

Comparison of a partially hydrolyzed infant formula with two extensively hydrolyzed formulas for allergy prevention: A prospective, randomized study

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The aim of this study was to compare the allergy-preventive effect of a partially hydrolyzed formula with two extensively hydrolyzed formulas, in infants with a high risk for development of allergic disease. High-risk infants from four Danish centres were included in the period from June 1994 to July 1995. Five-hundred and ninety-five high-risk infants were identified. High-risk infants were defined as having bi-parental atopy, or a single atopic first-degree relative combined with cord blood immunoglobulin E (IgE) ≥ 0.3 kU/l. At birth all infants were randomized to one of three different blinded formulas. All mothers had unrestricted diets during pregnancy and lactation and were encouraged to breast-feed exclusively. If breast-feeding was insufficient, one of the three formulas, according to randomization, was given during the first 4 months. It was recommended not to introduce cow's milk, cow's milk products, and solid foods until the age of 4 months. After the age of 4 months a normal unrestricted diet and conventional cow's milk-based formula were given when needed. All infants were followed-up prospectively with interview and physical examination at the age of 6, 12, and 18 months, and if any possible atopic symptoms were reported. If food allergy was suspected, controlled elimination/challenge procedures were performed in a hospital setting. Of 550 infants included in the study, 514 were seen at all visits and 36 were excluded owing to non-compliance. Of 478 infants who completed the study, 232 were exclusively breast-fed, 79 received an extensively hydrolyzed casein formula (Nutramigen), 82 an extensively hydrolyzed whey formula (Profylac), and 85 a partially hydrolyzed whey formula (Nan HA), during the first 4 months of life. These four groups were identical in regard to atopic predisposition, cord blood IgE, birthplace, and gender. Exclusively breast-fed children were exposed less to tobacco smoke and pets at home and belonged to higher social classes, whereas the three formula groups were identical concerning environmental factors. The frequency of breast-feeding was high; only eight (2%) children were not breast-fed at all. The three formula groups were identical in regard to duration of breast-feeding and age at introduction of formula and solid foods. No significant differences were found in the three groups of infants receiving formula milk regarding the cumulative incidence of atopic dermatitis or respiratory symptoms. The cumulative incidence of parental-reported cow's milk allergy was significantly higher in children fed partially hydrolyzed formula (Nan HA) compared with extensively hydrolyzed formula (Nutramigen or Profylac) at 12 and 18 months (NanHA, 7.1%; Nutramigen, 2.5%; Profylac, 0%; $p=0.033$). The cumulative incidence of confirmed cow's milk allergy was 1.3% (three of 232) in exclusively breast-fed infants, 0.6% (one of 161) in infants fed extensively hydrolyzed formula (Nutramigen or Profylac), and 4.7% (four of 85) in infants fed partially hydrolyzed formula (Nan HA). Partially hydrolyzed formula was found to be less effective than extensively hydrolyzed formula in preventing cow's milk allergy, 0.6% vs. 4.7% ($p=0.05$), but because of the small number of cases the results should be interpreted with caution. Compared with other similar studies the frequency of atopic symptoms was low, even though the dietetic intervention did not include either maternal diet during lactation or dietary restrictions to the children after the age of 4 months.

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Allergic disease has emerged as a common and increasing cause of illness in children. The occurrence of allergic disease may vary with age, and symptoms may disappear completely, possibly to be replaced by other symptoms. In infancy the main atopic symptoms are atopic dermatitis, gastrointestinal symptoms, and recurrent wheezing, whereas bronchial asthma and allergic rhinoconjunctivitis are the main problems later in childhood. Adverse reactions to foods, mainly cow's milk protein, are most common in the first years of life, whereas allergy to inhalant allergens generally occurs beyond this period.

Several previous prospective studies have shown a protective effect of feeding breast milk (1–9) or an extensively hydrolyzed casein – whey or soy formula (4–14) – on the incidence of atopic dermatitis and food allergy in high-risk infants, when the period of the diet included at least the first 4–6 months of life and solid foods were not introduced until the age of 4 months (15). The different study designs, study populations, definitions, diagnostic criteria, and control for confounders (environmental factors) in the previous studies do not allow a comparison of the effect of different dietary regimens and hypoallergenic hydrolyzed formulas. In a previous study we compared the preventive effect of an extensively hydrolyzed casein formula (Nutramigen) with an extensively hydrolyzed and ultrafiltrated whey formula (Profylac) and found no difference regarding prevention of cow's milk allergy until the age of 5 years (9, 16). Recently, data were published that indicated a protective effect of a partially hydrolyzed whey formula compared with conventional cow's milk-based formula and soymilk-based formula (3, 5, 6, 17–20).

In previous studies, the content of β -lactoglobulin has been analyzed by using enzyme-linked immunosorbent assay (ELISA), and was found to be 12 400 ng/ml in partially hydrolyzed formula (Nan HA) compared with 8.9 ng/ml in extensively hydrolyzed whey formula (Profylac), 0.91 ng/ml in extensively hydrolyzed casein formula (Nutramigen), and 0.9–150 ng/ml in breast milk (15, 21–23). In agreement with these results, several studies have indicated a positive correlation between sensitization/immunoglobulin E (IgE)-mediated reactions to hypoallergenic formula (HAF) (extensively hydrolyzed formula and partially hydrolyzed formula) in cow's milk allergic children and the degree of hydrolysis in these products and their residual content of high molecular-weight protein, as measured by skin-prick test (SPT) and specific IgE (21, 24).

However, comparative studies of the preventive effect of partially vs. extensively hydrolyzed formulas are still necessary (15) as only one such study has been published (25).

The primary aim of this study was to compare the allergy-preventive effect of a partially hydrolyzed whey formula (Nan HA) with two extensively hydrolyzed formulas (Nutramigen and Profylac) in a birth cohort of infants with a high risk for development of allergic disease. In addition, we wanted to confirm our previous finding of no difference in the allergy-preventive effect of the two different extensively hydrolyzed formulas, Nutramigen and Profylac. This paper deals with the effect of these formulas on development of cow's milk allergy, food allergy in general, and atopic symptoms.

Subjects and methods

Study population

The study population was a birth cohort from four different geographical areas in Denmark. High-risk infants born alive at Odense University Hospital, Viborg Hospital, Glostrup University Hospital, and Sønderborg Hospital, Denmark, in the period 01 June 1994 to 30 June 1995 were invited to participate in the study. Owing to a delay, one hospital (Glostrup) extended the inclusion period until 31 July 1995. All these infants had bi-parental atopic predisposition or severe single atopic predisposition and a raised cord blood IgE (CB IgE) ≥ 0.3 kU/l. The total number of infants born alive at these four hospitals in the study period was 11 632. A total of 595 high-risk infants (representing 5.1% of the total) were identified and informed of the study: 199 from Odense, 135 from Viborg, 103 from Glostrup, and 158 from Sønderborg.

