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Allergic manifestation 15 years after early intervention with hydrolyzed formulas – the GINI Study

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GINIplus 15 Study Group details are given in Appendix

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Abstract

Background: Data on the long-term impact of hydrolyzed formulas on allergies are scarce.
Objective: To assess the association between early intervention with hydrolyzed formulas in high-risk children and allergic outcomes in adolescence.
Methods: GINI trial participants (n = 2252) received one of four formulas in the first four months of life as breastmilk substitute if necessary: partial or extensive whey hydrolyzate (pHF-W, eHF-W), extensive casein hydrolyzate (eHF-C) or standard cow's milk formula (CMF) as reference. Associations between these formulas and the cumulative incidence and prevalence of parent-reported physician-diagnosed asthma, allergic rhinitis (AR) and eczema, as well as spirometric indices and sensitization, were examined using generalized linear models.
Results: Between 11 and 15 years, the prevalence of asthma was reduced in the eHF-C group compared to CMF (odds ratio (OR) 0.49, 95% confidence interval (CI) 0.26–0.89), which is consistent with the spirometric results. The cumulative incidence of AR was lower in eHF-C (risk ratio (RR) 0.77, 95% CI 0.59–0.99]) and the AR prevalence in pHF-W (OR 0.67, 95% CI 0.47–0.95) and eHF-C (OR 0.59, 95% CI 0.41–0.84). The cumulative incidence of eczema was reduced in pHF-W (RR 0.75, 95% CI 0.59–0.96) and eHF-C (RR 0.60, 95% CI 0.46–0.77), as was the eczema prevalence between 11 and 15 years in eHF-C (OR 0.42, 95% CI 0.23–0.79). No significant effects were found in the eHF-W group on any manifestation, nor was there an effect on sensitization with any formula.
Conclusion: In high-risk children, early intervention using different hydrolyzed formulas has variable preventative effects on asthma, allergic rhinitis and eczema up to adolescence.

Abbreviations
aOR, adjusted odds ratio; AR, hay fever/allergic rhinitis; aRR, adjusted relative risk; CI, confidence interval; CMF, standard cow´s milk formula; eHF-C, extensively hydrolyzed casein formula; eHF-W, extensively hydrolyzed whey formula; GINI, German infant nutritional intervention; IgE, immunoglobulin E; ITT, intention-to-treat; n.s., statistically not significant at 5%; OR, odds ratio; pHF-W, partially hydrolyzed whey formula; PP, per protocol; RR, relative risk.

Early nutritional intervention with certain cow′s milk protein hydrolyzate infant formulas, one of several proposed approaches for allergy prevention in high-risk infants, has been shown to be successful and is recommended by international scientific societies (1–5).

However, despite the existing evidence supporting their use, the benefits of hydrolyzed formulas with respect to allergy prevention have recently been questioned (6). The lack of a well-understood underlying biologic mechanism is one concern, as is the reported lack of efficacy of a partial hydrolyzate (7). It is possible that beneficial effects may arise because of the absence of certain allergy-inducing epitopes or alternatively because of the presence of certain peptide profiles as the result of the hydrolyzing process (8). Whether both or only one of these mechanisms is at work, or even whether other mechanisms may also play a role, such as those involving skin contact, remains to be discovered (9, 10).

Most (11–19) but not all (7) randomized, controlled trials that have compared different partially and/or extensively hydrolyzed formulas with standard cow′s milk formula (CMF) have confirmed some degree of preventative effect on allergies in the first 2–3 years of life. Results appear especially consistent for eczema (20) within the first year of life. Randomized, controlled trials of nutritional interventions with hydrolyzed formulas on allergic outcomes in adolescence, including asthma, hay fever/allergic rhinitis (AR) and eczema, are scarce (21) or have used a multifaceted approach that combined nutritional and aeroallergen avoidance measures (22–24). Data on the long-term effectiveness of these interventions are important because asthma and especially AR risk increase during adolescence. The German Infant Nutritional Intervention (GINI) study allows a longitudinal evaluation of the long-term impact of hydrolyzed formulas on allergic outcomes in 2252 children with a positive family history for allergic diseases, which were randomized at birth to three different hydrolyzed formulas or standard cow′s milk formula (25–28).

The aim of the present study was to assess the long-term relation between early use of certain formulas and the occurrence of asthma, AR and of eczema, including lung-function and sensitization up to adolescence.

Materials and methods

Study design and population

The present study reports on the 15-year follow-up of the GINI trial, which was initiated in 1995 to investigate the potential preventive effects of three different hydrolyzates (pHF-W[1] , eHF-W[2] and eHF-C[3]), compared to regular cow′s milk formula (CMF[4]), on allergy development in children at high risk of allergy. The design of the study, details of recruitment, randomization, blinding and allocation of the formulas, outcome definitions, and results from the one-, three-, six-, and ten-year follow-ups have been published previously (21, 25–28). In brief, between September 1995 and July 1998, a total of 2252 healthy term newborns at high risk of allergy were recruited in two regions in Germany (Wesel, Munich) and randomly allocated at birth to one of four blinded study formulas using a computer-generated list. High risk was defined as having at least one parent or biologic sibling with a history of allergic disease. These formulas were used during the first four months of life as a milk substitute only if exclusive breastfeeding was not possible (25).

