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

To the Editor,

Prevention of allergic disease in children has been on the agenda for many decades. Extensively hydrolyzed formulae are designed primarily for treatment of cow's milk allergy, and later these and partially hydrolyzed formulae were also recognized for prevention of atopic diseases, but their efficacy has been challenged repeatedly.[1–3] The German Infant Nutritional Intervention (GINI) study allows evaluation of long-term effects of hydrolyzed formulae on allergic diseases in high-risk children.[4,5]

Between 1995 and 1998, 2252 healthy term newborns with high risk of allergy were recruited at birth in Munich and Wesel (Germany) 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 to be fed during the first four months of life if exclusive breastfeeding was not possible.

At the 20-year follow-up (Appendix S1), intention-to-treat (ITT) and per-protocol (PP) analyses were performed considering information obtained by questionnaires from 1199 subjects and 548 subjects, respectively (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 ). In the PP analysis, effect sizes were similar but not statistically significant. For allergic rhinitis (AR), no significant differences in incidence or prevalence were observed. In the ITT analysis of eczema, the cumulative incidence was reduced in the eHF-C [relative risk (RR) = 0.61; 95% CI = (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 of eHF-C and pHF-W on the cumulative incidence were even stronger in the PP-analysis, but the effect of eHF-C on prevalence of eczema did not reach statistical significance. The mechanisms through which hydrolyzed formulae might affect allergic disease development are not well understood. As only certain formulae showed a protective effect, it might be speculated that the specific processes of hydrolyzation differ in their effectiveness regarding deterioration of potentially allergy-inducing epitopes.[5] Indirect effects on the immune systems of the formulas cannot be excluded, like different impact on the intestinal microbiome or metabolome or exposure to the skin.

Compared with results obtained up to 15 years of the GINI study,[5] the 20-year follow-up revealed similar effects for eczema incidence. While protective effects for AR observed at 15 years were no longer present, 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 cannot be explained by the reduction in eczema by certain hydrolyzed formulae in the first 3 years since the percentage of the total association explained by early eczema was 8.1% for eHF-C and 6.6% for pHF-W.

Potential limitations include the lack of objective data such as specific IgE and spirometry, different types of questionnaires over the years (parents versus subjects, paper versus online), higher participation of females and the high drop-out from the original study, which is not uncommon for such long-term birth cohorts. All aspects apply similarly to all study groups and therefore should not have substantially biased the results (Tables S1 and S2). The information on allergic diseases was collected by questionnaires only, using consistent questions since the first year of life, which allows an investigation of their course over the entire follow-up period.

It has been questioned whether hydrolyzed formulae should have a role in primary allergy prevention.[1–3] A recent nationwide observational study from France even found an increase in wheezing in infants who had received partially hydrolyzed formulae in short term.[6] In contrast, our randomized study demonstrates that the preventive effect is different with different hydrolyzed formulae and not entirely dependent on the degree of hydrolyzation. Accordingly, pooling of different types of hydrolyzed formulae is misleading.

## CONCLUSION

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 support the idea of preventive effects. Our findings confirm the concept that early nutritional intervention with certain hydrolyzed formulae, if