Only infants with a high risk for development of allergic disease were included. According to results of a previous study (9), we expected that 20% of these high-risk infants would develop cow's milk allergy during the first year of life if they were given ordinary cow's milk-based formula when exclusive breast-feeding was not possible during the first 6 months of life.

Our intention was to compare the effect of extensively hydrolyzed formulas with a partially hydrolyzed formula, particularly regarding the development of cow's milk allergy. In a previous study (9) the cumulative incidence of cow's milk allergy was found to be 5% and 20% in identically defined cohorts of a high-risk group, with and without dietary preventive measures, respectively. In that study no difference was detected con-

cerning the preventive effect of the two different extensively hydrolyzed formulas, Nutramigen and Profylac. As we wanted to confirm this finding, we included Nutramigen and Profylac in the present study. Therefore, power calculation was performed using a test between two samples with twice as many observations in one of the samples (Nutramigen and Profylac) than in the other (Nan HA). The test was one-tailed as the partially hydrolyzed formula was not expected to be more effective than extensively hydrolyzed formulas. Assuming that the partially hydrolyzed formula would be 50% as effective in preventing cow's milk allergy as extensively hydrolyzed formulas (9, 17), we estimated that a total of at least 200 infants in the formula groups was required to detect a difference with 76.2% probability, and a total of 300 infants was required to detect a difference with 90.5% probability and a level of significance at 5%. As we expected $\approx 50\%$ of the birth cohort to be breast-fed exclusively, and as we could not expect a 100% follow-up rate and total compliance, we planned to include at least 500 (optimally 600) high-risk infants in the study.

Methods

This study was a double-blind, placebo-controlled, randomized intervention study.

During pregnancy, a questionnaire concerning atopic predisposition was completed by a midwife/nurse/doctor after interview with each set of parents, and all the parents were informed about the study orally and in writing.

All mothers had an unrestricted diet during pregnancy and lactation, and were encouraged to breast-feed exclusively. If breast-feeding was not possible or insufficient, feeding an extensively hydrolyzed casein formula (Nutramigen), an extensively hydrolyzed whey formula (Profylac) or a partially hydrolyzed whey formula (Nan HA) was advised during the first 4 months of life.

At birth, all high-risk infants were randomized to one of these three formulas, according to the date of birth. Before the start of the project the project year was divided into consecutive periods of 3 days. By lot it was decided that children born on day 1 belonged to group A and children born on days 2 and 3 in each period then belonged to group B or C, respectively. Thus, one-third of these high-risk infants were placed in each of the three groups.

The three different formulas were blinded in identical cans named A, B, or C. The coding and distribution from original to identical cans were performed by an independent company, Nomeca,

allowed by the Danish Authorities to follow this type of procedure. It was recommended not to introduce cow's milk, cow's milk products (including conventional cow's milk-based formula), and solid foods until the age of 4 months. If there was a need for supplement during the first days of life until lactation was established, water was recommended, and only if there was a medical indication for supplement were the infants offered formula A, B or C. After the age of 4 months we recommended a normal unrestricted diet and conventional cow's milk-based formula, when a substitute formula was required. Furthermore, it was recommended to avoid daily exposure to tobacco smoke, furred pets, and damp housing conditions.

The parents of these high-risk infants were informed orally and in writing during pregnancy and at the hospital soon after birth. The infants were followed-up prospectively, and the diet, various environmental factors, and any symptoms, were recorded. At the age of 6, 12, and 18 months, and if possible atopic symptoms developed, all the infants underwent physical investigation by one of the authors, and the individual case histories and data were recorded for each child. Upon any suspicion of food allergy, controlled elimination/challenge procedures were performed. At the age of 18 months, and if the child had atopic symptoms, a SPT was performed, and specific IgE against milk, egg, peanut, birch, grass, mugwort, dog, cat, horse, house dust mites *Dermatophagoides pteronyssinus* and *D. farina*, *Alternaria alternata* and *Aspergillus fumigatus* were analyzed.

The blinded coding of the products was not revealed until all the data registration was complete. Product A was Nutramigen, B was Profylac, and C was Nan HA.

Definitions

High-risk infants. High-risk infants were identified when both parents had or had had doctor-diagnosed and treated atopic disease (asthma, hayfever, atopic dermatitis, allergic urticaria, and food allergy), or severe single atopic predisposition combined with CB IgE ≥ 0.3 kU/l. Severe atopic predisposition was present in the latter group when one parent and/or one or more older sibling had, or had had, atopic disease with perennial symptoms or symptoms from more than one organ system. We did not perform any testing (SPT, specific IgE) of parents or siblings.

Any atopic symptoms. Any atopic symptoms were defined as infants having one or more of the symptoms: asthma, atopic dermatitis, allergic rhinoconjunctivitis or at least two episodes of allergic urticaria.

Asthma. Asthma was defined as at least three episodes of wheezing, requiring bronchodilator treatment, and diagnosed by a physician.

Recurrent wheezy bronchitis. Recurrent wheezy bronchitis was defined as at least two episodes of respiratory infections associated with wheezing, requiring bronchodilator treatment, and diagnosed by a physician.

Atopic dermatitis. Atopic dermatitis was diagnosed if physical examination revealed areas of scaly, erythematous and itchy eczematous rash, primarily of the face, the scalp, and at the flexural folds. Only eczema with at least two locations in typical areas and chronically/relapsing with a duration of at least 3 months was recorded.

Allergic urticaria. Allergic urticaria was registered when a physician diagnosed at least two episodes caused by the same allergen.

Rhinoconjunctivitis. Rhinoconjunctivitis was diagnosed when typical symptoms lasted at least 1 month or recurred on exposure to the same allergen (diagnosed by a physician).

Food allergy. Food allergy was defined as reproducible reactions diagnosed by controlled elimination/challenge procedures.

Cow's milk allergy. Cow's milk allergy was diagnosed in cases of reproducible atopic symptoms (skin, gastrointestinal, and respiratory) temporally associated with cow's milk ingestion. The following criteria had to be fulfilled: (i) definite disappearance of symptoms after each of two dietary eliminations of cow's milk and cow's milk products; (ii) recurrence of identical symptoms after one challenge; and (iii) exclusion of lactose intolerance and coincidental infection.

All challenges were performed open, but with equivocal results the challenge was repeated and blinded.

Social status. Social status was registered, according to the social grouping system of the

Danish Social Research Institute, when the child was 18 months old. This grouping system resulted in five social classes (I–V), according to education and employment on which the family income was gained, with class V being the lowest.

Milk challenges

The children were maintained on a milk-free diet for at least 1–2 weeks before milk challenge. All challenges were performed in a controlled hospital setting and observed by a doctor and a nurse. The challenges were performed with cow's milk or conventional cow's milk-based formula (Allomin or NAN) starting with a dose of 5 ml followed by 10, 20, 40, and 80 ml at 30-min intervals. If no symptoms occurred after 3 h (a total cumulative dose of 155 ml) the child was sent home with instructions to take increasing amounts at home until a customary daily intake of milk was tolerated. When late reactions occurred, the patients were re-examined at the hospital as soon as possible, and when a reaction was suspected the last dose was repeated. Before and after challenge and on any adverse reaction, the patients were examined and symptoms and signs were noted.

