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Safety and efficacy of a new extensively hydrolyzed formula for infants with cow's milk protein allergy

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Cow's milk protein allergy (CMPA) is best treated by complete elimination of cow's milk from the diet. For infants with CMPA who cannot be breast-fed, formulas based on extensively hydrolyzed proteins or on amino acids are the preferred substitutes for cow's milk-based formulas. In this study, we compared the tolerance and growth of infants with CMPA who were fed a new extensively hydrolyzed formula containing lactose (eHF) with those who were fed an amino acid formula (AAF). This was a prospective, multi-center, randomized, reference-controlled study. Seventy-seven infants <12 months old with suspected CMPA were enrolled. In 66 of these, CMPA was confirmed by oral challenge in a double-blind, placebo-controlled food challenge (DBPCFC) or by a medical history of severe allergic reaction to cow's milk and a positive skin prick test. These infants were then tested for their reaction to eHF and AAF in a DBPCFC. All infants tolerated both formulas and were randomized to receive either eHF (n = 34) or AAF (n = 32) for 180 days. Growth (weight, length, and head circumference) and tolerance [skin, gastro-intestinal, and respiratory tract symptoms of allergy] were evaluated after 30, 60, 90, and 180 days. There were no significant differences between the two groups in any of the growth measurements. Length and head circumference were similar to Euro-growth standards, but weight was slightly lower. Gastro-intestinal and respiratory tract symptoms of allergy were also similar in the two groups. However, whereas SCORAD scores for atopic dermatitis remained constant throughout the study in infants-fed eHF, there was a slight decrease in those fed AAF. Infants-fed eHF had significantly fewer incidents of vomiting than infants-fed AAF and a significantly higher frequency of soft stools. The new eHF is safe and well tolerated in infants diagnosed with CMPA.

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The incidence of food allergy is around 5–10% in young children (1), with cow's milk protein being the leading cause of allergy in infants (2). By 3–5 yr of age, most infants develop tolerance to food allergens (1, 3), and by adulthood the incidence of food allergy is around 1–2% (1, 2). However, food allergies or sensitizations to foods, especially when characterized by early atopic eczema, are often predictive factors for later respiratory allergies such as allergic rhino-conjunctivitis and asthma (4–6). Thus, for about 50% of the infants with food allergies who will continue to be affected, this may not be a limited illness but may represent the beginning of

the so-called 'allergic march', an important burden in terms of later illness, quality of life, and healthcare costs.

The first line treatment for cow's milk protein allergy (CMPA) is the complete elimination of cow's milk from the diet (2, 7). However, in infants and young children, elimination of milk from the diet without an adequate replacement leads to an increased risk of growth impairment (7, 8). This is especially important in infants with both CMPA and atopic dermatitis (AD), who have been shown to be at even greater risk of growth retardation (9, 10).

Formulas based on extensively hydrolyzed proteins have been shown to be effective in reducing the incidence of CMPA (11) and are the recommended substitute for cow's milk-based formulas in infants with CMPA who cannot be breast-fed (12–14). Several studies have shown that highly allergic infants react to even the very low amount of residual allergens in these formulas (15–19), and currently the only recourse for these infants is an amino acid-based formula (AAF). Both of these formulas have a bitter taste (20, 21), and until recently it has not been possible to add lactose because sufficiently pure lactose, devoid of contaminating protein had been unavailable.

In this study, we tested a new extensively hydrolyzed infant formula (eHF) in which lactose that was not contaminated with intact milk protein was added to improve palatability. Lactose may also stimulate the growth of bifidobacteria and therefore have a beneficial effect on the microbiota of infants (11). We evaluated safety and tolerance of the new eHF in infants with CMPA by comparing growth and clinical symptoms of infants-fed eHF with those of infants-fed AAF. We also evaluated the cost of treatment with the two formulas. The study was performed according to international guidelines for demonstrating hypoallergenicity adopted by the American Academy of Pediatrics, the European Academy of Allergology and Clinical Immunology, and the European Society of Pediatric Gastroenterology Hepatology and Nutrition (12, 22, 23). These guidelines require that 90% of infants with CMPA not react to the new product (with 95% CI) in a double-blind placebo-controlled food challenge (DBPCFC), and that trials be conducted in at least two study centers with a minimum of six subjects in each. Furthermore, the new formula has to be demonstrated to promote normal growth and maintain normal nutritional status of infants.