The 15-year follow-up examination was approved by the local ethic committees. The parents and adolescents gave their written informed consent.

15-year follow-up

All of the original 2252 trial participants, who had not actively declined further participation in the study, were invited to the 15-year follow-up examination (2011–2013). Parents received information about the aims and procedures of the follow-up examination, a form for written informed consent, the main questionnaire, and a list of modules to which they could individually consent.

The parental questionnaire included questions on their child′s health, allergic symptoms, physician diagnoses of allergic diseases, and several covariates. The questions pertaining to the primary outcomes (details outlined in the online repository Data S1) were identical to those posed during the one to ten year follow-ups (21). Participants were invited to the study center for a physical examination that included skin inspection, anthropometric data assessments, blood sampling for specific immunoglobulin E (IgE), and spirometric lung-function measurements. All procedures were conducted according to the standardized protocols, and extensive quality control was ensured by monitoring the physical examinations.

Primary outcome assessment

The primary outcomes of the present analysis are cumulative incidence up to 15 years of age and period prevalence between 11 and 15 years of age of asthma, AR, and eczema. A positive reply indicating a ‘yes response’ between 11 and 15 years and/or treatment in the last 12 months was used to determine the period prevalence. Any such positive reply during the lifetime of the child was used to determine the cumulative incidence. Both cumulative incidence and period prevalence were individually calculated for asthma, AR, and eczema using parental reports of physician diagnoses, asked separately for each year of life.

Secondary outcome assessment

Sensitization was assessed using specific IgE measurements as in previous examinations (21). The level of specific IgE to the

[1]Partial whey hydrolyzate (pHF-W) Beba- HA

[2]extensive whey hydrolyzate (eHF-W) HIPP-HA (at that time identical to Nutrilon Pepti)

[3]extensive casein hydrolyzate (eHF-C) Nutramigen

[4]standard cow′s milk formula (CMF) Nutrilon Premium

most common food (FX5) and aeroallergens (SX1) were measured with the CAP System (Pharmacia, Freiburg, Germany) at the age of 15 years. If the screening test was positive, single allergens were subsequently tested (FX5: children's food, containing hen's egg, milk protein, codfish, soybean, peanut, and wheat; and inhalation mix SX1, containing dermatophagoides pteronyssinus, rye, timothy grass, mugwort, birch pollen, Cladosporium species, and cat and dog dander). Additionally, ragweed was tested as a single allergen. Sensitization was defined as positive if at least one specific IgE level was 0.35 kU/l or greater (i.e. CAP class 1 or higher). Sensitization to any food, aero- and pollen allergens was reported.

Spirometry measurements were performed in line with the ATS/ERS recommendations (29), before (baseline) and after bronchodilation with salbutamol. A pneumotachograph-type spirometer (EasyOne Worldspiro meter, ndd, Zürich, Switzerland) was used to obtain flow-volume curves. The procedures used are outlined in the online repository. GLI reference equations (30) were applied to calculate z-scores for the spirometric indices using the software provided at http://www.ers-education.org/guidelines/global-lung-function-initiative/tools.aspx.

Statistics

Intention-to-treat (ITT) and per protocol (PP) analyses were performed. The ITT population consists of all primarily randomized children (n = 2252). The PP population consists of all children who received study formula within the first four months of life and who complied with the study protocol (n = 988) (31).

Logistic regression analyses were performed and odds ratios (OR) are reported for the associations between the study formulas with the period prevalence of the primary outcomes between 11 and 15 years of age, and with sensitization at 15 years.

Cumulative incidence was estimated by the life table method (32) and analyzed by generalized estimation equations (33) using PROC GENMOD with complementary log–log link and independent correlation structure. To assess whether the treatment effect remained constant over time, an interaction term between time and formula group was included in the models. The results are presented as relative risks (RR).

When analyzing the PP populations, all models were adjusted for a fixed set of known risk factors: family history of the modeled outcome (AR, asthma or eczema, respectively), heredity of family allergy, sex, study region, parental education, and older siblings. Results of the adjusted models are given as adjusted OR or RR (aOR, aRR). Interactions with sex and study region were assessed, but no indication of statistical significance was apparent.

In a sensitivity analysis, the effect of attrition was evaluated by repeating the analyses including only children who participated in all follow-ups.