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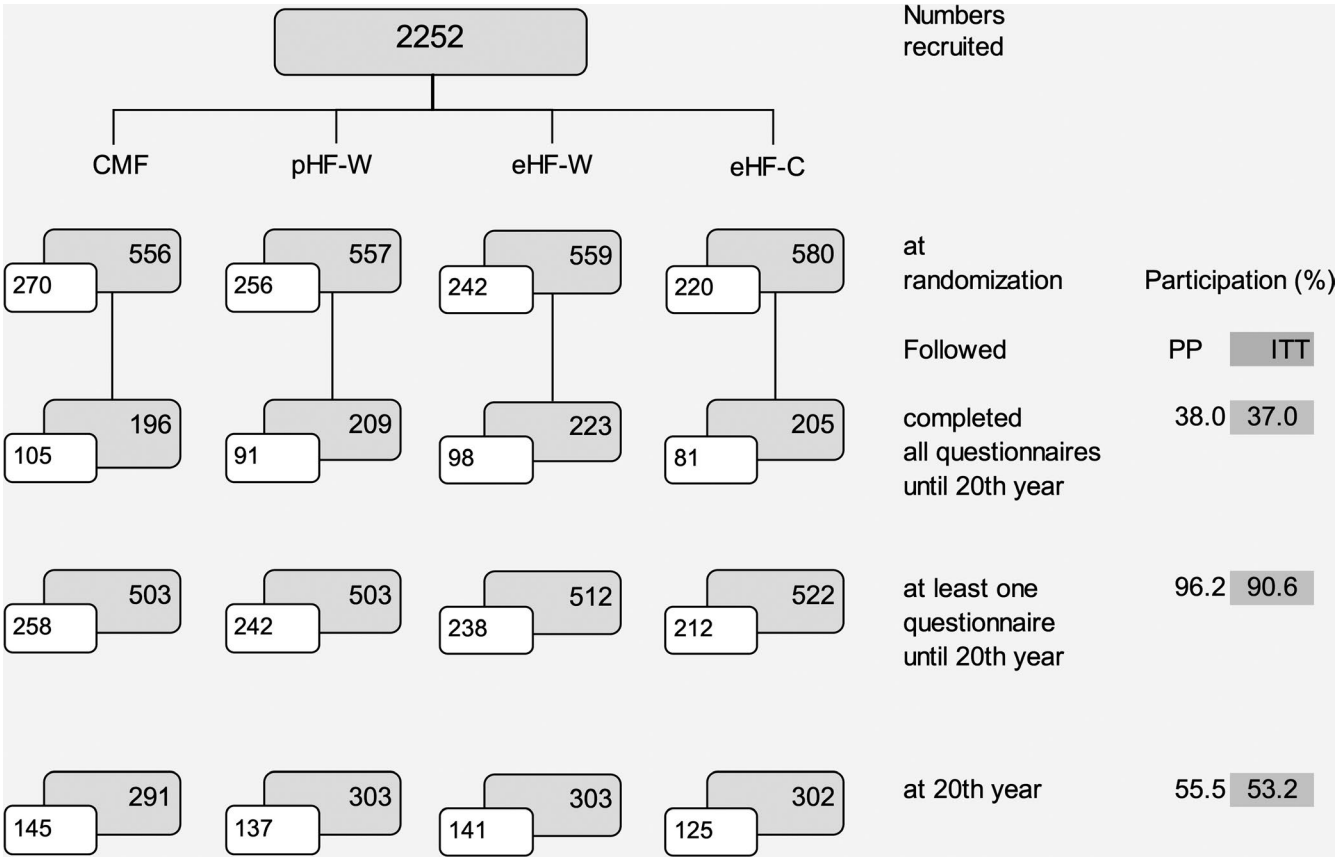


**FIGURE 1** Number of subjects and proportion of participation (%) in each study formula group included in the ITT (shaded boxes) and PP (open boxes) analysis at recruitment, with complete information from all follow-ups, at least one follow-up, and at the 20-year follow-up

exclusive breastfeeding is not feasible, has a preventive effect until adulthood for both eczema and allergic airway manifestation.


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## CONFLICT OF INTEREST

Dr. Gappa reports grants from Nestlé Vevey, Switzerland, during the conduct of the study. Dr. Filipiak-Pittroff has nothing to disclose. Dr. Libuda is member of the German National Breastfeeding Committee. Dr. von Berg reports grants and personal fees from Nestlé Vevey, Switzerland, and Nestlé Nutrition Institute, Germany, during the conduct of the study. Dr. Koletzko reports grants from Mead Johnson Company during the conduct of the study; personal fees from Nestle, Danone, Biocodex, Shire, AbbVie, R-Biopharm, Vifor, Pharmacosmos, Celgene, Thermo Fisher, Janssen, Pfizer, Takeda, Berlin-Chemie, Mead Johnson, grants from BioGaia outside the submitted work. Dr. Bauer has nothing to disclose. Dr. Heinrich has nothing to disclose. Dr. Schikowski has nothing to disclose. Dr. Berdel has nothing to disclose. Dr. Standl has nothing to disclose.


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**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 hydrolyzed formula groups, when compared to the cow's milk formula group. Intention-to-treat (ITT) and per-protocol (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[b] (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[b] (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[b] (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[a] (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[b] (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[a] (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[b] (95% CI) | 1 | 1.27 (0.72–2.26) | 0.90 (0.49–1.63) | 1.38 (0.76–2.50) |

(Continues)

**TABLE 1** (Continued)

| | CMF | pHF-W | eHF-W | eHF-C |
|---|---|---|---|---|
| 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[a] (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[b] (95% CI) | 1 | 0.72 (0.25–2.02) | 1.54 (0.63–3.79) | 0.77 (0.27–2.18) |

*Note:* CMF standard cow's milk formula (Nutrilon Premium), pHF-W partially hydrolyzed whey (Beba-HA), eHF-W extensively hydrolyzed whey (HIPP-HA, at that time identical with Nutrilon Pepti), eHF-C extensively hydrolyzed casein formula (Nutramigen). 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. 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. Bold values and bold CI represent significant effects; bold values but not bold CI indicate strong effects with loss of significance.