In patients with equivocal results, double-blind placebo-controlled challenges (DBPCC) were performed with one placebo and one active formula, following a previously described and accepted procedure (26). An extensively hydrolyzed casein formula (Nutramigen) with cherry juice was used as placebo and Nutramigen+ cherry juice+milk in different concentrations as the active formula. The procedure was as described for open challenges.

Hypoallergenic infant formulas

The formulas used in this study were as follows.

Nutramigen. Nutramigen (Mead Johnson Nutritionals, Evansville, IN, USA) is an extensively hydrolyzed casein formula with a MW <1500Da.

Profylac. Profylac (ALK, Hørsholm, Denmark) is an extensively hydrolyzed, ultrafiltrated whey formula with a MW <8000 Da.

Nan HA. Nan HA (Nestlé, Geneva, Switzerland), is a partially hydrolyzed whey formula with a MW in the range of 5000–40 000 Da (15, 21, 22).

Ethics

The study was approved by the human research ethics committee. All the parents were supplied with information orally and in writing, and the parents gave informed consent before entering their children in the study.

Statistics

Data management and statistical analyses were performed using the PC programme SPSS, version 8.0, for Windows. For comparison of groups the chi-square test or Fisher's exact test was used. All calculations on p-values were based on two-tailed tests and the p-value of significance was 0.05. Application of statistical methods and calculations were discussed with an experienced statistician.

The aim of the study was to compare the allergy-preventive effect of partially and extensively hydrolyzed formulas, and thus it was not relevant to follow the intention-to-treat principle.

Results

Study population, nutrition, confounding

Parents of the 595 high-risk infants were informed (orally and in writing) about the study soon after the birth of their child. The parents of 19 infants did not want to participate; three infants were born prematurely [gestational age (GA) < 34 weeks]; and 23 infants received, for some reason, supplements of another formula (Profylac, Nutramigen, Pregestimil or conventional cow's milk-based formula) during the first few days of life. Thus, 550 high-risk infants were included in the study: 151 from Sønderborg, 199 from Odense, 91 from Glostrup and 109 from Viborg.

Thirty-six children dropped out during the study: 12 before the first visit at 6 months, a further 12 between 6 and 12 months, and 12 between 12 and 18 months of age. Data were available for children, who did not drop out before the first visit, at 6 months of age. Of those

who dropped out after the age of 6 months, 15 were exclusively breast-fed, one had formula A, two had formula B, four had formula C, and two had ordinary cow's milk-based formula during the first 4 months of life.

A total of 514 (93%) children completed the follow-up until 18 months of age. Twelve children were investigated only twice: 11 at 6 and 18 months of age, and one at 12 and 18 months of age. Only children investigated at the age of 18 months were included in the analyses. Of these 514 children, 36 were excluded: four did not fulfil the inclusion criteria and 32 were excluded because of non-compliance to the dietary measures. Seventeen were fed breast milk and Nutramigen, Profylac or Nan HA between 0 and 4 months of age. Six had two different blinded products A/B/C (two A+B, and four B+C). Probably because of the taste, these six infants would not accept the formula to which they were originally allocated (two to A and four to B) and therefore their parents tried another product (two used B and four used C), but one infant would accept neither A nor B. Ten had A/B/C and supplements of conventional cow's milk-based formula or Nan HA before the age of 4 months; one of these infants also had both products A and B. The group of 32 children who were excluded owing to dietary non-compliance were breast-fed for a shorter duration, had solid foods introduced at an earlier age, and were exposed more to tobacco smoke than the children who were compliant with the dietary measures. The children who were excluded did not differ from the rest of the children with regard to gender, atopic predisposition, birthplace, or any of the other environmental factors.

Thus, a total of 478 high-risk infants completed the study. Two-hundred and thirty-two infants were breast-fed exclusively during the first 4 months (16 weeks) of life. Of the infants who were not exclusively breast-fed, 79 received product A, 82 product B, and 85 product C, in

Table 1. Gender, atopic predisposition, and cord blood immunoglobulin E (CB IgE)

	Total (n=478)	BF (n=232)	Nutramigen (n=79)	Profylac (n=82)	Nan HA (n=85)
Double-disp.	161 (34)	80 (34)	30 (38)	18 (22)	33 (39)
Single disp + CB IgE ≥ 0.3 kU/l	317 (66)	152 (66)	49 (62)	64 (78)	52 (61)
CB IgE median	0.50	0.50	0.41	0.50	0.50
Boys	269 (56)	125 (54)	45 (57)	49 (60)	50 (59)
Girls	209 (44)	107 (46)	34 (43)	33 (40)	35 (41)

Results are expressed as n (%).

BF, exclusively breast-fed children; Double-disp., biparental atopic disposition; Single disp; severe single parental atopic disposition.

Table 2. Environmental factors in breast-fed high-risk infants (BF) compared with formula-fed high-risk infants

	Total (n=478)	BF (n=232)	Formula (n=246)
Tobacco smoke			
No	301 (63)	168 (72)**	133 (54)
At home	177 (37)	64 (28)**	113 (46)
Mother	102 (21)	30 (13)**	72 (29)
Pets			
No	298 (62)	154 (66)	142 (58)
At home	180 (38)	76 (33)*	104 (42)
Day care	87 (18)	34 (15)	53/230 (23)
Day care	340 (71)	161 (69)	179 (75)
Before 6 months	29	15	14
n.i.	10	3	7
Housing conditions			
Flat	102 (22)	49 (21)	53 (22)
House	370 (78)	179 (77)	191 (78)
n.i.	6	4	2
Social class**			
I	54 (11)	41 (18)	13 (5)
II	45 (9)	23 (10)	16 (9)
III	99 (21)	56 (24)	43 (18)
IV	176 (37)	79 (34)	97 (40)
V	100 (21)	31 (13)	69 (28)
n.i.	4	2	2

Results are expressed as n (%).

n.i., no information

*BF compared with formula (A/B/C) $p = 0.040$, chi-square test.**BF compared with formula (A/B/C) $p < 0.001$, chi-square test.

different amounts and duration during the first 4 months of life, all according to the initial randomization.

The four groups were identical with regard to atopic predisposition, cord blood IgE, and gender (Table 1). Environmental factors are shown in Table 2. Children breast-fed exclusively were exposed less to tobacco smoke and to pets at home, and belonged to higher social classes than the three formula groups, whereas the three formula groups were identical in regard to environmental factors.

The frequency of breast-feeding was high: only eight (2%) children were not breast-fed at all; 35

(7%) were breast-fed for a short period of less than 1 month; and 370 (77%) children were breast-fed for at least 3 months (Table 3). The three formula groups (A, B, and C) were identical regarding the duration of breast-feeding (Table 3). Only eight children were exclusively formula fed from birth. Only 11 (2%) children had solid food before the age of 4 months.