To investigate a potential effect of the early nutritional intervention on lung function, analyses of covariance were performed on the z-scores and indices of spirometry (continuous variables). Age, height, and sex were included as covariates in the ITT and PP analyses (in PP in addition to the fix set of covariates listed above), and differences in least-square means were tested. When the assumption of normality was not met, the outcomes were transformed using a suitable function. In a sensitivity analysis, physician-diagnosed asthma was included as a covariate.

For analyses examining participation, multiple logistic regression models were performed and odds ratios (OR) are given. P values <0.05 were considered statistically significant, and estimates of OR and RR are given with 95% confidence intervals (95% CI). Statistical analyses were performed using the statistical software SAS for Windows, Release 9.2/9.3 (SAS Institute, Cary, NC, USA).

Results

Study population and participation

Questionnaire participation from the time of randomization up to 15 years of age for the ITT and PP populations is shown in Fig. 1. The mean age of the participants was 15.1 years. Response rates for the 15-year questionnaire were 61.1% in the ITT population (1377/2252) and 66.0% in the PP population (652/988), and did not differ significantly across the formula groups (Table S1). Of the 1377 children in the ITT population and 652 children in the PP population who responded to the 15-year questionnaire, 1056 (76.7%) and 492 (75%), respectively, also answered all prior questionnaires at one, two, three, four, six, and ten years (n.s. across the formula groups).

As observed in the six- and ten-year follow-ups (21, 27), participation at 15-years was influenced by study region, birth order, and parental education, but not by formula group, family history of eczema, family history of asthma, and double heredity of familial allergy (Table S1).

Clinical outcomes

Results on cumulative incidence, prevalence, and effect sizes relative to CMF are given in Fig. 2a–c and in Table 1.

Asthma

Constant (not significant) effects in cumulative incidence over time for pHF-W and eHF-W were observed, that is proportional curves in comparison with CMF. However, the effect for the eHF-C group changed after 10 years of age, and the increase between 11 and 15 years was reduced (Fig. 2a). The interaction term between time and eHF-C compared to the time-trend for CMF was significant (P = 0.036). The prevalence of asthma in the CMF group was 9.9%, and the prevalence was reduced in the eHF-C group in the ITT (OR 0.49, 95% CI 0.26–0.89) and PP (OR 0.47, 95% C, 0.19–1.17) populations, although the latter was not significant.

Allergic rhinitis

A significant reduction in AR cumulative incidence was observed for eHF-C in the ITT population (OR 0.77, 95% CI 0.59–0.99), but this effect was weaker and not significant in the PP population. The prevalence between 11 and 15 years was

