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Long-term effects of hydroyzed formulae on atopic diseases in the GINI study

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Abstract:	Clinical Implication Results from 20-year follow-up of the GINI study suggest that hydrolyzed formulae that reduced eczema in early childhood have a long-term effect on eczema and asthma in young adults. Such hydrolyzed formulae have a role in allergy prevention in high risk infants.

Cover Letter to Editor only

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08/13/20

Dear Dr. Leung

Please find enclosed our manuscript entitled: „ Long-term effects of hydroyzed formulae on atopic diseases in the GINI study“ by Monika Gappa and Birgit Filipiak-Pittroff et al.

Hydrolyzed formulae were designed for prevention of atopic diseases in children, but their efficacy and long-term effects have been challenged repeatedly. The German Infant Nutritional Intervention (GINI) study allows long-term evaluation of the effect of hydrolyzed formulae on allergic diseases in high risk children.
In this double-blind, placebo-controlled trial, children were allocated at birth to one of three hydrolyzed formulae or a cow's milk-based formula as reference. The present manuscript reports the findings of the 20-year follow-up of the GINI study.
We found that certain hydrolyzed formulae that reduced eczema in early childhood have a long-term preventive effect on eczema and asthma in young adults. Therefore, such hydrolyzed formulae have a role in allergy prevention in high risk infants.
Our findings support the hypothesis that use of certain hydrolyzed formulae, if exclusive breastfeeding is not feasible, still has a role in allergy prevention until young adulthood.

We are convinced that our report will make an important contribution to the field and will be of particular interest to the readership of *JACI – in practice*. The study has been performed in accordance with the latest version of the Declaration of Helsinki and has been approved by the local ethics committees. All the authors listed fulfil the criteria for authorship and have read and approved the manuscript that is enclosed. The work described has not been submitted elsewhere for publication, in whole or in part.

We are looking forward to hearing from you.

Sincerely,

Dr. Marie Standl

Long-term effects of hydroyzed formulae on atopic diseases in the GINI study

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Clinical Implication

Results from 20-year follow-up of the GINI study suggest that hydrolyzed formulae that reduced eczema in early childhood have a long-term effect on eczema and asthma in young adults. Such hydrolyzed formulae have a role in allergy prevention in high risk infants.

Key words

Allergy prevention, double-blind randomized trial, hydrolyzed infant formulas, nutritional intervention, 20-year follow-up of birth cohort

Family history of atopy is the single most important risk factor for developing allergic diseases. Therefore, prevention of allergic disease in the offspring of at-risk families has been on the agenda for many decades. Hydrolyzed formulae are designed to prevent manifestation of atopic diseases, but their efficacy and long-term effects have been challenged repeatedly (1–3). The German Infant Nutritional Intervention (GINI) study allows long-term evaluation of the effect of hydrolyzed formulae on allergic diseases in high risk children (4,5).

In the original double-blind trial 2,252 healthy term newborns with a positive family history for allergic diseases in at least one first degree relative were recruited at birth in Munich and Wesel (Germany) between 1995 to 1998, and randomized to one of three hydrolyzed formulae [partially hydrolyzed whey (pHF-W); extensively hydrolyzed whey (eHF-W); extensively hydrolyzed casein (eHF-C)] or a formula based on intact cow's milk (CMF) as reference. These formulae were fed during the first four months of life as a breast milk substitute only if exclusive breastfeeding was not possible. All subjects were followed by questionnaires at 1, 2, 3, 4, 6, 10, 15 and now 20 years of age (response numbers in Figure 1). Here, we report the 20-year follow-up, which was approved by the local ethics committees. Written informed consent was obtained.

Main outcomes were defined using yearly questions on doctor diagnoses of eczema, asthma and allergic rhinitis/hay fever (AR), covering the timeframe since the previous follow-up. At 20 years, questionnaires were completed by the study participants themselves (by their parents up to the 15-year follow-up). Any positive response during lifetime was used to determine cumulative incidence. A positive response and/or disease specific treatment in the last 12 months was defined as prevalence between 16 and 20 years.