A total of 246 children had supplements of one of the three formulas during the first 4 months of life. In 73 (30%) of these children, formula was introduced during the first week of life and a further 52 (21%) had formula introduced during the first month. Table 4 shows the age of introduction of formula. The median age of introduction of formula was 1 month in children in the three formula groups, without any significant difference between the three groups. In 238 of 246 infants, data concerning the amount of formula given to the children were available. As seen in Table 5, 62 children had formula only a few times, 72 had formula every day as a supplement to breast-feeding, and 104 children were fed formula exclusively for a period of at least 1 week. The three formula groups were identical concerning age of introduction of formula and amount of formula given to the children.

Solid foods were introduced at a later age in the group of children who were exclusively breast-fed than in formula-fed infants (median age 5.0 months vs. 4.0 months, $p < 0.01$), but there was no difference between groups A, B or C.

Development of atopic symptoms

Table 6 shows the cumulative incidence of atopic symptoms from birth until 18 months of age. Children who were exclusively breast-fed had a significantly lower cumulative incidence of recurrent wheezy bronchitis than the formula groups at 6 months ($p = 0.01$), 12 months ($p = 0.004$), and 18 months ($p = 0.001$) of age. No significant

Table 3. Duration of breast-feeding

Duration (months)	All (n=478)	Nutramigen (n=79)	Profylac (n=82)	Nan HA (n= 85)
0	8 (2)	2 (2)	2 (2)	4 (5)
> 0	470 (98)	77 (97)	80 (98)	81 (95)
≥ 1	435 (91)	65 (79)	67 (82)	72 (85)
≥ 2	396 (83)	52 (66)	59 (72)	54 (64)
≥ 3	370 (77)	44 (56)	48 (59)	47 (55)
≥ 4	319 (67)	30 (38)	35 (43)	39 (46)
≥ 5	182 (38)	16 (20)	21 (26)	20 (24)
≥ 6	85 (18)	9 (11)	10 (12)	10 (12)
Median	4.5	3.5	3.5	3.8

Results are expressed as n (%).

differences were found in the incidence of asthma, rhinoconjunctivitis or atopic dermatitis. The cumulative incidence of parental-reported adverse reactions to foods (food allergy), the greatest number being against cow's milk, was highest in group C (Nan HA). The cumulative incidence of parental-reported cow's milk allergy was significantly higher in group C infants compared with infants of groups A and B at the age of 12 months (A, 2.5%; B, 0; C, 7.1%; $p = 0.033$) and 18 months (A, 2.5%; B, 0; C, 7.1%; $p = 0.033$). At the age of 12 months, the cumulative incidence of parental-reported food allergy was significantly higher in group C compared with groups A and B (A, 3.8%; B, 1.2%; C, 11.8%; $p = 0.009$). One-hundred and twenty-five children had formula milk introduced to their diet during the first 4 weeks of life. Among these children, the cumulative incidence of parental-reported food

allergy was significantly higher in group C compared with groups A and B at 12 months (groups A and B: 4% [three of 78] vs. group C: 15% [seven of 47]; $p = 0.040$) and at 18 months (groups A and B: 4% [three of 78] vs. group C: 17% [eight of 47]; $p = 0.020$). Groups A and B were identical regarding the cumulative incidences of atopic symptoms and reported food allergies. The 32 children who fulfilled the inclusion criteria, but who were excluded owing to 'dietary breaks', did not differ in regard to the cumulative incidence of atopic symptoms.

Figures 1 and 2 show the time-course of cumulative incidences and point prevalence of any atopic symptoms, atopic dermatitis, parental-reported food allergy, and parental-reported milk allergy. In both, for cumulative incidences and point prevalence, there is a tendency that the Nan HA group had higher frequencies of