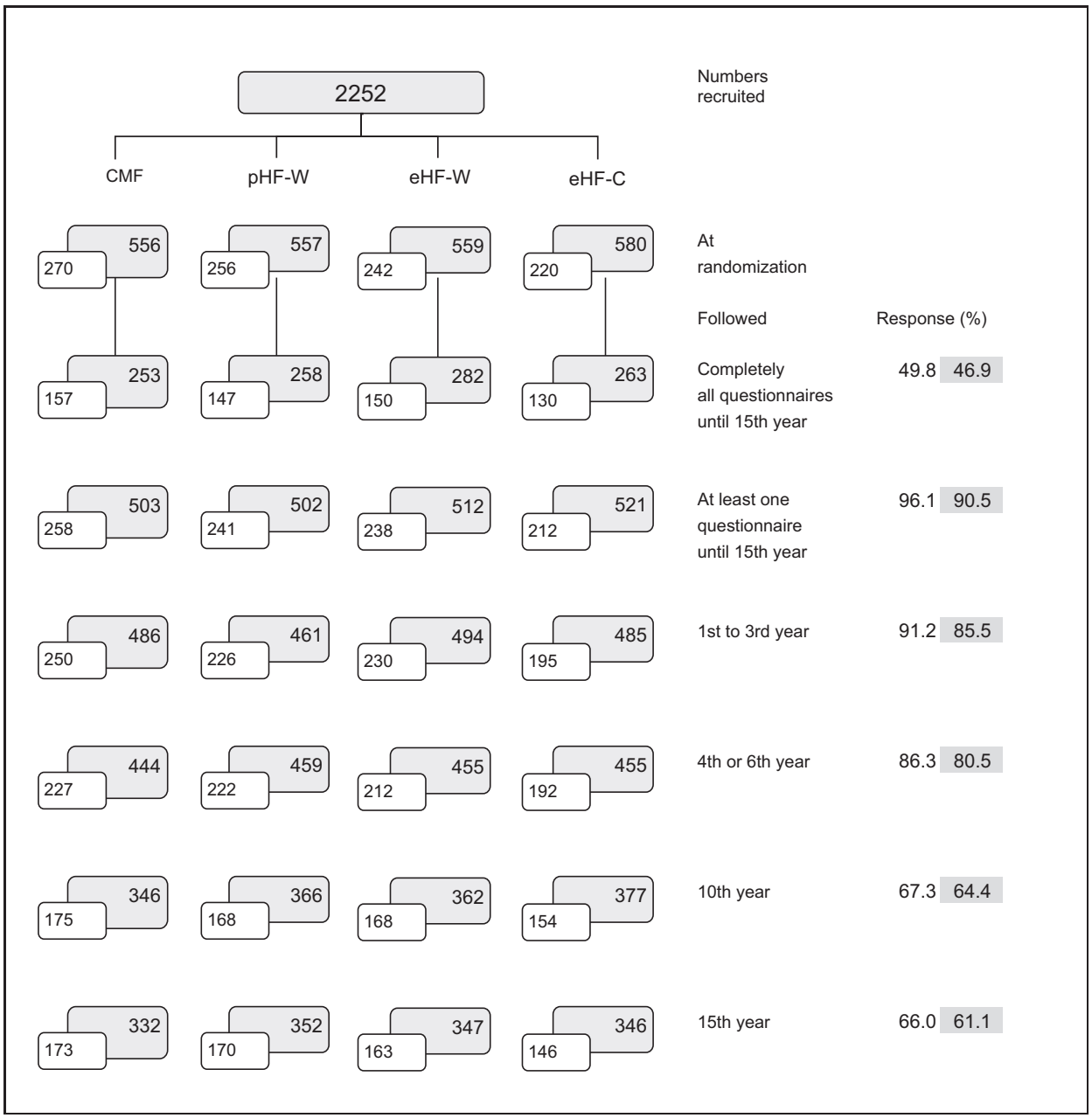

Figure 1 GINI study profile from birth to 15 years. Number of children followed and participation (%) for the ITT (shaded boxes) and PP (open boxes) population are shown.

significantly reduced for pHF-W (OR 0.67, 95% CI 0.47–0.95) and eHF-C (OR 0.59, 95% CI 0.41–0.84) in the ITT population. However, in the PP population the effect for pHF-W was weaker and no effect was observed for eHF-C. The eHF-W group did not differ from the CMF group.

Eczema

The cumulative incidence was significantly reduced in the eHF-C (OR 0.60, 95% CI 0.46–0.77) and pHF-W (OR 0.75, 95% CI 0.59–0.96) groups in the ITT population. Stronger effects were observed in the PP population. The numbers needed to treat to prevent one case of eczema up to 15 years of age for the pHF-W, eHF-W, and eHF-C formulas were 7, 11, and 6, respectively, in the PP population, and 14, 30, and 8 in the ITT population.

The prevalence between 11 and 15 years in the eHF-C (OR 0.42, 95% CI 0.23–0.79) and pHF-W (OR 0.58, 95% C, 0.33–1.02) groups was reduced in the ITT population, although the latter failed to reach statistical significance. In

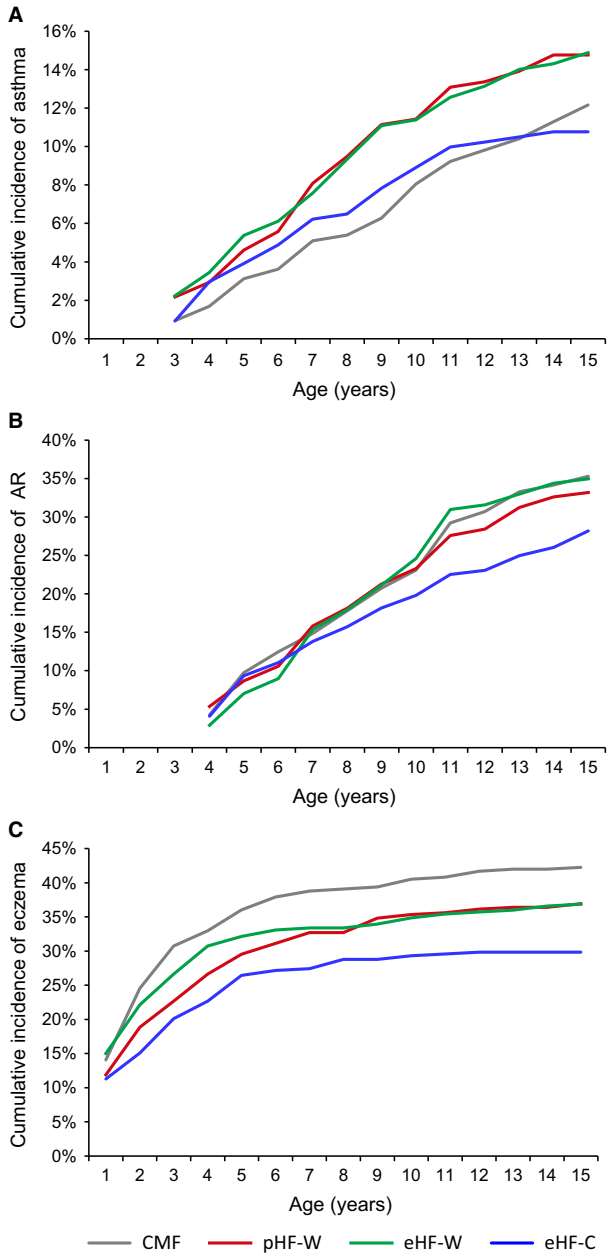

Figure 2 Cumulative incidence curves of the allergic manifestations asthma, hay fewer/allergic rhinitis and eczema until 15 years of age for the three different hydrolyzate formula groups and the cow's milk formula group (ITT population).

the PP population, the ORs for the prevalence estimates in the eHF-C and pHF-W groups were lower than 0.5 but not statistically significant. No significant effect was found in the eHF-W group.

