = 2252) because all of them were contacted based on the annual questionnaires.

Variations in the strength of the effects at 1 and 3 years in the present article compared with the 2 previous articles[21,22] are likely to be explained by a slightly different analysis. First, the outcome is not quite identical: in the 2 previous articles the outcome definition was based on clinical diagnosis, whereas in the present article we used the parental report of a physician's diagnosis during the first, second, third, fourth, fifth, and sixth years from the yearly questionnaires. The concordance between these 2 outcome definitions was checked at age 3 years, when clinical diagnosis and parental report of physician's diagnosis from

TABLE III. Prevalence of physician-diagnosed allergic diseases in the fourth to sixth years: relative risk and adjusted relative risk from marginal log-binomial models with generalized estimating equations by study formula in comparison with cow's milk feeding

	Prevalence (%)	CMF	pHF-W, RR (95% CI)	eHF-W, RR (95% CI)	eHF-C, RR (95% CI)
ITT AM*	21.6	1	0.82 (0.67-1.02)	0.85 (0.69-1.04)	0.82 (0.66-1.01)
AD	11.4	1	0.83 (0.61-1.12)	0.85 (0.63-1.15)	0.75 (0.55-1.03)
Rhinitis	6.6	1	0.87 (0.57-1.33)	0.66 (0.42-1.02)	0.82 (0.54-1.24)
Asthma	2.7	1	1.60 (0.74-3.45)	2.16 (1.02-4.58)	1.98 (0.92-4.29)
PP AM*†	20.0	1	0.71 (0.53-0.95)	0.61 (0.45-0.85)	0.62 (0.45-0.87)
AD‡	10.3	1	0.53 (0.34-0.84)	0.60 (0.39-0.93)	0.48 (0.29-0.78)
Rhinitis†	7.0	1	0.95 (0.55-1.63)	0.51 (0.26-1.02)	0.75 (0.40-1.41)
Asthma§‖	2.8	1	1.64 (0.59-4.53)	1.07 (0.34-3.38)	2.22 (0.77-6.41)

RR, Relative risk.
*Defined by any of physician-diagnosed AD, urticaria, food allergy/intolerance, asthma, and rhinitis.
†Adjusted for family history of AD, hay fever, and asthma, heredity of family allergy, sex, education, exposure to tobacco smoke, pets in the household, siblings, and study region.
‡Adjusted for family history of AD, sex, education, exposure to tobacco smoke, pets in the household, siblings, and study region.
§Adjusted for family history of asthma, heredity of family allergy, sex, education, exposure to tobacco smoke, pets in the household, siblings, and study region.
‖Estimated by using a logistic model because of low prevalences.

questionnaires were simultaneously available. This showed a moderate agreement, as measured by using the κ coefficient. The effect sizes for the preventive effects of hydrolyzed formulas were also similar. However, because of smaller variances, the preventive effects for AD and AM were more often significant when the diagnosis was based on parent-reported physician's diagnosis. Nevertheless, the similar pattern of the effects allows consistent implications.

Second, the analyzed populations are slightly different. In the ITT population more parents who did not comply with the feeding regimen (noncompliant) filled in the annual questionnaires. This is especially true for the eHF-C group, in which the fraction of noncompliance is 5% higher than in the pHF-W group. Interestingly, the percentage of AD in the noncompliant children randomized to the eHF-C group is much higher than in the pHF-W and CMF groups, which also might contribute to some of the slight differences in the 3-year results. The PP population comprises all compliant children independent of completeness of follow-up.

The results found with eHF-W in this 6-year analysis are equally interesting and puzzling. Until 3 years, this formula showed only a small preventive effect that never reached significance,[21] whereas in the present analysis it demonstrated a preventive late-onset effect on AD and AM in the PP population similar to that of the other 2 hydrolysates and a considerable, although not significant, effect on rhinitis. Although we had no real explanation for the ineffectiveness in the first 3 years,[21] this late-onset effect now is similarly difficult to understand. We did several sensitivity tests (genetic heredity of rhinitis and distribution of dropouts) but could not find any bias that might explain these results. We suggest interpreting these results with caution until we have analyzed the 10-year results of the GINI study, when allergic rhinitis will have developed to the most important atopic manifestation in childhood.

Similar to the results at 3 years,[21] there is no preventive effect of any of the formulas on the cumulative incidence of asthma at 6 years. In contrast, a relative risk of 1.1 or greater for the 3 hydrolysates (Table III) indicates that the physician's diagnosis of asthma is less frequently given to children fed with CMF than with hydrolysates. However, because of the few cases and the large CIs in both the ITT and PP analyses (Table III), a statement on the quality of the effect that the hydrolysates have on asthma does not seem appropriate.

Studies on the long-term effect of hydrolyzed infant formulas are rare. In the study by Vandenplas et al,[9] pHF-W showed a significant preventive effect on "any allergic symptoms" up to 5 years when compared with regular CMF. The comparison between unrestricted infant diet and eHF-C in combination with rigid recommendations for late introduction of solid foods revealed a significantly reduced cumulative food allergy until 4 years in the intervention group, which did not reach significance by 7 years.[19] No long-term data of studies that compared different hydrolyzed infant formulas with regular CMF[15] or breast milk[16] are available. Our study is the first and largest randomized trial ever conducted that followed high-risk children who were supplemented with different hydrolyzed infant formulas in the first 4 months of life up to the age of 6 years.

As mentioned in the 2 previous articles,[21,22] it was not the goal of the GINI study to compare the hydrolysates with breast-feeding as the gold standard for infant nutrition. Instead, we wanted to evaluate, in case of formula feeding (for whatever reason), which formula would be the best alternative to reduce the risk for AMs.

The major strength of the GINI study is the large scale of the study, the independency of the industry, and now the long-term follow-up until the age of 6 years. The major limitation of this study, however, as of other cohort studies, is that the follow-up was not complete. At least 1 questionnaire was answered in 90% of the 2252 randomized children and in 96% of the PP population. The 6-year questionnaire was answered by 75% and 80%, respectively. We carefully investigated whether participation was selective and might therefore bias the results. Less educated parents, parents from Wesel, or parents with more than 3 children participated less often until the sixth year of the child, but this was equally pronounced in all study formula groups. When controlling for parental education, number of siblings, and residency in Wesel, the relative risks remained nearly unchanged.

It might also be regarded as a limitation that for the present article a physician's diagnosis rather than a clinical diagnosis at visits in the study center, as in the 2 previous articles,[21,22] was used for the outcome determination. However, the type of instrument had no major influence on clinical implications.

In conclusion, the data of the 6-year follow-up of the GINI study confirm a persistent preventive effect of eHF-C and pHF-W in the ITT and PP analyses on AM and AD and, in addition, a late-onset effect of eHF-W in the PP analysis.

We thank the children and their families for continuous participation in the study, the GINIplus study group (see Appendix) for their excellent work, and the sponsors for financing the study.

Clinical implications: Early nutritional intervention with certain hydrolyzed infant formulas in high-risk children has a long-term preventive effect on AD until the age of 6 years.

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APPENDIX

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