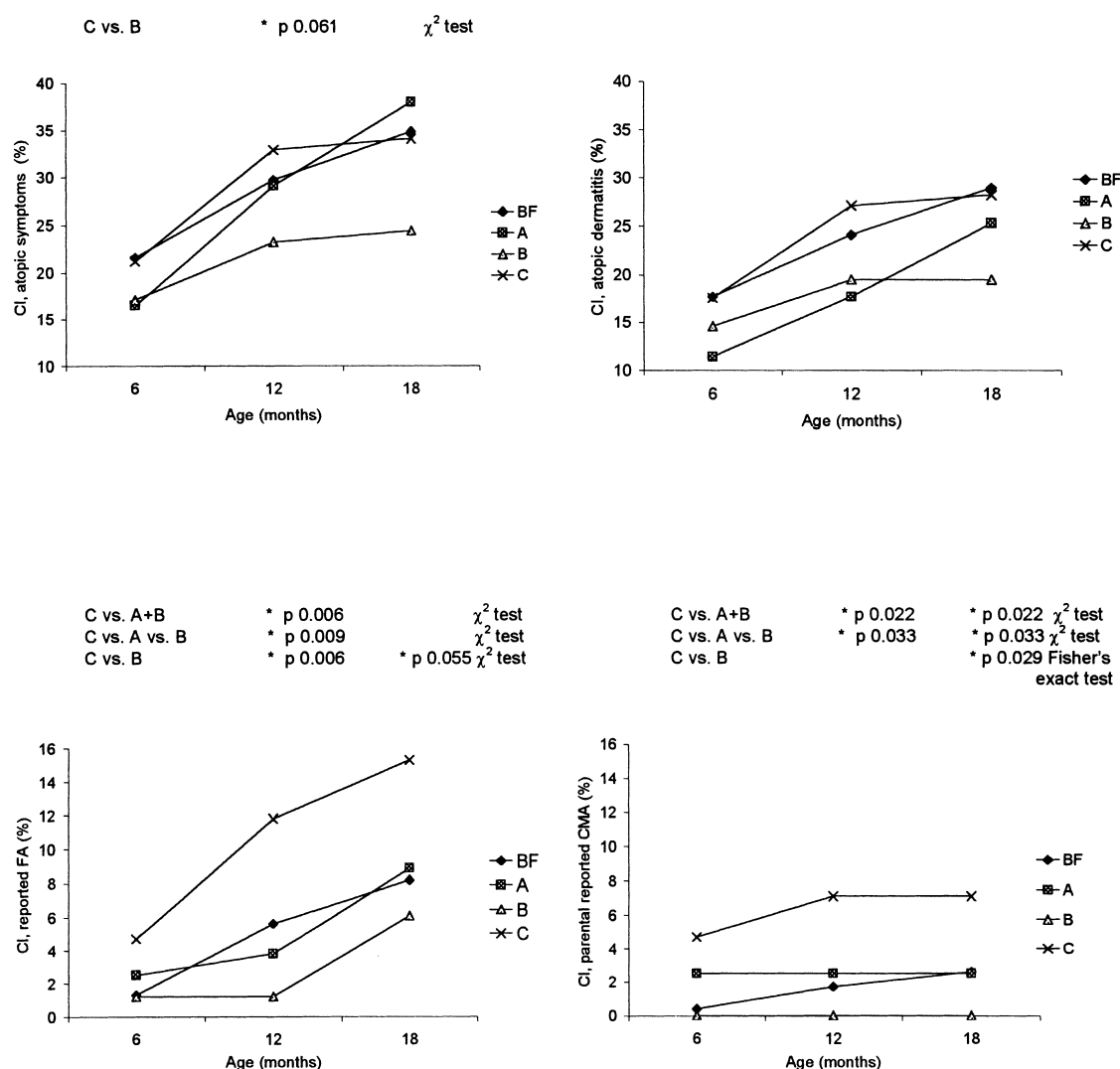


Fig. 1. Cumulative incidence (CI) (%) of atopic symptoms, atopic dermatitis, parental-reported food allergy (FA), and parental-reported cow's milk allergy (CMA) in exclusively breast-fed children (BF), and children fed Nutramigen (A), Profylac (B) and Nan HA (C) until 6, 12, and 18 months of age. Atopic symptoms: asthma, rhinoconjunctivitis, atopic dermatitis, and urticaria.

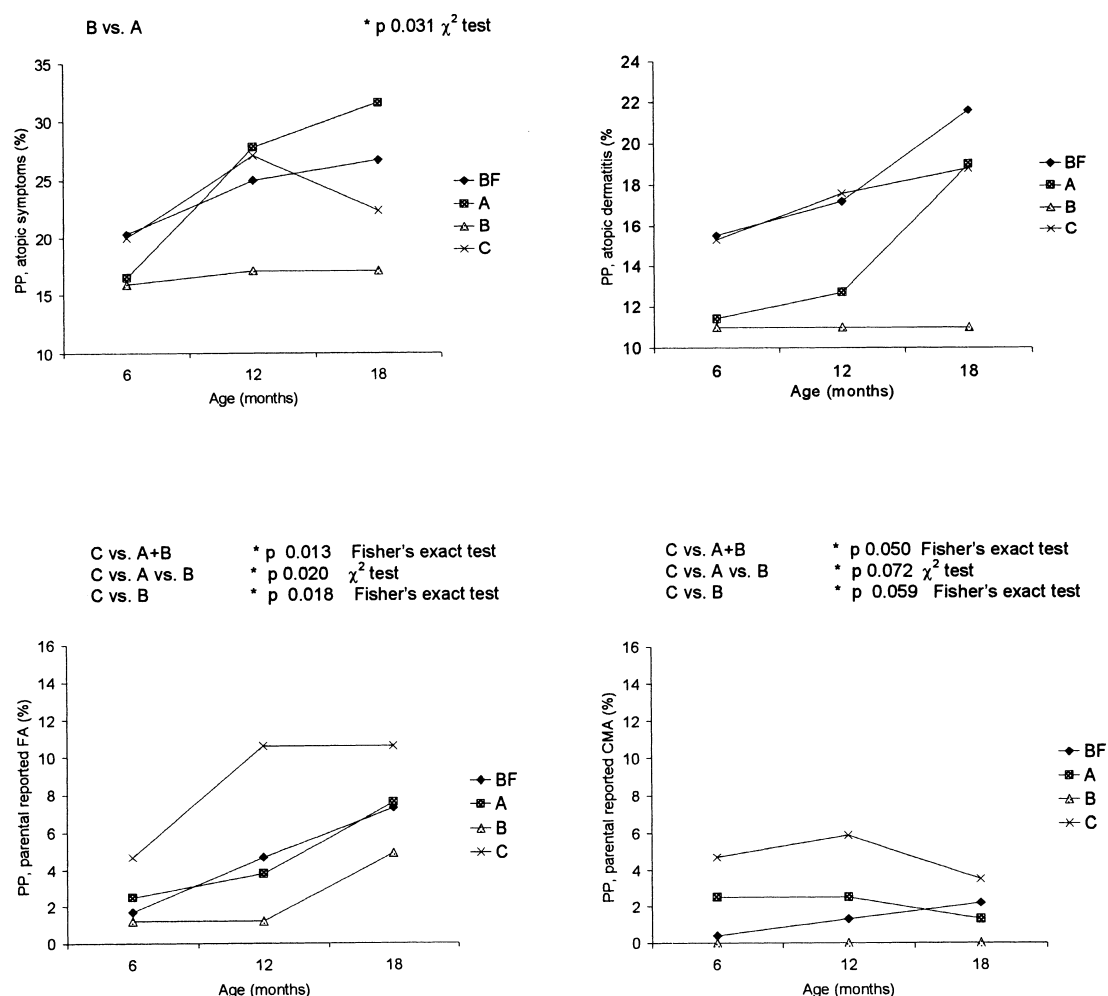


Fig. 2. Point prevalence (PP) (%) of atopic symptoms, atopic dermatitis, parental-reported food allergy (FA) and parental-reported cow's milk allergy (CMA) in exclusively breast-fed children (BF), and children fed Nutramigen (A), Profylac (B), and Nan HA (C) at 6, 12, and 18 months of age. Atopic symptoms: asthma, rhinoconjunctivitis, atopic dermatitis, and urticaria.

symptoms, although this was only significant for parental-reported food allergy.

Development of food allergy

Controlled elimination/challenge procedures with cow's milk were performed in 65 children: open-controlled challenges in 62 and double-blind, placebo-controlled food challenges in three. Nine of the 62 open challenges and none of the double-blind, placebo-controlled food challenges were positive. One of the nine positive open challenges could not be confirmed by a double-blind, placebo-controlled food challenge. The remaining eight had unambiguous positive open challenges and double-blind, placebo-controlled food challenges were not performed. No placebo reactions were recorded.

Ten children underwent controlled elimination/challenge procedures with egg, two with wheat, and one with codfish. Six had positive reactions

against egg, one had a positive reaction against wheat, and none had positive reactions against codfish. Two of the reactions against egg were confirmed by DBPCC; the remaining four had unambiguous reactions upon open challenges. No placebo reactions were recorded.

Thus, eight children had confirmed cow's milk allergy, six had food allergy against egg, and one food allergy against wheat (Table 7). Two children experienced positive challenges against both milk and egg, and a further one had reactions against milk, egg, and wheat. Reactions against vegetables, fruits, nuts, and additives are not diagnosed by controlled elimination/challenge procedures and were therefore referred to as parental-reported reactions.

All but one of the children with cow's milk allergy had at least two different atopic symptoms, and the child with only one symptom had severe atopic dermatitis. All children with cow's milk allergy had an onset of symptoms before

Table 4. Age at introduction and duration of feeding with the following formulas: Nutramigen, Profylac, and Nan HA

Age at introduction (months)	Duration (months)	Formula*			
		Any (n=246)	Nutramigen (n=79)	Profylac (n=82)	Nan HA (n=85)
0	4	73 (30)	22 (28)	23 (28)	28 (33)
>0-<1	3-4	52 (21)	16 (20)	17 (21)	19 (22)
≥1-<2	2-3	48 (20)	19 (24)	13 (16)	16 (19)
≥2-<3	1-2	40 (16)	10 (13)	16 (20)	14 (16)
≥3-<4	<1	33 (13)	12 (15)	13 (16)	8 (9)
Median age at introduction		1.0	1.3	1.4	1.0

*Data are expressed as n (%).