Spirometry

Spirometry measurements were performed in 66% of the participants at the 15-year follow-up examination. Participation did not differ across formula groups. The spirometry results are given in Table 2. Lung volume indices (FVC, FEV1) did not significantly differ by formula group, but all flow indices were consistently highest in the eHF-C group. Particularly, indices of small airway function (FEF50, FEF25, and FEF25/75) were significantly higher in the eHF-C group compared to the CMF group. After bronchodilation, all indices increased in all formula groups, and the differences between formula groups weakened, suggesting partly reversible effects in the small airways (not shown). Adjusting for asthma did not change the results. The analyses based on z-scores yielded comparable findings (not shown).

Sensitization at 15 years

Sensitization data at 15 years were available for 963 and 449 children in the ITT and PP populations, respectively. Participation did not differ across formula groups.

Allergic sensitization, defined as at least one positive specific IgE value to any of the tested allergens, was 51.5% in the ITT population and 51.9% in the PP population. Most children were sensitized to aeroallergens (50.4% in the ITT population), specifically to pollen (40.7%). Only 12% of children were sensitized to food allergens. Similar proportions were observed across the analysis populations (ITT, PP) and formula groups (Table 3).

Sensitivity analyses

Sensitivity analyses, restricted to children who completed all questionnaires (at one, two, three, four, six, ten and 15 years of age), yielded comparable point estimates to those observed in the original analyses (not shown).

Discussion

The 15-year follow-up study of the GINI cohort provides evidence that the interventional use of certain hydrolyzate formulas as breastmilk substitutes in the first four months of life in high-risk children is associated with less eczema from birth until the age of 15 years. Furthermore, for the first time during this longitudinal follow-up, some beneficial associations between the use of certain formulas and respiratory allergies emerged.

Of the three hydrolyzates used in this study, only the eHF-C and pHF-W were associated with a lower occurrence of an allergic disease from early childhood up to adolescence. There is hardly any effect on any allergic outcomes in the eHF-W formula group, which is consistent with previous reports from the GINI cohort (21, 25).

Although the size of the preventative effect is always slightly higher for eHF-C, the most striking and consistent effect for both the eHF-C and pHF-W formulas is the reduced development of eczema from birth onwards. Even though eczema peaks in the first 3 years of life and thus the preventive effect on eczema has an early ceiling, as shown in the previous publications of the GINI study (21, 25), the documentation of this effect as being well sustained and with-

Table 1 Cumulative incidence from birth to 15 years and period prevalence between 11 and 15 years