Intention-to-treat (ITT), and per protocol (PP) analyses were performed. The ITT population consists of all primarily randomized children (n=2,252). The PP population comprises all children who received study formula within the first four months of life and complied with the study protocol (n= 988). Details of design, sample size, recruitment, outcome definitions, and follow-up have been published previously (5,6) and statistical methods are described in Online Repository.

At the 20-year follow-up, information on the main outcomes were available for 1,199 subjects in the ITT-analysis and 548 subjects in PP-analysis (Figure 1). Asthma prevalence between 16 and 20 years was significantly lower in the eHF-C group [adjusted odds ratio (aOR)=0.46; 95% confidence interval (CI)=(0.24-0.87)], and in the pHF-W group [aOR=0.44; 95%CI=(0.23-0.85)], compared to CMF (Table 1). In the PP population, effect sizes were similar but not significant. For AR, no significant differences in incidence or prevalence were observed in the ITT-analysis or PP-analysis. In the ITT-analysis of eczema, the cumulative incidence was reduced in the eHF-C [relative risk (RR)=0.61;

95%C=(0.47-0.78)] and the pHF-W [RR=0.73; 95%CI=(0.57-0.94)] groups and the prevalence between 16 and 20 years in the eHF-C group [aOR=0.49; 95%CI=(0.25-0.94)] compared to the CMF group. The effects on the cumulative incidence were even stronger in the PP analysis, but the effect of eHF-C on prevalence did not reach significance.

The 20-year follow-up of the GINI study group found a significant beneficial long-term effect of certain hydrolyzed formulae on allergic manifestations. For eczema incidence, this effect is similar to results obtained up to the age of 15 years for eHF-C and pHF-W (5). Moreover, asthma prevalence from 16-20 years was significantly reduced in pHF-W in the ITT analysis, which was not observed previously. Mediation analysis showed that this preventive effect on asthma in young adults cannot be explained by the observed reduction in eczema by certain hydrolyzed formulae in the first 3 years (6): the percentage of the total association that was explained by early eczema was 8.1% for eHF-C and 6.6% for pHF-W.

For AR, the protective effect observed at age 15 years was no longer present at 20 years. This observation could not be explained by the use of immunotherapy (data not shown) or other factors related to non-random loss-to-follow-up (Online Repository Tables E1 and E2). As the 20-year follow-up of the GINI study was a solely questionnaire-based survey, no testing for specific IgE antibodies or spirometry were performed as in previous follow-ups. Further limitations are the use of different questionnaire types compared to previous follow-ups (online version, paper version and shortened paper version; Online Repository Table E2) and a 10% higher participation among females (Online Repository Table E1). Therefore, the prevalence estimates should be compared with previous periods with caution. We cannot exclude that the participants interpreted questions on physician's diagnosis of AR differently, although this is very unlikely to differ between intervention groups and should apply to asthma and eczema similarly. It could also be hypothesized, that other factors become more important in the onset of AR in late adolescence or young adulthood. Additionally, drop-out rates from the original population until 20-year follow-up was high; however, this is not unusual in long-term follow-up studies and rates were similar across all study groups.

It has repeatedly been questioned whether hydrolyzed formulae should have a role in primary allergy prevention (1,7). Recently, the American Academy of Pediatrics (8) stated that there is not sufficient evidence to support the use of hydrolyzed formulae for prevention of atopic disease. A recent nationwide study in France even found an increase in hyperreactive airway disease in infants who had received partially hydrolyzed formula in short-term (9). In contrast, our study demonstrates that the preventive effect is different with different formulae and not entirely dependent on the degree of denaturation of the protein. Accordingly, pooling of different types of hydrolyzed formulae is misleading.

In summary, in the GINI study, both, eHF-C and pHF-W reduced prevalence of asthma after puberty in a high-risk population and retained their effect on eczema until adulthood while eHF-W did not provide preventive effects. Our findings support the hypothesis that use of certain hydrolyzed formulae, if exclusive breastfeeding is not feasible, still has a role in allergy prevention until adulthood for both, cutaneous and airway manifestation of allergic diseases.

