

Long-term effects of hydrolyzed protein infant formulas on growth—extended follow-up to 10 y of age: results from the German Infant Nutritional Intervention (GINI) study^{1–6}

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ABSTRACT

Background: Previous analysis in a prospective, population-based cohort reported reduced weight gain in children fed extensively hydrolyzed casein (eHF-C) formula during the first year of life but showed no effect on growth between 1 and 6 y of life. No studies have been conducted in children up to the age of 10 y.

Objective: The objective was to investigate potential differences in body mass index (BMI) over the first 10 y of life between infants fed within the intervention period of the first 16 wk of life with partially hydrolyzed whey (pHF-W), extensively hydrolyzed whey (eHF-W), eHF-C, or cow-milk formula (CMF) and infants exclusively breastfed (BF) within the intervention period.

Design: This was a prospective, randomized, double-blind trial in full-term neonates with atopic heredity in the German birth cohort German Infant Nutritional Intervention (GINI) followed through the first 10 y of life. Analyses of absolute and World Health Organization (WHO)-standardized BMI trajectories for 1840 infants [pHF-W ($n = 253$), eHF-W ($n = 265$), eHF-C ($n = 250$), CMF ($n = 276$), and BF ($n = 796$)] were conducted according to intention-to-treat principles.

Results: Except for the previously reported slower BMI gain in infants fed with eHF-C formula within the first year of life, no significant differences in absolute or WHO-standardized BMI trajectories were shown between the pHF-W, eHF-W, eHF-C, CMF, and BF groups thereafter up to the age of 10 y.

Conclusions: Extension of the follow-up period from 6 to 10 y for this randomized controlled trial showed no long-term consequences on BMI for the 4 infant formulas considered. These data need to be confirmed in future studies. *Am J Clin Nutr* 2011;94(suppl):1803S–7S.

INTRODUCTION

Our group previously showed short-term effects on growth within the first years of life, but no long-term effects on growth, of feeding hydrolyzed formulas in a randomized controlled trial of infants fed with either partially hydrolyzed whey (pHF-W), extensively hydrolyzed whey (eHF-W), extensively hydrolyzed casein (eHF-C), or a standard cow-milk formula (CMF) and infants exclusively breastfed (BF) for the first 16 wk of life and followed from birth to 6 y of age (1).

Compared with all other formula groups, the eHF-C group showed a lower weight gain in the first year of life [difference in trajectories: -0.2 body mass index (BMI) z scores] even in

comparison with the exclusively breastfed group. This growth retardation was not due to a different growth pattern in length. No other feeding group showed this weight retardation within the first year of life or at any time during the whole follow-up period from birth to age 6 y.

At 5–6 y of age, body fatness normally declines to a minimum, a point called adiposity rebound, before increasing again into adolescence and adulthood (2). Because weight gain may have been affected by either early, late, or a steep adiposity rebound at ≈ 6 y of age, potential long-term differences may not have been visible in the previous analyses of the German Infant Nutritional Intervention (GINI) cohort, which was restricted to a follow-up until age 6 y.

The GINI study is an ongoing, prospective birth cohort study with follow-up data now extended to the age of 10 y. Thus, the aim of the present analysis was to investigate whether the feeding regimen in early infancy has long-term consequences on growth beyond the sixth year of life.

SUBJECTS AND METHODS

Study design and population

The GINI is an ongoing birth cohort study initiated to prospectively investigate the influence of nutrition intervention during

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³ Members of and institutions affiliated with the GINI-plus study group are listed before the References.

⁴ This study does not necessarily reflect the views of the Commission of the European Community and in no way anticipates its future policy in this area.

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infancy, and air pollution and genetics on allergy development. The intervention comprised nutritional advice promoting exclusive breastfeeding for ≥ 4 mo and a randomized trial on the effect of hydrolyzed formula compared with conventional CMF in the prevention of allergies. Infants were born between September 1995 and July 1998 in either Munich or Wesel and were followed for the development of atopic manifestation for up to 10 y. Details of the design, recruitment, and follow-up of this intervention study have been published previously (3–6).