		CMF	pHF-W	eHF-W	eHF-C
ITT, number of followed children (n = 2252)		n = 556	n = 557	n = 559	n = 580
Asthma Cumulative	%	12.2	14.8	14.9	10.8
incidence, 3 to 15 years	RR (95% CI)	1	1.26 (0.85–1.88)	1.29 (0.87–1.93)	0.91 (0.60–1.40)
Prevalence, 11 to	%	9.9	8.8	11.1	5.0
15 years, n = 1338	OR (95% CI)	1	0.88 (0.52–1.49)	1.15 (0.69–1.89)	**0.49 (0.26–0.89)**
AR† Cumulative	%	35.3	33.2	35.0	28.2
incidence, 4 to 15 years	RR (95% CI)	1	0.90 (0.70–1.16)	0.95 (0.74–1.22)	**0.77 (0.59–0.99)**
Prevalence, 11 to	%	30.7	22.8	27.2	20.6
15 years, n = 1331	OR (95% CI)	1	**0.67 (0.47–0.95)**	0.84 (0.60–1.18)	**0.59 (0.41–0.84)**
Eczema Cumulative	%	42.3	36.9	36.9	29.8
incidence, birth to 15 years	RR (95% CI)	1	**0.75 (0.59–0.96)**	0.84 (0.66–1.07)	**0.60 (0.46–0.77)**
Prevalence, 11 to	%	10.6	6.5	11.3	4.7
15 years, n = 1336	OR (95% CI)	1	0.58 (0.33–1.02)	1.08 (0.66–1.77)	**0.42 (0.23–0.79)**
PP, number of followed children (n = 988)		n = 270	n = 256	n = 242	n = 220
Asthma Cumulative	%	13.5	16.8	13.4	12.3
incidence, 3 to 15 years	RR (95% CI)	1	1.29 (0.75–2.21)	1.01 (0.57–1.79)	0.94 (0.52–1.71)
	aRR* (95%CI)	1	1.22 (0.70–2.12)	1.05 (0.59–1.87)	0.94 (0.51–1.72)
Prevalence, 11 to	%	9.9	9.8	9.7	4.9
15 years, n = 633	OR (95% CI)	1	0.99 (0.48–2.02)	0.98 (0.47–2.03)	**0.47** (0.19–1.17)
	aOR* (95% CI)	1	0.95 (0.46–1.97)	1.02 (0.48–2.15)	**0.47** (0.19–1.18)
AR†Cumulative incidence,	%	32.8	34.7	32.9	31.4
4 to 15 years	RR (95% CI)	1	1.04 (0.72–1.51)	0.95 (0.66–1.37)	0.91 (0.62–1.33)
	aRR* (95%CI)	1	0.99 (0.68–1.44)	0.90 (0.62–1.30)	0.88 (0.60–1.30)
Prevalence, 11 to	%	26.6	22.2	26.1	25.0
15 years, n = 632	OR (95% CI)	1	0.79 (0.48–1.30)	0.97 (0.60–1.60)	0.92 (0.55–1.53)
	aOR* (95% CI)	1	0.78 (0.47–1.32)	0.94 (0.56–1.56)	0.91 (0.54–1.54)
Eczema Cumulative	%	42.0	32.6	35.1	25.7
incidence, birth to 15 years	RR (95% CI)	1	**0.62 (0.44–0.89)**	0.77(0.54–1.09)	**0.47 (0.32–0.70)**
	aRR* (95%CI)	1	**0.58 (0.40–0.84)**	0.71 (0.49–1.03)	**0.45 (0.30–0.67)**
Prevalence, 11 to	%	10.2	4.9	10.9	5.0
15 years, n = 626	OR (95% CI)	1	**0.45** (0.19–1.08)	1.07 (0.53–2.18)	**0.46** (0.18–1.14)
	aOR* (95% CI)	1	**0.43** (0.18–1.04)	1.02 (0.49–2.12)	**0.43** (0.17–1.08)

Relative risks (RR), adjusted RR (aRR*), odds ratios (OR), and adjusted OR (aOR*) for the three different hydrolyzate formula groups, when compared to the cow's milk formula group (ITT and PP population).

*Adjusted for family history of disease, heredity of family allergy, sex, study region, siblings, and parental education.

†Hay fever/allergic rhinitis.

Bold values and bold CI were used for significant effects, bold values for strong effects with loss of significance.

out any rebound up to 15 years is an important finding for the children, their families, and the health economy (34). For eHF-C the prevalence of eczema between 11 and 15 years in the ITT population is significantly reduced, while for pHF-W the reduction of >40% just fails to reach statistical significance. The fact that the ORs for the prevalence estimates (ORs <0.5) in the PP analysis are not significant could be explained by reduced power due to the small numbers in the PP analysis.

Using a longitudinal design, this study is the first to show associations between hydrolyzed formulas with asthma and allergic rhinitis, diseases which typically occur later in childhood than eczema. Children who belonged to the eHF-C formula group had a lower likelihood of being asthmatic between the age of 11 and 15 years, an effect that was confirmed by spirometry. Measures for distal airway function seemed to be especially positively affected by the eHF-C formula. This is important to note because small-airways dysfunction can already be present in patients with mild asthma (35). The associations between eHF-C and pHF-W with AR found in the ITT population were not apparent in the PP population. This is surprising, because one would expect stronger effects in the PP population. On the other hand, the number of subjects in each formula group is smaller in the PP population and this could affect instability in the estimation of effects. Nevertheless, although the association between early formula feeding and the emergence of a late-onset preventative effect on respiratory allergies is interesting, it should be interpreted with caution until further results confirm these findings.

Table 2 Spirometry parameters. Means of measured values from linear models for the three different hydrolyzate formula groups and the cow's milk formula group (ITT and PP population).