Subjects and methods

Study population

Healthy infants, < 12 months old, who had been previously diagnosed with CMPA were enrolled. The inclusion criteria were: birth at term (gestational age between 37 and 42 wk); birth weight between 2500 and 4000 g; and a maximum intake of breast milk of twice per day. Infants were excluded if they had any malformations, congenital cardiovascular, kidney, liver, central nervous system or metabolic diseases, serious gastro-intestinal (GI) tract diseases other than CMPA, or lactose intolerance.

Study design

This was a prospective, controlled, clinical trial conducted in three centers in Germany. Upon enrolment, CMPA was verified in all infants either by their response to a double-blind, placebo-controlled oral challenge with cow's milk and a skin prick test. All oral challenges were performed under the supervision of trained medical staff in a DBPCFC as previously described (24). Briefly, placebo or doses of 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, and 100.0 ml of fresh, pasteurized cow's milk were administered and infants were observed for at least 48 h. Provocation was stopped if clinical symptoms were observed. All infants were fed an AAF *ad libitum* for 1–2 days prior to challenge, and antihistamines were withdrawn at least 3 days prior to challenge, but topical corticosteroids (1% hydrocortisone or 0.3% betamethasone) were allowed twice a day.

All infants with confirmed CMPA entered a two-phase trial (Fig. 1). In phase A, allergenicity of the new eHF was compared to that of a control formula (AAF) in a DBPCFC study with a crossover design. Two or more days following challenge with cow's milk, infants were randomized to an eHF diet followed by an AAF diet or the reverse sequence. Following each challenge, infants were observed for any reaction to the formulas for 48 h. Each challenge period lasted a minimum of 2 days and was separated by a minimum of 2 days of wash-out during which infants were fed AAF. Residual allergic reaction to cow's milk and to AAF and eHF was also assessed by skin prick tests to confirm the absence of allergy to both formulas.

Phase B was a randomized, open trial in which the growth, allergy-related symptoms, tolerance, volume of formula intake, and metabolic status of infants were assessed. Infants with no adverse food reactions to eHF and

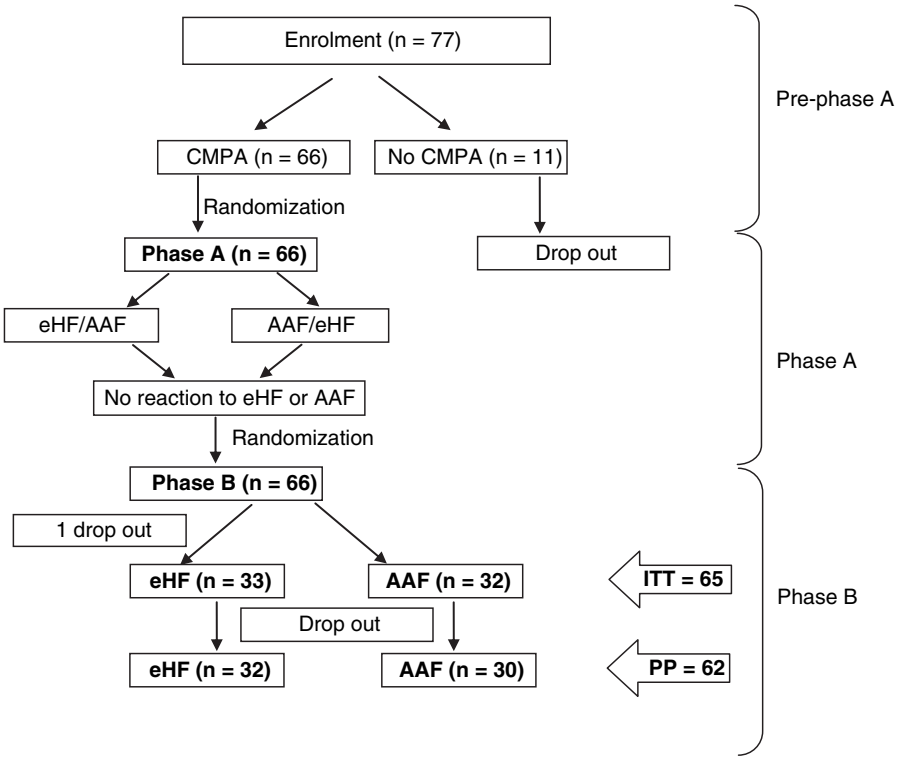

Fig. 1. Study design.

AAF were randomized to receive either of these formulas *ad libitum* for 180 days. Infants in the AAF group received this formula throughout phase B, whereas infants in the eHF group were gradually introduced to this formula as follows: ¾ AAF and ¼ eHF on day 1; ½ AAF and ½ eHF on day 2; ¼ AAF and ¾ eHF on day 3; and eHF exclusively starting on day 4. Infants older than 120 days on enrolment were allowed rice-based cereal (Alete, Nestlé, Switzerland) prepared with AAF. Cow's milk-free oligoantigenic foods (free of eggs, soy, wheat, fish, nuts, citrus, etc.) were also allowed upon the recommendation of the physician.