In brief, 2252 full-term neonates at risk of atopy because of family history were randomly assigned at birth to 1 of 3 hydrolyzed infant formulas or a regular CMF. The main components of the 4 study formulas per 100 mL were Beba-HA (Nestlé, Vevey, Switzerland) for pHF-W; HIPP_HA (HIPP, Pfaffenhofen, Germany), at that time identical to Nutrilon Pepti (Nutricia/Numico, Zoetermeer, Netherlands) for eHF-W; Nutramigen (Mead Johnson, Dietzenbach, Germany) for eHF-C; and Nutrilon Premium (Nutricia/Numico) for standard CMF. This composition is listed in **Table 1**.

Regardless of random assignment at birth to 1 of the 4 formula groups, exclusive breastfeeding for the first 16 wk, with the introduction of solid foods postponed until after the end of the fourth month, was recommended. This was also true for the breastfeeding group, which was naturally established because of compliance with this general feeding recommendation during the intervention period of the first 16 wk of life.

At the end of the intervention period of 16 wk this randomized trial comprised an interventional arm of 1172 infants who were fed study formula, and a noninterventional arm of 889 infants who were exclusively breastfed. These infants were followed up annually for 3 y, again at 6 y, and at 10 y by both physical examination and detailed questionnaire. At the age of 4 y, follow-up was conducted by questionnaire.

From the 2252 infants randomly assigned at birth, 191 did not complete the trial up to the end of the intervention period of 16 wk for various reasons, which were mainly problems with the study formula (41%) and time constraints (34%). For details on the number of infants included and randomly assigned at birth to the 5 study groups and the number of infants followed during the intervention period, see the previous publication (1) and the flowchart in **Figure 1**. In addition, the analyzed study population among each study group from birth to age 10 y is listed in **Figure**

TABLE 1
Composition of study formula per 100 mL¹

	pHF-W	eHF-W	eHF-C	CMF
Protein (g)	1.6	1.6	1.9	1.4
Casein-whey ratio	0:100	0:100	100:0	40:60
Fat (g)	3.4	3.6	3.4	3.6
Carbohydrates (g)	7.4	6.9	7.4	7.1
Lactose	5.1	2.6	0	7.1
Other	2.3	4.3	7.4	0
Energy (kcal)	67	67	68	66

¹ pHF-W, partially hydrolyzed whey; eHF-W, extensively hydrolyzed whey; eHF-C, extensively hydrolyzed casein; CMF, cow-milk formula.

1. Only annual follow-ups are listed; follow-ups at days 3–10, weeks 4–6, and months 6–7 were omitted to save space and reduce the complexity of the flowchart, but of course these were incorporated into the statistical analysis model. The final study population comprised those 1840 of the 2252 infants randomized at birth who completed the intervention period of the first 16 wk and had known anthropometric data in the intention-to-treat analyses [pHF-W ($n = 253$), eHF-W ($n = 265$), eHF-C ($n = 250$), CMF ($n = 276$), and BF ($n = 796$)]. At the end of follow-up at age 10 y, the groups comprised 118, 123, 110, 127, and 448 children, respectively.

Weight and length were measured by pediatricians during physical examinations of each child at birth, at days 3–10, at weeks 4–6, and at months 3–4, 6–7, 10–12, 21–24, 43–48, and 60–64 of life to monitor normal growth and a healthy development of the child. These data were recorded in the well-baby check-up books and were available for this study. At the 10-y follow-up, weight and length measurements were collected during invited physical examinations by study pediatricians. The study protocol was approved by the local ethics committees (Bavarian General Medical Council and Medical Council of North Rhine Westphalia), and written consent was obtained from all participating families.