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Conflict of interest

AvB has received speakers' fees from Nestlé Nutrition Institute. The Research Institute at the Marien-Hospital has received a grant from Nestlé Vevey, Switzerland. SK received research support from BioGaia, Mead Johnson and Nestec Nutrition, and received honoraria or consultant fees from Abbvie, BerlinChemie, Celgene, Danone, Janssen, Mead Johnson, Nestlé, Pharmacosmos, Pfizer, Takeda, Vifor. LL is member of the German National Breastfeeding Committee. MG received research funding from Boehringer and Nestlé Vevey, Switzerland; and received honoraria for lectures and consultant fees from Astra Zeneca, Boehringer, Chiesi, GSK, Novartis, Pari and Sanofi.

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Figure Caption

Figure 1 Participant number at each follow-up of the GINI study for the ITT (shaded boxes) and PP (open boxes) populations

Table 1 Cumulative incidence from birth to 20 years and period prevalence between 16 and 20 years. Relative Risks (RR), adjusted RR (aRR), Odds Ratios (OR) and adjusted OR for the three different hydrolyzate formula groups, when compared to the cow's milk formula group. ITT and PP population.

		CMF	pHF-W	eHF-W	eHF-C
ITT, number of followed children (N=2252)		556	557	559	580
Asthma Cumulative incidence, 3 to 20 years	%	16.2	16.1	17.6	14.1
	RR (95% CI)	1	1.06 (0.73-1.54)	1.16 (0.81-1.68)	0.90 (0.61-1.31)
Prevalence, 16 to 20 years, N=1184	%	10.1	5.0	7.1	5.4
	OR (95% CI)	1	**0.47 (0.25-0.89)**	0.67 (0.38-1.21)	**0.50 (0.27-0.95)**
	aOR[2] (95% CI)	1	**0.44 (0.23-0.85)**	0.64 (0.35-1.16)	**0.46 (0.24-0.87)**
AR[+] Cumulative incidence, 4 to 20 years	%	42.3	39.2	39.9	37.5
	RR (95% CI)	1	0.90 (0.71-1.15)	0.92 (0.73-1.17)	0.83 (0.65-1.06)
Prevalence, 16 to 20 years, N=1169	%	24.0	22.9	22.4	23.8
	OR (95% CI)	1	0.94 (0.64-1.38)	0.91 (0.62-1.34)	0.99 (0.67-1.45)
	aOR[2] (95% CI)	1	0.94 (0.64-1.39)	0.97 (0.65-1.44)	1.00 (0.68-1.48)
Eczema Cumulative incidence, birth to 20 years	%	44.0	37.5	40.3	32.1
	RR (95% CI)	1	**0.73 (0.57-0.94)**	0.86 (0.68-1.10)	**0.61 (0.47-0.78)**
Prevalence, 16 to 20 years, N=1176	%	9.5	6.4	9.5	5.4
	OR (95% CI)	1	0.64 (0.35-1.19)	0.99 (0.57-1.73)	**0.54** (0.28-1.02)
	aOR[2] (95% CI)	1	0.60 (0.32-1.13)	0.94 (0.53-1.66)	**0.49 (0.25-0.94)**
PP, number of followed children (N=988)		270	256	242	220
Asthma Cumulative incidence, 3 to 20 years	%	16.9	17.4	15.5	16.3
	RR (95% CI)	1	1.10 (0.66-1.84)	0.93 (0.55-1.59)	0.97 (0.57-1.66)
	aRR[1] (95%CI)	1	1.05 (0.62-1.79)	0.97 (0.56-1.66)	0.95 (0.55-1.64)
Prevalence, 16 to 20 years, N=539	%	7.7	3.7	6.5	5.7
	OR (95% CI)	1	**0.46** (0.16-1.36)	0.84 (0.34-2.09)	0.73 (0.27-1.95)
	aOR[2] (95% CI)	1	**0.45** (0.15-1.36)	0.80 (0.31-2.08)	**0.68** (0.25-1.87)
AR[+] Cumulative incidence, 4 to 20 years	%	40.4	41.1	36.8	40.4
	RR (95% CI)	1	1.03 (0.73-1.45)	0.88 (0.62-1.25)	0.95 (0.67-1.34)
	aRR[1] (95%CI)	1	1.02 (0.72-1.45)	0.86 (0.61-1.23)	0.97 (0.68-1.37)
Prevalence, 16 to 20 years, N=535	%	21.4	25.7	20.9	26.7
	OR (95% CI)	1	1.27 (0.73-2.22)	0.97 (0.54-1.72)	1.33 (0.75-2.36)
	aOR[2] (95% CI)	1	1.27 (0.72-2.26)	0.90 (0.49-1.63)	1.38 (0.76-2.50)
Eczema Cumulative incidence, birth to 20 years	%	42.0	33.2	39.3	27.2
	RR (95% CI)	1	**0.63 (0.44-0.91)**	0.83 (0.58-1.18)	**0.49 (0.33-0.72)**
	aRR[1] (95%CI)	1	**0.59 (0.41-0.86)**	0.78 (0.54-1.12)	**0.47 (0.31-0.70)**
Prevalence, 16 to 20 years, N=538	%	6.4	5.2	10.1	5.7
	OR (95% CI)	1	0.79 (0.29-2.18)	1.63 (0.68-3.90)	0.88 (0.32-2.43)
	aOR[2] (95% CI)	1	0.72 (0.25-2.02)	1.54 (0.63-3.79)	0.77 (0.27-2.18)