Visits took place at randomization and after approximately 28 (Visit 1), 60 (Visit 2), 90 (Visit 3), and 180 days (Visit 4). At each visit, anthropometric measurements and all symptoms of CMPA, including skin, respiratory and GI tract manifestations were recorded. Blood samples were drawn from infants, right before challenge with cow's milk and at Visits 1 and 3. Parents kept records of the amount of formula consumed every day for the first week. Thereafter, they recorded the infants' daily formula intake, supplementary food/drink, stool characteristics (frequency, colour, and consistency), behavior (restlessness for longer than 30 min), and GI health (flatulence and frequency of vomiting and spitting up) for the 3 days preceding each visit.

This study was approved by the appropriate ethical committees of each institution: Ethical Committee Charité (E/Kn, 15/09/98), Berlin University Hospital; Ethik Kommission der Medizinischen Fakultät (Nr 1176, 13/10/98), University Hospital Bochum; and Ärztekammer Nordrhein (Nr 98223, 18/11/98), Marien-Hospital, Wesel. Written consents were obtained from the parents before challenge testing and randomization. This study was performed according to Good Clinical Practice guidelines, and conformed to the Declaration of Helsinki.

Formulas

The control formula, AAF (Neocate, SHS, UK), was an amino acid-based formula, that had previously been shown to be adequate in the management of CMPA (15, 16, 25). It contained amino acids (2.74 g protein equivalent/100 kcal), carbohydrates (maltodextrin), vegetable fats, and added vitamins and minerals. The study formula, eHF (Althera, Nestlé, Switzerland), was a new formula based on extensively hydrolyzed and ultrafiltered whey protein (2.74 g protein/100 kcal, median peptide size 362 Da; 99.7% of peptides < 2400 Da). It also contained hydrolyzed lactose and maltodextrin as carbohydrate sources, vegetable fats, and added vitamins and minerals. Its composition complied with the guidelines of CODEX and the European Regu-

lation for Infant Formulas (23). *In vitro* tests (ELISA and mast cell degranulation assays) indicated the hypoallergenicity of the eHF (26). All formulas and instructions for preparation were supplied to the study centre by the sponsor. Hospital dieticians prepared and blinded the formulas in phase A.

The average cost of treating infants with AAF or eHF was calculated based on the average daily intake, the percentage of the formula powder dilution, and the average German retail pharmacy prices of the formulas.

Safety and tolerance measures

Atopic dermatitis was evaluated by the investigator using SCORAD index according to established criteria (27). These included topology (localization and extent of concerned skin area), intensity (extent of erythema, edema, crust excoriations, lichenification, and dryness), and the degree of itching and loss of sleep. Investigators also evaluated GI and respiratory tract symptoms of CMPA (such as vomiting, flatulence, diarrhea, constipation, colic's, nausea, and wheezing), other manifestations of skin disorders (such as eczema, yeast infection, dry skin, impetigo, urticaria, and itching), and occurrences of acute gastroenteritis, upper respiratory tract infections (bronchitis, pneumonia, and purulent rhinitis) and otitis media.

Weight was measured on beam scales to the nearest gram, recumbent length on measuring boards to the nearest centimeter, and head circumference using steel measuring tapes to the nearest millimeter. Formula intake, GI health and behavior were evaluated based on parents' records.

Investigators assessed all adverse events (AE) for severity and relatedness to the treatment. AE were considered to be serious if they were fatal, life-threatening, caused permanent harm, or required/extended inpatient treatment of subjects.

Blood analysis

Titers of total and cow's milk-specific immunoglobulin E (IgE) were determined by fluorescence enzyme immunoassay using the CAP system, PCS (Phadia, Uppsala, Sweden) as described previously (28). Serum IgE concentrations above 0.35 kU/l (the detection limit) indicated sensitization. Other blood parameters were analyzed using standard methods, and plasma amino acid concentrations were determined as described previously (29).

Statistical analysis

To fulfill the criterion that a therapeutic formula has to be tolerated by 90% of infants with a confidence of 95%, 61 subjects were required to complete the oral challenge test with both formulas. Assuming a 20% dropout rate, a minimum of 76 subjects had to be recruited. According to the guidelines, normal growth also had to be demonstrated in a minimum of 28 subjects per formula group, and in at least two study centers with six or more subjects in each center.