Outcome definition

BMI in absolute terms is defined as weight in kilograms divided by length in meters squared. Anthropometric measures (≤ 10 measurements/infant over the entire follow-up period) could not always be conducted at the exact age of the designated time schedule, of course, but varied, in particular during the first 2 y of life. Thus, because there were up to 7 measurement occasions for each infant, there were weight and height measurements at almost every month during this early period of life. After the second life-year, only 3 measurement occasions were available up to the 10-y follow-up and therefore, anthropometric measures between the ages of 2 and 3 y, between the ages of 4 and 5 y, and between the ages of 7 and 9 y are either scarce or not available at all.

To ease numeric comparison of our results with other and future growth studies and growth references, absolute BMI was transformed to SD scores (z scores) (9). Transformation was conducted according to the new sex- and age-specific World Health Organization Child Growth Standards for 0–5-y-old children (≤ 1856 d) and according to the World Health Organization

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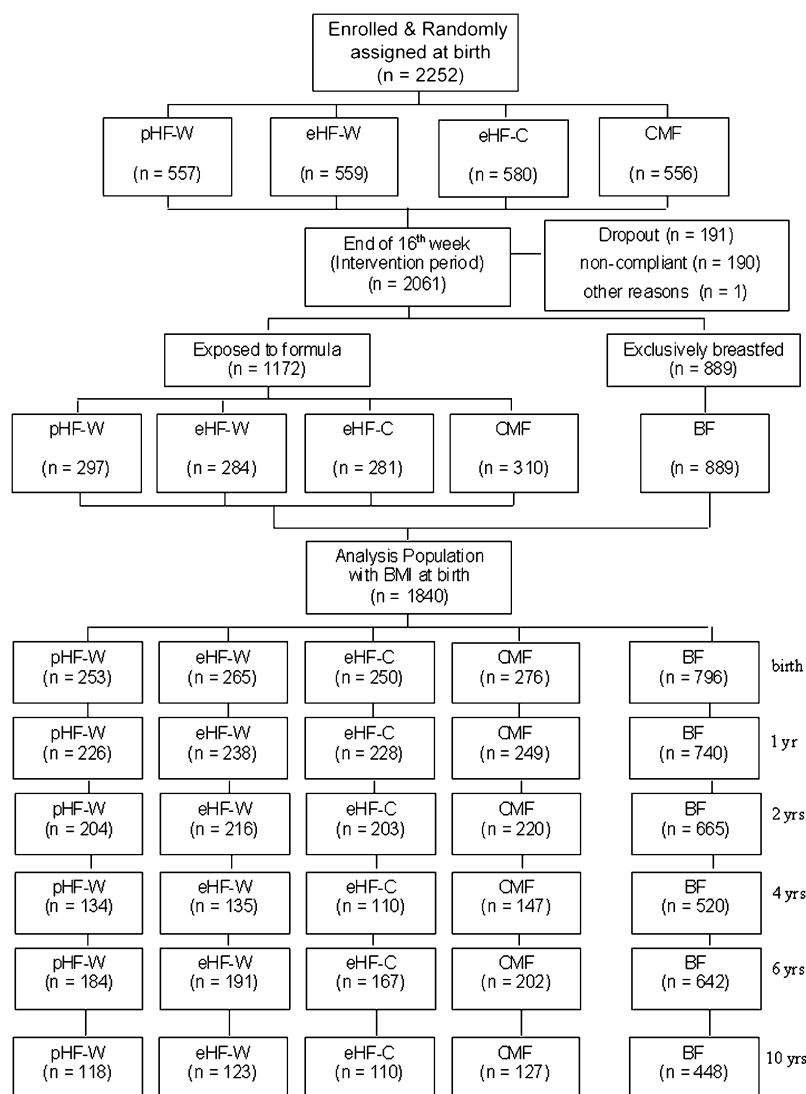


FIGURE 1. Flowchart of the study population during the intervention period (birth to week 16) and follow-up of the analysis population from birth to age 10 y. pHF-W, partially hydrolyzed whey; eHF-W, extensively hydrolyzed whey; eHF-C, extensively hydrolyzed casein; CMF, cow-milk formula; BF, breastfed.