+ hay fever/allergic rhinitis

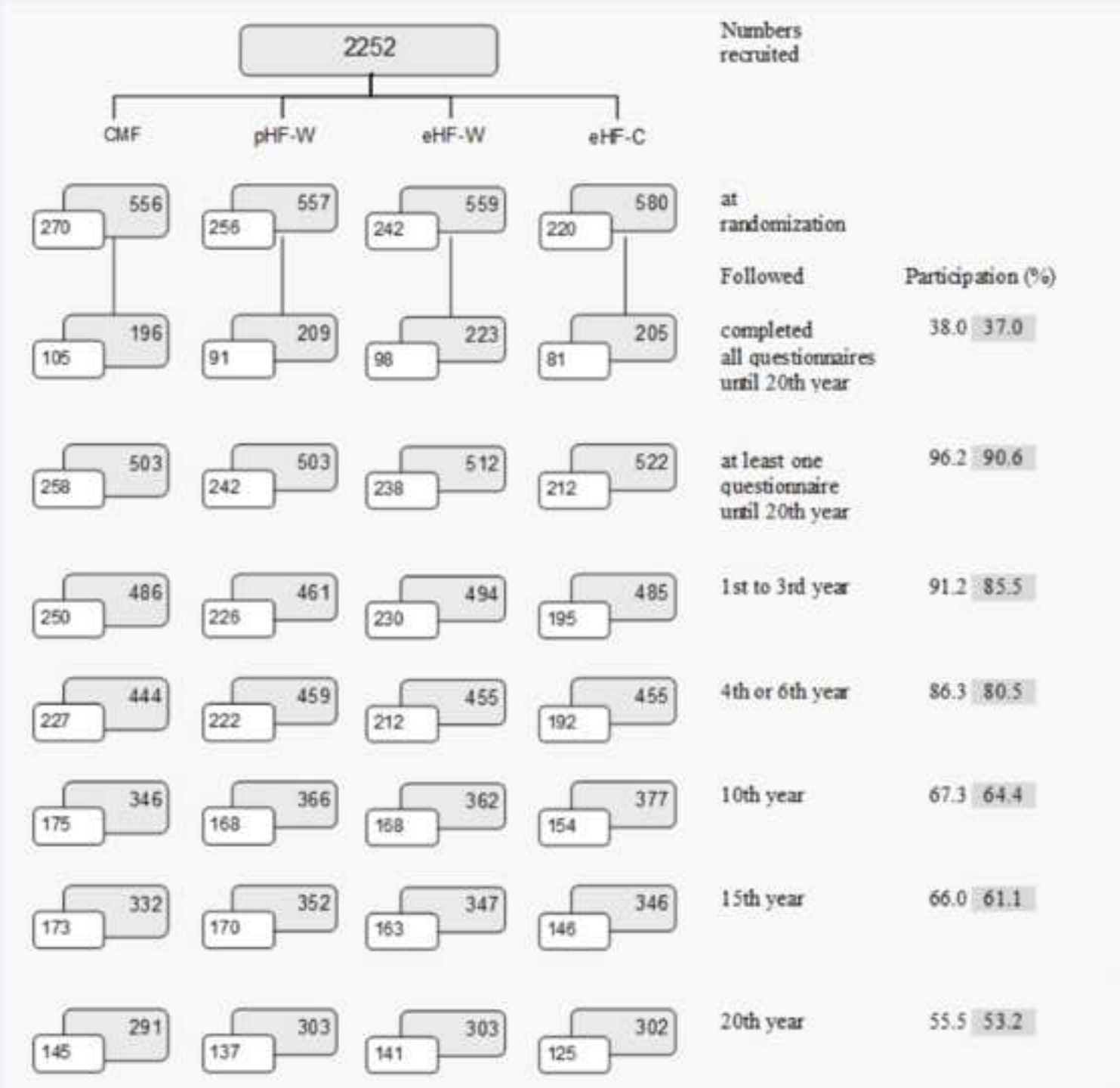
1 adjusted for parental history of disease, heredity of family allergy, sex, study region