Comparison of the two study groups was performed by *t*-test, or by chi-squared test in situations where no initial value was present or relevant (e.g. for stool characteristics and behavior). For anthropometry, the initial value (point 0 or 1) was entered as a co-variable in ANCOVA to estimate the treatment effect. Skewed data were either log-transformed or tested by non-parametric Mann–Whitney or Spearman's rank correlation tests. *Z*-scores were calculated using the Euro-Growth references (30). P-values for SCORAD scores were adjusted for multiple testing as described previously (31). AE were analyzed using the intention-to-treat (ITT) population. All other data were analyzed on the per protocol (PP) population unless otherwise indicated.

Results

Study population

Seventy-seven infants with symptoms of CMPA were initially recruited to the study. In 66 of these infants, CMPA was confirmed and they were enrolled in the randomized double-crossover trial (phase A). None of the 66 infants showed any allergic reaction to either eHF or AAF and were subsequently randomized in phase B to receive either eHF (n = 34) or AAF (n = 32). Four subjects (6%) dropped out during the 6-month period of phase B. As very little follow-up information was available for one of these infants, it was not included in the ITT population (n = 65). The other three (one from the eHF group and two from the AAF group) were withdrawn from the study by the parents without an explanation (PP population, n = 62).

Infants in both study groups had comparable baseline characteristics (Table 1). The majority of subjects had dietary restrictions at the time of enrolment and were receiving a special formula. Very few (4/62) had received any complementary foods before 4 months of age. Approximately, 75% of subjects (70% in the AAF group and 81% in the eHF group) had AD of moderate severity at baseline.

Table 1. Baseline characteristics of infants in the per protocol population. Numbers or mean values (SD) are shown

Characteristics	eHF (n = 32)	Amino acid formula (n = 30)
Sex: male/female	13/19	12/18
Study Centre Berlin/Bochum/Wesel	18/5/9	16/5/9
Gestation age in weeks	39.4 (1.4)	39.6 (1.1)
Year of mothers birth	1969 (4)	1968 (4)
Schooling of mother (years)	11.0	11.3 (1.7)
Age at randomization (days)	250 (75)	236 (91)
Duration of breast-feeding (weeks)		
0–2	6	6
3–16	7	5
≥17	17	21
SCORAD score at randomization	16.4 (14.4)	19.4 (16.1)
Prevalence† (%) of		
Vomiting	3	0
Flatulence	6	3
Colic	6	3
Stuffy nose	6	17
Immunoglobulin E†§ in kU/l (median 10–90th percentile)	0.62 (<0.35–66.8)	0.65 (<0.35–61.3)

Legend: †at enrolment, §cow's milk-specific.

Outcomes

All 66 infants with confirmed CMPA tolerated both the AAF and eHF in the DBPCFC of phase A, showing with 95% CI, that more than 90% of the infants with CMPA tolerated both formulas. Skin prick test confirmed the absence of allergy to AAF and eHF in all infants.

Relative to Euro-Growth standards (30), *Z*-scores for length, and head circumference of both groups were close to 0 at all time points, but weight was close to –0.5. There were no significant differences between the two groups in *z*-scores for weight ($p \geq 0.5$), length ($p \geq 0.1$), and head circumference ($p \geq 0.1$) at the end of the study.

SCORAD scores remained constant throughout the trial period in the eHF group and tended to decrease in the AAF group (Fig. 2). Although at visit 3 the difference in SCORAD scores were significantly lower in the AAF group compared to the eHF ($p < 0.005$), the differences at the other visits were not significant ($p > 0.1$ at visit 1 and 3 and $p > 0.05$ at visit 2).

Infants in the two groups had similar stool characteristics. There were a few reports by parents of unusual stool colours, including some black stools, mostly in the AAF group. Although black stools could indicate GI bleeding, the presence of blood in stools was not reported and therefore the medical significance is unknown. In addition, the frequency of soft stools was significantly higher in infants in the eHF group compared to those in the AAF group (66% vs. 47%, *t*-test, $p < 0.05$). Flatulence,

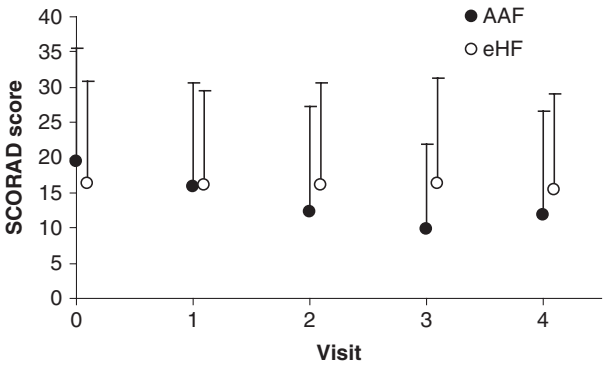

Fig. 2. Mean (+SD) SCORAD scores in infants during the trial. Values on visit 0 refer to measurements taken at randomization (per protocol population).

periods of unrest, and spitting up occurred at similar frequencies in the two groups (chi-squared test, $p > 0.1$). On the other hand, vomiting was reported in more infants in the AAF group compared to the eHF group (8/30 vs. 1/32, chi-squared test, $p < 0.01$).