1 year of age: six before 6 months, one at 9 months, and one at 11 months of age. Three children with cow's milk allergy were exclusively breast-fed during the first 4 months of life, another one was breast-fed but had supplements of Nutramigen during the first few days of life, and four had Nan HA in varying amounts during the first 4 months of life. All these children had solid foods introduced after the age of 4 months. The total cumulative incidence of cow's milk allergy was eight of 478 (1.7%): three of 232 (1.3%) in children exclusively breast-fed, one of 161 (0.6%) in children fed extensively hydrolyzed formula (Nutramigen, one of 79 = 1.3%; or Profylac, none of 82) and four of 85 (4.7%) in children fed a partially hydrolyzed formula (Nan HA). Thus, the cumulative incidence of confirmed cow's milk allergy was significantly higher among infants fed partially hydrolyzed formula compared with infants fed extensively hydrolyzed formula ($p=0.05$, Fisher's exact test). No difference was found between the Nutramigen and the Profylac group.

All the children with cow's milk allergy tolerated Nutramigen and/or Profylac but not Nan HA.

Discussion

In this prospective study we compared the allergy-preventive effect of an extensively hydrolyzed casein formula (Nutramigen), an extensively hydrolyzed and ultrafiltrated whey formula (Profylac), and a partially hydrolyzed whey formula (Nan HA). Only children with a high risk for development of atopic disease were included. Identical methods and diagnostic criteria were used in all centres, where the investigations were performed by pediatricians with special knowledge and experience of allergic diseases in children. Ideally a control group of infants fed a conventional cow's milk-based formula should have been included. However, for ethical reasons it was not possible to allocate high-risk infants to a

conventional cow's milk-based formula in Denmark. For the same reasons it was not possible to randomize Danish infants to breast-feeding or formula feeding. All mothers were encouraged to breast-feed, but in instances where breast-feeding was not possible or insufficient, the infants were given one of the three blinded products, according to randomization. The inclusion of a total birth cohort of 1 year and the method of randomization takes into account seasonal variations and possible variations caused by the day of the week on which the children were born.

The compliance and follow-up rate (93%) were high and most of the children followed the dietary allergy-prevention program, as recommended. The frequency of breast-feeding was high and most (67%) of the children were breast-fed for longer than 4 months. Thus, very few children were formula fed exclusively and most of the children in the three formula groups received

Table 5. Number (%) of children fed the formulas Nutramigen, Profylac or Nan HA in varying amounts during the first 4 months of life

	Any (n=246) (n=238)*	Nutramigen (n=79) (n=78)*	Profylac (n=82) (n=79)*	Nan HA (n=85) (n=81)*
Seldom†	62 (26)	21 (27)	17 (21)	24 (28)
Partly††	72 (30)	21 (27)	22 (27)	29 (34)
Exclusively§ in a period	104 (44)	36 (46)	40 (49)	28 (33)
Exclusively from age:				
0-1 months	42 (18)	15 (19)	15 (19)	12 (15)
1-2 months	27 (11)	10 (13)	7 (9)	10 (12)
2-3 months	20 (8)	6 (8)	10 (13)	4 (5)
3-4 months	15 (6)	5 (6)	8 (10)	2 (3)
Median		1.5	1.8	1.4

Data are expressed as n (%).

*Data were available only for these children.

†Seldom, only formula as a supplement a few times during the whole 0-4-month period.

††Partly, formula used daily, but only as a supplement to breast feeding until 4 months of age. Breast feeding contributed to the nutrition of the child with an amount corresponding to at least one daily meal.

§Exclusively, or almost exclusively formula feeding from a certain age until 4 months of age. These children may have had a minor amount of breast milk during this time-period as well as supplements of formula for a period before.

Table 6. Cumulative incidence of atopic symptoms in children from 0–18 months of age fed Nutramigen, Profylac, or Nan HA, or exclusively breast-fed (BF) from 0–16 weeks of age

	BF (n=232)	Nutramigen (n=79)	Profylac (n=82)	Nan HA (n=85)	Excluded (n=32)
Asthma	21 (9.1)	11 (13.9)	5 (6.1)	5 (5.9)	4 (12.5)
Wheezy bronchitis	50 (21.6)*	27 (34.2)	29 (35.4)	30 (35.3)	10 (31.3)
Rhinoconjunctivitis	8 (3.4)	4 (5.1)	2 (2.4)	3 (3.5)	0
Atopic dermatitis	67 (28.9)	20 (25.3)	16 (19.5)	24 (28.2)	7 (21.9)
Urticaria	4 (1.7)	4 (5.1)	1 (1.2)	2 (2.4)	1 (3.1)
Any symptom§	81 (34.9)	30 (38.0)	20 (24.4)	29 (34.1)	11 (34.3)
Food allergy¶	19 (8.2)	7 (8.9)	5 (6.1)	13 (15.3)	1 (3.1)
Milk	6 (2.6)	2 (2.5)	0	6 (7.1)†‡	0
Egg	4 (1.7)	4 (5.1)	1 (1.2)	1 (1.2)	0
Fish	2 (0.9)	0	0	1 (1.2)	0
Citrus	6 (2.6)	1 (1.3)	0	0	0
Peanut	0	0	0	0	0
Others	11 (4.7)	4 (5.1)	4 (4.9)	7 (8.2)	1 (3.1)

Data are expressed as n (%).

*Breast-fed vs. formula-fed infants: $p = 0.001$ (chi-square test).

†Nan HA vs. Nutramigen vs. Profylac; $p = 0.033$ (Fisher's exact test).

‡Nan HA vs. Nutramigen + Profylac; $p = 0.022$ (Fisher's exact test).

§Any symptom (asthma, rhinoconjunctivitis, atopic dermatitis, urticaria).

¶Parental-reported food allergy.

formula (in varying amounts) as a supplement to breast-feeding. In 30% (73 of 246) of the children in the three formula groups, formulas were introduced during the first week of life. No differences of age at introduction or amount of formula used were found between the three formula groups. The median age for introduction of formula was 1 month.

The children exclusively breast-fed differed significantly from the children in the formula groups in regard to age at introduction of solid foods, exposure to tobacco smoke at home and maternal smoking, exposure to pets at home, and social status. No differences were found among the three formula groups.

Children who were exclusively breast-fed had a lower incidence of wheezy bronchitis than did children in the three formula groups. This difference may be explained, in part, by differences in environmental factors, especially exposure to tobacco smoke, and socio-economic status. The group of breast-fed children was not comparable with formula-fed children.