2 adjusted for parental history of disease, heredity of family allergy, sex, study region, education and cigarette smoking of young adult, actual pets and type of questionnaire

Bold values and bold CI represent significant effects; bold values but not bold CI indicate strong effects with loss of significance

Figure No.1: Participant number at each follow-up of the GINI study for the ITT (shaded boxes) and PP (open boxes) populations

Click here to access/download;Figure No.;Figure1.png



Long-term effects of hydroyzed formulae on atopic diseases in the GINI study

Online Repository

Methods

Study population

Between September 1995 and June 1998, a total of 2,252 healthy term newborns with high risk of allergy were recruited from 16 maternity wards in two regions of Germany (rural Wesel and urban Munich) for the prospective, double-blind intervention trial. High risk was defined as having at least one parent or biologic sibling with a history of allergic disease. If parents agreed to participate, the newborns were randomly allocated at birth to one of four blinded study formulae using a computer-generated list. These formulae were used during the first four months of life as a milk substitute only if exclusive breastfeeding was not possible.

In addition to completing yearly self-administered questionnaires regarding the child's health, nutrition, and living conditions, the parents kept weekly diaries for the first 6 months and participated in structured interviews and physical examinations at regular intervals at the study center until the age of 3 years. Non-compliance was defined as not following the milk-feeding recommendations.

Follow-up

All subjects were followed until the 20th year. Self-administered questionnaires were sent to the parents at their child's 1st, 2nd, 3rd, 4th, 6th, 10th and 15th birthdays. At the age of 20, the study participants themselves were asked to complete an online questionnaire. It was also possible to receive a paper version or to use a shortened version. Information on the study participants' health, allergic symptoms and treatment, physician diagnoses of allergic diseases and several lifestyle factors were collected.

Primary outcomes

Data on the primary outcome was collected in the 20-year-questionnaire using the following question: "Did a doctor diagnose you with any of the following diseases [asthma, eczema, hay fever, allergic rhinitis] in the past 5 years, and if so, at what age [16, 17, 18, 19, 20]?". These questions correspond to those used in the previous questionnaires administered to the parents. Hay fever and perennial allergic rhinitis were asked as one question until the 6-year follow-up and then separately as two questions. For analyses, answers were combined.

The questions about treatment were: "Have you been treated for [asthma, hay fever, allergic rhinitis, atopic eczema] in the past 12 months?" In the long version of questionnaire, the question on treatment was a sub-question of the question of ever heaving the specific allergic disease (asthma, hay fever, allergic rhinitis,

atopic eczema) in the past 5 years, whereas in the 10- and 15-year questionnaire the main question was “Did your child ever have [asthma, atopic eczema, hay fever]?”

Statistical analysis

Cumulative incidence was estimated by the life table method and analyzed by generalized estimation equations using PROC GENMOD in SAS. A complementary log-log link and independent correlation structure was used. The results were presented as relative risks (RR) for the specified contrasts: hydrolyzed formula in comparison with cow’s milk formula. To determine associations between feeding groups and period prevalence, logistic regression analyses were performed and odds ratios (OR) are reported.