The frequency with which at least one AE occurred was similar in the eHF and AAF groups (54% vs. 55% in the ITT population and 53% vs. 60% in the PP population). The most common AE affected the GI and respiratory tracts and the skin, and they were not related to the study product. None of the symptoms was assessed as serious.

Infants in the two groups consumed similar amounts of formula (609 ± 175 ml/day of AAF vs. 590 ± 213 ml/day of eHF for visits 1–4). The average costs of treating an infant with AAF or eHF for 1 month was 318 and 149€, respectively.

Total IgE titers at visit 3 were similar in the two formula groups (Mann–Whitney test, $p > 0.1$). There was also a significant correlation between IgE titers and SCORAD scores in both groups at this time (Spearman's $r_s = 0.64$, $p < 0.0005$ for both groups; AAF: $r_s = 0.55$, eHF: $r_s = 0.75$).

Blood analyses showed good nutritional status of infants in both groups (data not shown). There were no differences between the two groups in the time interval between feeding and blood sampling for the determination of amino acid concentration. There was no significant difference in the post-prandial plasma amino acid concentration between the two groups and no infant had values in the toxic range.

Discussion

Infants with allergies tend to have lower growth in infancy compared with healthy infants (8, 32), which has been partly attributed to inappropriate

food eliminations without proper substitutions (3, 8). The presence of sustained allergic inflammation, which may lead to poor use or loss of nutrients in allergic infants, has also been put forth as a possible explanation (8, 10, 33). Thus, modification of hypoallergenic formulas to promote increased intake could be an approach to ameliorate problems with intake and compliance and, therefore, growth in infants with CMPA.

Formulas based on extensively hydrolyzed proteins or on amino acids have been used to manage CMPA. However, these formulas have a strong bitter taste (20, 21), which may contribute to reduced intake and as a result may affect growth of infants. The addition of lactose in eHF may not only improve the palatability of these formulas and, thus, increase intake in infants with CMPA, but it is also thought to be important for the absorption of calcium (12, 34). Additionally, lactose may have a beneficial effect on the gut microbiota of infants by stimulating the growth of bifidobacteria species (12, 35), which are prevalent in the gut of breast-fed infants and have been suggested to confer beneficial health effects (36, 37).

Although numerous studies evaluating infant formulas for hypoallergenicity have been reported, few have been performed following the rigorous guidelines defining hypoallergenicity (12, 22, 23). Using these international guidelines, we have shown that eHF is safe and tolerated well by infants with CMPA. Growth (weight, length, and head circumference) and symptoms of allergy and tolerability in infants fed eHF were similar to those of infants-fed AAF, a formula considered to be the best treatment for highly sensitive infants (12). The mean *Z*-scores for length and head circumference of infants in both formula groups were similar to the Euro-Growth standards indicating that they grew normally. On the other hand, the weight *Z*-scores of infants in both groups were slightly lower than these standards.

However, it is possible that the weight of infants in our study may have improved as infants got older, and that the follow-up period in our study may have been too short to see this change. In a similar study, Seppo et al. showed that although infants with CMPA who were fed formulas based on hydrolyzed proteins had lower weight compared to standards, after 4 years of follow-up infants had caught up in weight (38). This suggests that the decrease in weight relative to standards is unlikely to be a permanent effect.

SCORAD scores remained constant throughout the study among infants in the eHF group, indicating eHF-stabilized AD although it did not eliminate all its symptoms. The absence of an alleviation of AD symptoms in the eHF-fed infants may be explained by the moderate intensity of AD in the infants enrolled in the study. Significant improvements or subtle effects in these moderate symptoms may not be easily visible. On the other hand, infants in the AAF group tended to show a decrease in SCORAD scores, which may suggest differences in the hypoallergenicity of these formulas. However, the difference in SCORAD scores between the two groups was not significant at most of the visits.

Our results show that the new eHF is safe and well-tolerated in infants diagnosed with CMPA.

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