	CMF		pHF-W		eHF-W		eHF-C	
	Mean*	$P^{\dagger}$	Mean*	$P^{\dagger}$	Mean*	$P^{\dagger}$	Mean*	$P^{\dagger}$
ITT	$n = 220$		$n = 234$		$n = 233$		$n = 231$	
FEV1/FVC	0.8643	Ref	0.8541	0.139	0.8642	0.935	0.8743	0.073
PEF [l/s]	7.0394	Ref	7.0083	0.477	7.1212	0.513	7.1412	0.299
FEF75 [l/s]	2.1783	Ref	2.1662	0.671	2.1902	0.854	2.2530	0.233
FEF50 [l/s]	4.3511	Ref	4.2954	0.457	4.3862	0.710	4.5636	0.020
FEF25 [l/s]	6.1179	Ref	6.0519	0.346	6.1748	0.639	6.3002	0.066
FEF25/75 [l/s]	3.8449	Ref	3.8025	0.487	3.8681	0.800	3.9943	0.068
PP	$n = 112$		$n = 114$		$n = 110$		$n = 95$	
FEV1/FVC	0.8600	Ref	0.8562	0.605	0.8673	0.374	0.8773	0.044
PEF [l/s]	6.9690	Ref	7.0774	0.593	7.2916	0.030	7.2039	0.085
FEF75 [l/s]	2.1064	Ref	2.1961	0.403	2.2261	0.220	2.2647	0.080
FEF50 [l/s]	4.2864	Ref	4.3092	0.922	4.5068	0.092	4.5960	0.018
FEF25 [l/s]	6.0488	Ref	6.0664	0.995	6.3504	0.031	6.3663	0.022
FEF25/75 [l/s]	3.7721	Ref	3.8353	0.646	3.9573	0.118	4.0214	0.035

*Means for original values adjusted for age, sex, height, and weight, in PP additionally for family history of asthma, heredity of family allergy, study region, siblings, and parental education

†Tests for the comparison with cow's milk formula (CMF) on original values or after normalizing using suitable function. FEV1 = forced expiratory volume in one second. FVC = forced vital capacity. PEF = peak expiratory flow. FEF25, FEF50, FEF75 = forced expiratory flow rates 25, 50 and 75% of exhaled FVC. FEF25/75 = mean flow rate between 25% and 75% of FVC.

One of the critical arguments against the use of hydrolyzed formulas for allergy prevention is a lack of understanding of the mechanisms driving the beneficial effects. As 80–90% of respiratory allergic diseases in children and young adolescents are associated with sensitization to aeroallergens (mainly pollen and house dust mites), it is reasonable to hypothesize that IgE might be involved. However, we did not observe any association between specific IgE measured in serum and hydrolyzates at 15 years, which is consistent with our findings at six and ten years (21, 27) and with reports from the literature (17–19). Therefore, IgE does not appear to be involved in the observed findings in this study. Although we cannot exclude the possibility that, due to selective participation in blood sampling, IgE may still play a role, other (immune-) mechanisms should be considered. It can be speculated that the observed differential effects of the individual formulas may be driven by the absence of allergy-inducing epitopes, the presence of certain peptides that promote the development of tolerance via activation of T regulatory cells (36), or differential changes on the mucosal level in the gut, skin, or respiratory tract.

The differential effects of the formulas imply that all hydrolyzed formulas are not equal. Instead, each formula has to be considered as its own individual intervention. This is an important message that needs to be considered when evaluating which formula(s) should be provided to a child. Our results suggest that, apart from characteristics such as molecular weight profile and basic protein (whey or casein), other factors seem to be relevant to the biologic functionality of hydrolyzed formulas. Recent studies indicate that the peptide profiles of different commercially available cow′s milk protein hydrolyzates provide a descriptive and distinct signature as a result of the hydrolyzing process, which changes the peptide sequences (8). Through the use of new technologies, our previously reported hypothesis (21), that is the individual hydrolyzing process for each formula changes its biologic functionality, is now confirmed. This new knowledge may help explain the currently poorly understood differential effects of the three hydrolyzed formulas used in the GINI trial.

As reported data on the preventive potential of hydrolyzed formulas are inconsistent, an updated systematic review, which includes the study by Lowe et al. (7), should be used to guide feeding practices for high-risk children who need breastmilk supplementation. In the study by Lowe, although the same pHF-W formula was used, no preventive effect on allergy was observed up to 6–7 years of age. We appreciate the publication of null-findings to avoid publication bias and to learn from the differences between studies, which may help explain why inconsistent results have been observed. However, based on the results reported in this study, we currently feel that – at least for eczema – there is sufficient evidence to recommend feeding certain hydrolyzed formulas to children at high risk who need breastmilk supplementation (37).

The main strengths of this study are the original large sample size of 2252 children, randomization and allocation concealment, and a fair participation at 15 years of age (ITT 61.1%, PP 66.0%), which are the formula groups. Identical questions were posed for the primary outcomes in all follow-ups, from one up to 15 years of age (21). The current study nevertheless also has important limitations, such as the unblinding of the formulas when the youngest child turned three years old, as extensively discussed in our previous publication (21). Another potential source of bias is that the

Table 3 Sensitization†. Point prevalence at 15-year follow-up. Odds ratio or adjusted odds ratio (aOR*) from logistic regression for the three different hydrolyzate formulas in comparison with cow's milk formula (ITT and PP population).