The incidence models were adjusted for a fixed set of risk factors and confounders: family history of the modelled outcome (eczema, asthma and allergic rhinitis, respectively), heredity of family allergy, sex and study region. For adjusting the period prevalence, the list of confounders was extended to type of questionnaire, subject’s level of education, their cigarette smoking, and the current keeping of pets. Results of the adjusted models were given as adjusted OR or RR (aOR, aRR).

To examine whether early eczema (in the first 3 years) mediates the associations between the study formulae and asthma at age 16 to 20 years, mediation analyses were performed. Logistic regression models for early eczema and asthma were combined to obtain direct and indirect effects using odds ratios for mediation analysis for dichotomous outcomes using SAS macros.

For participation analyzes, multiple logistic regression models were performed and aOR were given. Possible interactions between the study formulae and possible risk or confounding factors as well as previous manifestations of allergic diseases were examined.

P values less than 0.05 were considered statistically significant and estimates of OR and RR are given with 95% confidence intervals (95%CI). Statistical analyses were done using the statistical software SAS for Windows, Release 9.4 (SAS Institute, Cary; NC).

Table E1 Association between possible factors and participation (%) at 20 years expressed as adjusted OR*

		ITT (N=2252)			PP (N=988)		
		n	**%**	**aOR* (95%CI)**	**n**	**%**	**aOR* (95%CI)**
Total		1199	53.2	-	548	55.5	-
Study formula	CMF	291	52.3	1	145	53.7	1
	pHF-W	303	54.4	1.09 (0.86-1.38)	137	53.5	0.99 (0.70-1.41)
	eHF-W	303	54.2	1.07 (0.85-1.36)	141	58.3	1.19 (0.83-1.69)
	eHF-C	302	52.1	0.99 (0.78-1.25)	125	56.8	1.13 (0.79-1.62)
Sex	Male	567	48.3	1	262	50.0	1
	Female	632	58.6	**1.51 (1.28-1.78)**	286	61.6	**1.57 (1.22-2.03)**
Parental history of eczema	No	862	53.5	1	419	57.4	1
	Yes	337	52.7	0.95 (0.79-1.14)	129	50.0	0.75 (0.56-1.00)
Parental history of asthma	No	873	53.1	1	406	56.6	1
	Yes	326	53.5	1.00 (0.82-1.20)	142	52.4	0.86 (0.65-1.15)
Double heredity of family allergy	No	827	52.8	1	389	55.5	1
	Yes	372	54.3	1.01 (0.84-1.22)	159	55.5	1.01 (0.76-1.34)
Study region	Munich	662	56.8	1	267	58.4	1
	Wesel	537	49.4	**0.74 (0.63-0.88)**	282	52.9	0.79 (0.61-1.02)

* Results from multiple logistic regression models including all variables listed in the table. None of the interaction terms examined in a previously step were significant.

Bold values and bold CI represent significant effects

Table E2 Distribution of risk factors and confounders across study formula groups

	Total	CMF	pHF-W	eHF-W	eHF-C	p-value
ITT, number of followed children	1199	292	303	303	302	
Female sex	52.7	51.2	52.1	52.1	55.3	0.764
Study region Munich	55.2	57.0	52.2	54.5	57.3	0.543
High parental education	78.9	78.4	76.9	80.2	80.1	0.714
Type of questionnaire (reference: online)						
paper	11.7	8.6	12.9	13.5	11.6	0.515
short	17.6	18.6	15.5	18.5	17.9	
Active smoking*	15.7	13.4	15.2	15.5	18.5	0.380
Current pets*	29.1	27.2	27.1	31.0	31.1	0.541
PP, number of followed children	548	145	137	141	125	
Female sex	52.2	50.3	49.6	51.8	57.6	0.566
Study region, Munich	48.7	50.3	43.8	53.9	46.4	0.352
High parental education	74.5	74.5	72.3	75.9	75.2	0.911
Type of questionnaire (reference: online)						
paper	12.6	11.0	14.6	9.9	15.2	0.787
short	20.4	20.0	19.0	23.4	19.2	
Active smoking*	18.1	14.5	14.6	20.6	23.2	0.158
Current pets*	31.0	27.6	26.3	33.3	37.6	0.163

* not included in the shortened version of the questionnaire