Growth Standards for School-Aged Children and Adolescents for children aged >1856 d (7, 8).

Statistical analysis

Piecewise linear random coefficient models were applied to assess subject-specific (individual) and population-averaged (mean) growth curves (trajectories) and period-specific change between 0–8, >8–16, >16–52, >52–104, >104–312 wk, and past the 312th week of life. Partitioning of the time axis to period-specific segments is based on previous literature (10), the intention to assess both the short- and long-term effects on change in weight, length, BMI, and BMI (z scores) over time for the 4 formula-feeding groups and the breastfeeding group, and the additional measurement at the 10-y follow-up around the 512th week.

Details of this longitudinal model and its interpretation are described in our previous publication (1). In brief, the model applied here is a longitudinal version and random coefficient variant of the sometimes-called “B-Spline regression model.” In

the presented case there are 6 specified time segments for which simultaneous linear regression lines are fitted to model an overall nonlinear curve by linear curve segments. The model used here has, in addition, a random intercept term and a random slope of the variable age. These random components allowed assessment of the individual heterogeneity of the mean of the outcome at birth (random intercept) and the individual slopes (random slopes of age) around the population-averaged growth curve within each time period.

The time dependence of the repeatedly measured anthropometric data for each person is also accounted for by this model because the measurements are modeled as nested within persons. Thus, the model allows modeling of the outcome over time at the exact time-varying ages when the measurements were actually taken, and can use all available data for each infant, even in the case of intermittently missing measurements. The inclusion of interactions between the period-specific age variables and 4 of the 5 indicator-coded variables for the study groups (reference BF group) allows the period- and study group-specific population-



averaged growth curves to be estimated for each of the study groups over time.

The statistical significance of differences between the growth curves of the study groups was assessed by 95% prediction bands. If an average trajectory of a study group did not run within the prediction band of another group, there was a significant difference between these growth curves with a 5% probability error.

Because weight and length are known to be higher for boys, sex was included as a main effect, and was adjusted for. In addition, analyses of the BMI *z* score were adjusted for any maternal smoking during pregnancy, low parental education, and urban or rural study area.

Moreover, with the use of a 2-factor analysis of variance or logistic regression model with interaction, we checked that those children who participated in the 10-y follow-up were not statistically different at birth in terms of maternal age, parental education, or percentage of maternal smoking during pregnancy among the formula groups from those who dropped out of the cohort before the 10-y follow-up (data not shown).

Descriptive analyses were conducted with the use of SAS statistical software, version 9.1.3 (SAS Institute, Cary, NC) (11). Longitudinal piecewise linear random coefficient analyses were performed with the software for multilevel modeling MLwiN, version 2.02 (Centre for Multilevel Modelling, Bristol Institute of Public Affairs, University of Bristol, Bristol, United Kingdom) (12).

RESULTS

There were no significant differences in BMI (*z* scores) among different feeding groups after the first year of life to the extended 10-y follow-up. The trajectories of each feeding group were within the 95% confidence band around the average trajectory of the eHF-C group after 1 y of life for the whole study period up to 10 y, as shown in **Figure 2**. This was true even though the analysis was adjusted for sex, maternal smoking during pregnancy, low parental education, and urban or rural study area.

There were no substantial or significant differences between any of the formula-fed or breastfed groups with respect to weight and length (*z* scores) (data not shown). The previously published result of a lower BMI *z* score trajectory within the first year of life for the eHF-C group was confirmed with the extended follow-up data to 10 y. Unadjusted analyses and analyses with absolute BMI as an outcome did not show substantially different results (data not shown).