[a]Adjusted for parental history of disease, heredity of family allergy, sex, study region.

[b]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.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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# Immunological changes in peripheral blood following nasal allergen challenge in subjects with allergic rhinitis pre- and post-peptide immunotherapy: An open-label clinical study

To the Editor,

We investigated immunological changes in peripheral blood samples obtained from individuals with allergic rhinitis (AR), triggered by exposure to cat allergen, who were receiving peptide immunotherapy. Ten cat-sensitized AR individuals received cat-peptide antigen desensitization (Cat-PAD; Circassia Pharmaceuticals PLC, Oxford, UK),[1] an experimental peptide immunotherapy for AR; participants were treated with four intradermal injections (at 4-week intervals) of 6 nmol Cat-PAD.

To measure the clinical response to therapy, nasal allergen challenge (NAC)[2] was performed on each participant at three study visits: screening (V1), pre-treatment (V1A), and post-treatment (V3) (Figure 1A). Peripheral blood samples and Total Nasal Symptom Scores (TNSS)—clinical symptoms—were sequentially collected prior to and following NAC at two visits: V1A and V3 (Figure 1A,B, and Table S1). Comparing blood differential cell counts and transcriptomic profiles between V1A and V3 allowed us to investigate cellular and molecular associations with the induction of immune tolerance by peptide immunotherapy.

TNSS at V3 was significantly reduced at 15 minutes, and 1, 2, and 4 hour post-NAC, compared with V1A: We defined the early-phase response (EPR) as symptoms 0–6 hour post-NAC and the late-phase response (LPR) as symptoms 7–12 hour post-NAC, based on the change in the mean value of TNSS at V1A (Figure 1C). In an additional analysis, each sum of TNSS over 0–12 hour post-NAC (Total.TNSS.Sum) and during EPR (EPR.TNSS.Sum) at V3 was significantly lower compared with V1A while the change in LPR (LPR.TNSS.Sum) narrowly missed statistical significance ($p = .0502$, likely due to limited sample size, only four participants had a subsequent LPR after EPR or a prolonged response) (Figure 1D).

In analyses of complete blood count (CBC) data, frequencies of immune cells (*ie*, neutrophils, lymphocytes, and monocytes) at V3 were significantly different from at V1A (Figure 1E). A high neutrophil/lymphocyte ratio (NLR) indicates systemic inflammation or infection: We identified a significant NLR reduction at baseline, and 1 and 2 hour post-NAC, at V3 compared with V1A. This is consistent with a previous study of baseline NLR being higher in patients with moderate-severe AR compared with healthy, non-allergic participants.[3] Interestingly, in the Pearson correlation test of the quantitative difference between V1A and V3, higher lymphocyte count at 1 hour post-NAC at V3 had a strong correlation with the sum of reduced TNSS through time points post-NAC at V3: Total.TNSS.Sum ($r = -.7927$). Moreover, the relationship was stronger ($r = -.8819$) when TNSS during EPR was considered (Figure 1F and Figure S1).

Considering each participant's regular exposure (on at least two separate occasions and for a total duration of at least 8 hours per week) to a cat in their daily lives throughout the study as persistent AR, the significant changes in TNSS and immune cell frequency were associated with Cat-PAD intervention, rather than due to seasonal changes. The mechanisms of action of Cat-PAD may be characterized by a reduction in systemic immune responses of immune cells in peripheral blood.

Immune gene expression profiling with the NanoString nCounter assay (NanoString, Seattle, WA) was used to delineate RNA-level changes and help understand the changed direction of any immune responses.

First, enrichment pathway analysis (Enrichr) of significantly differentially expressed genes in the comparison between visits indicated a reduction in Th2-biased immune responses and a return of the balance with Th1 cells following peptide treatment (Figure S2): Lower expression of immune genes at V3 was associated with IL-2–, IL-4–, IL-6–, granulocyte-macrophage colony-stimulating factor and B-cell receptor signaling pathway–mediated signaling events; conversely, higher expression of immune genes at V3 was associated with T-cell receptor signaling in naïve $CD4^+$ and $CD8^+$ T cells and IL-12–mediated signaling events.

Second, two of six interleukin genes with detectable RNA expression in blood had significant transcript differences in the comparison: Although it is a precursor needing cleavage to be active, *IL-1β*,