The aim of the present study was to compare the allergy-preventive effect of three different

hydrolyzed formulas – particularly regarding the allergen-specific effect on the development of cow's milk allergy. Therefore this work does not include a comparison with exclusively breast-fed children and does not include an evaluation of different risk factors for the development of atopic disease. The three formula groups were comparable in regard to factors other than the formula used, and therefore we used the chi-square test or Fisher's exact test for comparison of the groups. All calculations of p-values were based on two-tailed tests. Both in a previous and in the present study we found no differences in the incidences of any atopic symptoms, parental-reported food allergy or confirmed cow's milk allergy in the two groups receiving extensively hydrolyzed formula, which allowed us to combine these two groups in the statistical analyses. As the aim was to compare the allergy-preventive effect of different hydrolyzed formulas and not to compare the effect on the populations, it was not relevant to follow the intention-to-treat principle (27).

No significant differences were found regarding cumulative incidence of atopic symptoms. It

Table 7. Food allergy

	Total (n=478)	BF (n=232)	Nutramigen (n=79)	Profylac (n=82)	Nan HA (n=85)
Milk	8 (1.7)	3 (1.3)	1 (1.3)	0	4 (4.7)*
Egg	6 (1.3)	4 (1.7)	2 (2.5)	0	0
Wheat	1 (2.1)	1 (0.4)	0	0	0

*Nan HA compared with Nutramigen + Profylac; $p = 0.05$ (Fisher's exact test).

Reactions were confirmed by controlled elimination/challenge procedures. Results represent the cumulative incidence from 0–18 months and are expressed as n (%). BF, exclusively breast-fed.

is well known that asthma and allergic rhinitis most commonly develop later in childhood. Previous controlled studies (11, 14, 16, 28) have not shown any effect of a dietary allergy-prevention program on the development of asthma and/or inhalant allergy. Similar results in this study support these findings. However, in one Finnish study (29) of unselected newborns followed-up to 17 years of age, breast-feeding was associated with a lower 'prevalence of respiratory allergy' up to 17 years of age compared with children fed conventional cow's milk-based formula, but this study was neither randomized nor controlled for confounders.

As in our previous study (9) we found no difference regarding development of food allergy between the two groups receiving extensively hydrolyzed formula. Thus, for the statistical analyses we made comparisons between all three formula groups and between the partially hydrolyzed formula group and the two extensively hydrolyzed formula groups combined. The cumulative incidence of parental-reported food allergy at 12 months and cow's milk allergy at 12 and 18 months were significantly lower in children fed extensively hydrolyzed formula (Profylac or Nutramigen) compared with partially hydrolyzed formula (Nan HA). Among children who were given formula before 1 month of age, the cumulative incidence of parental-reported food allergy until the age of 12 and 18 months was lower in children fed extensively hydrolyzed formula compared with partially hydrolyzed formula.

In all children with suspected adverse reactions to foods, controlled elimination/challenge procedures were performed. Food allergy was found in very few children (12 of 478 = 2.5%). Thus, cow's milk allergy was confirmed in a total of eight of 478 (1.7%) children, with three of 232 (1.3%) in the group of exclusively breast-fed children. The cumulative incidence of cow's milk allergy until the age of 18 months was one of 161 (0.6%) in the children receiving extensively hydrolyzed formula (Nutramigen or Profylac) and thus significantly lower than four of 85 (4.7%) in the children who were fed partially hydrolyzed formula (Nan HA) ($p = 0.05$). Six children had allergy against egg and one had allergy against wheat, but no reactions against other foods were documented. Owing to the low incidence of allergy, the results regarding differences between groups should be interpreted with caution. Compared with some other studies (17–19) the incidence of cow's milk allergy in high-risk infants is low. This difference may be the result of a high frequency of breast-feeding or because of methodological differences.

The low incidence of atopic symptoms and food allergy in this study is in accordance with our previous report (16) although the duration of the dietary restriction in the present study was shorter – 4 months compared with 6 months. Another study with comparable results (11, 14) included extensively hydrolyzed formula and a restrictive diet for a long period – up to 2–3 years for some foods. Based on the present knowledge, restrictive preventive diets after the age of 4–6 months does not seem to be scientifically based.

Only one recent Swedish study, by Oldæus et al, compared the effect of an extensively and a partially hydrolyzed formula (25). In that study most children were breast-fed for a long time-period and formulas were introduced late (median 3.3–3.8 months). Compared with conventional cow's milk-based formula, a preventive effect (regarding the cumulative incidence of atopic symptoms) for up to 18 months was found for both the partially hydrolyzed formula and the extensively hydrolyzed formula, although a greater effect was reported in the group receiving extensively hydrolyzed formula at 6 and 9 months. Concerning cow's milk allergy, no significant differences were reported.

The methods used in our study seem, to a great extent, comparable with those of the Swedish study (25). However it is noteworthy that the partially hydrolyzed formula used in the Swedish study was a special, non-commercial product and is probably not comparable with the partially hydrolyzed formula used in our study, Nan HA, with respect to the degree of hydrolysis.

In the Swedish study (25), the cumulative incidence of atopic symptoms until the age of 18 months was 51%, 64%, 84%, and 67% in high-risk infants who were fed extensively hydrolyzed formula (Nutramigen), partially hydrolyzed formula, routine milk formula or breast milk, which is rather high compared with the corresponding figures in our study: 38%/24%, 34%, and 35% in the extensively hydrolyzed formula (Nutramigen/Profylac), partially hydrolyzed formula (Nan HA) and breast-fed groups, respectively. In the Swedish study it was concluded that most of the differences in morbidity in the four groups emerged during the first 3–6 months. In our study an early onset of food allergy was found, whereas the cumulative incidence of both atopic dermatitis and respiratory symptoms increased during the study period without any significant differences between the study groups (Figs 1 and 2).

In contrast to the Swedish study (25), and also to other previous studies (4–6, 10–13, 28), the present study included no dietary restrictions to

the pregnant/lactating mother, and the infants were advised normal infant feeding without any special dietary restrictions after the age of 4 months. Despite the less extensive diet and shorter duration of the dietary period in this study, we found very low frequencies of atopic symptoms and food allergy, especially cow's milk allergy. Thus, preventive dietary restrictions for the infants – comprising only avoidance of cow's milk proteins and solid foods – during the first 4 months of life seems to be sufficient, and dietary restrictions for the pregnant/lactating mother seem unnecessary.

Owing to a very high frequency of breast-feeding, especially in high-risk infants, studies on the preventive effect of HAF should include a very large study population in order to reach appropriate statistical power. Thus, a high frequency of breast-feeding in our study influenced the number of formula-fed infants.

Conclusion

In this prospective, double-blind, randomized study, only a few children developed cow's milk allergy and other food allergy. In general, a low incidence of cow's milk allergy was found in breast-fed as well as hypoallergenic formula-fed infants, probably owing to a very high frequency of breast-feeding. Partially hydrolyzed formula was less effective than extensively hydrolyzed formula for preventing cow's milk allergy. Because of the small number of cases these results should be interpreted with caution. No difference was seen as regards development of respiratory allergy. Compared with other similar studies the frequency of atopic symptoms was low, even though the dietetic intervention did not include restrictions to the maternal diet during lactation or dietary restrictions to the children after the age of 4 months. A dietary prevention programme including only the first 4 months of life seems sufficient.