DISCUSSION

The extension of the data analysis up to the age of 10 y did not show statistically significant long-term associations between growth and infant feeding with partially or extensively hydrolyzed formula (pHF-W, eHF-W, and eHF-C), CMF, or breast milk (BF). The only other randomized trial on the long-term effects of hydrolyzed formulas on growth (follow-up period up to age 4 y) did not find a significant difference between an eHF-W formula and a soy-based formula (13).

The planned 15-y follow-up of the present study may shed light on the interesting finding that the infants fed pHF-W formula did not increase in standardized BMI between the 6-y and the 10-y follow-up to the same extent that all other feeding groups did. Although the differences at 10 y are not significant, this potential leveling effect should be investigated further.

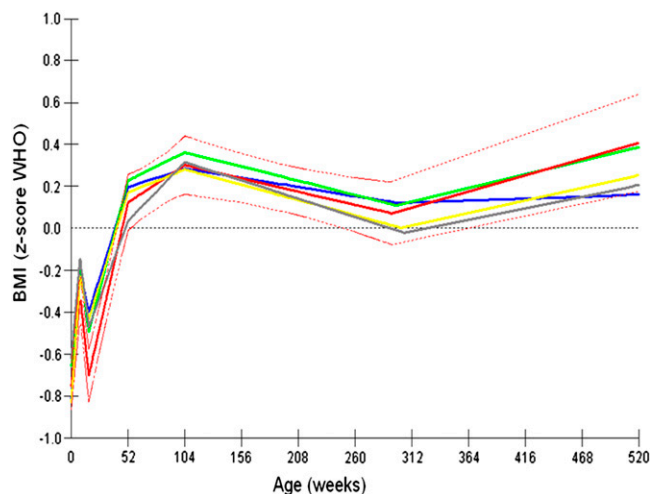


FIGURE 2. Period-specific development of World Health Organization (WHO)-standardized BMI for boys in intention-to-treat analyses between birth and 10 y of life. Study groups were as follows: partially hydrolyzed whey ($n = 253$, blue line); extensively hydrolyzed whey ($n = 265$, green line); extensively hydrolyzed casein (eHF-C; $n = 250$, red line); cow-milk formula ($n = 276$, yellow line); exclusively breastfed for 16 wk ($n = 796$, gray line). The red dashed lines indicate the 95% prediction band around the trajectory of the eHF-C study group. For details on the number of children followed over time, see the flowchart depicted in Figure 1. The statistical model applied is a piecewise linear random-effects model. For details, see “Statistical analysis” in Subjects and Methods.

As expected, the previously published short-term effect of a lower BMI (*z* score) trajectory within the first year of life for the eHF-C group was shown with this extended follow-up data. In the previous publication we discussed potential reasons for the retarded weight gain in the eHF-C group (1). In brief, the eHF-C hydrolysates from pure casein may have a lower biological value with a lower nitrogen use and a higher urinary amino acid concentration in infants, most likely because of amino acid imbalances, as some previous studies suggest (14–16). The lower biological value of casein may be related to its lower cysteine content and may not be sufficient to meet the requirements and the lower nitrogen requirement, and greater aminoaciduria may be a consequence of that.

Thus, weight gain may be reduced within the first year of life in infants fed pure casein formula eHF-C for the above-mentioned reasons, despite the fact that this formula has the highest protein content of the analyzed formulas in our study (17). This association with weight retardation in the eHF-C group should also be viewed in light of the protective association with eczema observed up to 6 y of age (5). However, the nutritional adequacy of the eHF-C formula and potential reasons for this weight gain retardation should be investigated in more detail.

In conclusion, extension of the follow-up period from 6 to 10 y for this double-blind, randomized controlled trial confirms previously published results that showed no long-term consequences on BMI for the 4 infant formulas considered. Further studies are needed to confirm that extensively hydrolyzed formulas do not result in higher BMI values in late childhood and adolescence.

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