	CMF		pHF-W		eHF-W		eHF-C	
ITT	*n* = 227		*n* = 239		*n* = 250		*n* = 247	
	%	OR	%	OR (95%CI)	%	OR (95%CI)	%	OR (95%CI)
Sensitization	51.5	1	48.5	0.89 (0.62–1.28)	51.6	1.00 (0.70–1.44)	54.3	1.12 (0.78–1.60)
Food	11.0	1	11.7	1.07 (0.61–1.90)	12.0	1.10 (0.63–1.94)	13.0	1.20 (0.69–2.10)
Aero	51.1	1	46.4	0.83 (0.58–1.19)	51.2	1.00 (0.70–1.44)	52.6	1.06 (0.74–1.53)
Pollen	39.6	1	39.3	0.99 (0.68–1.43)	43.2	1.16 (0.72–1.50)	40.5	1.04 (0.72–1.50)
PP	*n* = 113		*n* = 118		*n* = 119		*n* = 99	
	%	aOR*	%	aOR* (95%CI)	%	aOR* (95%CI)	%	aOR* (95%CI)
Sensitization	48.7	1	50.8	1.11 (0.65–1.90)	52.1	1.11 (0.65–1.89)	56.6	1.41 (0.80–2.47)
Food	11.5	1	14.4	1.34 (0.61–2.93)	11.8	0.98 (0.43–2.20)	13.1	1.15 (0.50–2.64)
Aero	48.7	1	49.2	1.05 (0.61–1.78)	51.3	1.08 (0.63–1.84)	54.5	1.29 (0.74–2.27)
Pollen	38.9	1	41.5	1.16 (0.67–2.01)	43.7	1.18 (0.68–2.03)	36.4	0.87 (0.49–1.56)

*Adjusted for heredity of family allergy, sex, study region, siblings, and parental education.
†At least 1 specific IgE level ≥ 0.35 kU/l (i.e. CAP class ≥ 1).

assessment of the formula effect is based on a parental report of a physician's diagnosis, as is the case for most epidemiological studies, and not on a clinical examination in the study centers. The inclusion of objective health assessments (skin examination, spirometry, and atopic sensitization) is a further strength, but the data should be interpreted with caution because the consent to lung-function testing and blood sampling was self-selective with response rates of 67% and 70%.

Conclusion

The results of the 15-year follow-up of the GINI study confirm that the previously reported preventative effect of the eHF-C and pHF-W formulas on eczema are sustained until adolescence without the existence of a rebound phenomenon. During the last years of follow-up, fewer emergences of AR and asthma were related to the use of certain hydrolyzates, mainly eHF-C. The effect in the eHF-C formula group on asthma is consistent with the spirometric results. The findings with respect to the respiratory allergies should be interpreted with caution until confirmed in future studies. None of the formulas had an influence on IgE sensitization.

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Author contributions

AvB contributed to the conception and design of the cohort study, was coordinator of the study and responsible for study execution in Wesel, interpreted the data, and drafted the manuscript. BFP performed statistical analysis, contributed to interpretation, and drafted parts of the manuscript. HS contributed to design of 15-year follow-up, spirometry measurement, interpretation of spirometry, and drafting the manuscript. UH contributed to data collection and clinical examination in Munich. EL contributed to data management since 6-year follow-up. MSu contributed to data management since 10-year follow-up. MSch contributed to study execution and data collection since 10-year follow-up. IB contributed to the design of 10- and 15-year follow-up. MSt contributed to the design of 15-year follow-up and data management. UK contributed to the design since 6-year follow-up and interpretation of the data. BH contributed to the design of 15-year follow-up, interpretation, and drafting the manuscript. JH contributed to the conception and design of the cohort study, interpretation, and drafting the manuscript. CPB contributed to the conception and design of the cohort study, interpretation, and drafting the manuscript. SK contributed to the conception and design of the cohort study, interpretation, and drafting the manuscript. DB is principal investigator of the GINI study, contributed to the conception and design of the cohort study, interpretation and drafting the manuscript, and agreed to be accountable for all aspects

of the work. All authors without contribution to drafting revised the manuscript critically for important intellectual content. All authors approved the final manuscript for publishing.

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Conflicts of interest

AvB has received speakers' fees from Nestlé Nutrition Institute. The Research Institute at the Marien-Hospital has received grant from Nestlé Vevey, Switzerland. SK has received research support from Mead Johnson; has board memberships with MSD, Nestlé, Danone, Merk, Nutricia; is a consultant for Boehringer Ingelheim, MSA, Merck, Abbvie, Danone; has received speakers' fees by Centocor, MSD, Danone, Merck, Vifor, Nestlé Nutrition Institute, Euroimmun, Thermo-Fischer (Phadia), Abbvie, Schär, Hipp, Falk and travel support by MSD; the institution has received grants from Nestlé Nutrition, Mead Johnson, Thermosphere, Euroimmun, INOVA, R-Biopharm, Schär. The rest of the authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1 Supplementary information on study methods, primary outcomes and procedure of lung-function assessment

Table S1 Association between possible factors and participation (%) at 15 years expressed as adjusted OR (aOR*)

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Appendix A

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