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References

1. CHANDRA RK, PURI S, CHEEMA PS. Predictive value of cord blood IgE in the development of atopic disease and role of breast-feeding in its prevention. *Clin Allergy* 1985; 15: 517–22.
2. MISKELLY FG, BURR ML, VAUGHAN-WILLIAMS E, et al. Infant feeding and allergy. *Arch Dis Child* 1988; 63: 388–93.
3. VANDENPLAS Y, DENEYER M, SACRE L, LOEB H. Preliminary data on a field study with a new hypo-allergenic formula. *Eur J Pediatr* 1988; 148: 274–7.
4. CHANDRA RK, SHAKUNTALA P, HAMED A. Influence of maternal diet during lactation and use of formula feeds on development of atopic eczema in high risk infants. *BMJ* 1989; 299: 228–30.
5. CHANDRA RK, SINGH G, SHRIDHARA B. Effect of feeding whey hydrolysate, soy and conventional cow milk formula on incidence of atopic disease in high risk infants. *Ann Allergy* 1989; 63: 102–6.
6. CHANDRA RK, HAMED A. Cumulative incidence of atopic disorders in high risk infants fed whey hydrolysate, soy, and conventional cow milk formulas. *Ann Allergy* 1991; 67: 129–32.
7. LUCAS A, BROOKE OG, MORLEY R, COLE TJ, BAMFORD MF. Early diet of preterm infants and development of allergic or atopic disease: randomized prospective study. *BMJ* 1990; 300: 837–40.
8. HALKEN S, HØST A, HANSEN LG, ØSTERBALLE O. Effect of an allergy prevention programme on incidence of atopic symptoms in infancy. A prospective study of 159 'high risk' infants. *Allergy* 1992; 47: 545–53.
9. HALKEN S, HØST A, HANSEN LG, ØSTERBALLE O. Preventive effect of feeding high-risk infants a casein hydrolysate formula or an ultrafiltrated whey hydrolysate formula. A prospective, randomized, comparative clinical study. *Pediatr Allergy Immunol* 1993; 4: 173–81.
10. ZEIGER RS, HELLER S, MELLON MH, et al. Effect of combined maternal and infant food-allergen avoidance on development of atopy in early infancy: a randomized study. *J Allergy Clin Immunol* 1989; 84: 72–89.
11. ZEIGER RS, HELLER S, SAMPSON HA. Genetic and environmental factors affecting the development of atopy through age 4 in children of atopic parents: a prospective randomized controlled study of food allergen avoidance. *Pediatr Allergy Immunol* 1992; 3: 110–27.
12. ARSHAD SH, MATTHEWS S, GANT C, HIDE DW. Effect of allergen avoidance on development of allergic disorders in infancy. *Lancet* 1992; 339: 1493–7.
13. HIDE DW, MATTHEWS S, MATTHEWS L, et al. Effect of allergen avoidance in infancy on allergic manifestations at age two years. *J Allergy Clin Immunol* 1994; 93: 842–6.
14. ZEIGER RS, HELLER RS. The development and prediction of atopy in high-risk children: follow-up at age seven years in a prospective randomized study of combined maternal and infant food allergen avoidance. *J Allergy Clin Immunol* 1995; 95: 1179–90.
15. BUSINCO L, DREBORG S, EINARSSON R, et al. Hydrolysed cow's milk formulae. Allergenicity and use in treatment and prevention. An ESPACI position paper. *Pediatr Allergy Immunol* 1993; 4: 101–11.
16. HALKEN S, HØST A, JACOBSEN HP, HANSEN LG, ØSTERBALLE O. Prevention of food allergy in high-risk infants until the age of 5 years. A prospective dietary intervention study. *Allergy* 1995; 26 (Suppl.): 49(Abstract).
17. VANDENPLAS Y, HAUSERIES B, VAN DEN BORRE C, SACRE L,

- DAB I. Effect of a whey hydrolysate prophylaxis of atopic disease. *Ann Allergy* 1992; 68: 419–24.
18. VANDENPLAS Y, HAUSERIES B, VANDEN BORRE C, et al. The long-term effect of a partial whey hydrolysate formula on the prophylaxis of atopic disease. *Eur J Pediatr* 1995; 154: 488–94.
19. CHANDRA RC. Five-year follow-up of high-risk infants with family history of allergy who were exclusively breast-fed or fed partial whey hydrolysate, soy, and conventional cow's milk formulas. *J Pediatr Gastroenterol Nutr* 1997; 24: 380–8.
20. MARINI A, AGOSTI M, MOTTA G, MOSCA F. Effects of a dietary and environmental prevention programme on the incidence of allergic symptoms in high risk atopic infants: three years follow-up. *Acta Paediatr* 1996; 85 (Suppl. 414): 1–22.
21. OLDAEUS G, BJÖRKSTEN B, EINARSSON R, KJELLMAN N-IM. Antigenicity of cow's milk hydrolysates intended for infant feeding. *Pediatr Allergy Immunol* 1991; 2: 156–60.
22. MÄKINEN KILJUNEN S, SORVA R. Bovine beta-lactoglobulin levels in hydrolysed protein formulas for infant feeding. *Clin Exp Allergy* 1993; 23: 287–91.
23. HØST A, HUSBY S, HANSEN LG, ØSTERBALLE O. Bovine beta-lactoglobulin in human milk of atopic and non-atopic mothers. Relationship to maternal intake of homogenized and unhomogenized milk. *Clin Exp Allergy* 1990; 45: 547–51.
24. RAGNO V, GIAMPIETRO PG, BRUNO G, BUSINCO L. Allergenicity of milk protein hydrolysate formulae in children with cow's milk allergy. *Eur J Pediatr* 1993; 152: 760–2.
25. OLDAEUS G, ANJOU K, BJÖRKSTEN B, MORAN JR, KJELLMAN N-IM. Extensively and partially hydrolysed infant formulas for allergy prophylaxis. *Arch Dis Child* 1997; 77: 4–10.
26. NIGGEMANN B, WAHN U, SAMPSON HA. Proposals for standardization of oral food challenge tests in infants and children. *Pediatr Allergy Immunol* 1994; 5: 11–4.
27. HOLLIS S, CAMPBELL F. What is meant by intention to treat analysis? Survey of published randomised controlled trials. *BMJ* 1999; 319: 670–4.
28. HIDE DW, MATTHEWS S, TARIQ S, ARSHAD SH. Allergen avoidance in infancy and allergy at 4 years of age. *Allergy* 1996; 51: 89–93.
29. SAARINEN UM, KAJOSAARI M. Breast-feeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. *Lancet* 1995; 346: 1065–9.