

ORIGINAL ARTICLE

A non-hydrolyzed, fermented milk formula reduces digestive and respiratory events in infants at high risk of allergy

M Morisset¹, C Aubert-Jacquin², P Soullaines³, D-A Moneret-Vautrin¹ and C Dupont³

¹Médecine Interne, Immunologie Clinique et Allergologie, CHU de Nancy, EA 3999 'Allergic Diseases: Diagnostic and Therapy', Nancy University, Nancy, France; ²Danone Research, Villefranche-sur-Saône, France and ³Université Paris-Descartes, Service d'Explorations Fonctionnelles Digestives Pédiatriques, Paris, France

Background/Objectives: To determine the impact of a not hydrolyzed fermented infant formula containing heat-killed *Bifidobacterium breve* C50 and *Streptococcus thermophilus* 065 (HKBBST) on the incidence of allergy-like events during the first 2 years of life in children at high risk of atopy.

Subjects/Methods: This multicenter, randomized, double-blind, controlled study included infants at high risk of atopy. Infants used HKBBST or a standard infant formula (SIF) since birth until 1 year of age, and were followed at 4, 12 and 24 months after birth. Skin prick tests (SPTs) for six foods and six aeroallergens were systematically performed and adverse events (AEs) were recorded. In case of potentially allergic AE (PAAE), allergy could be further tested by SPT, patch tests and quantification of specific IgEs. If cow's milk allergy (CMA) was suspected, an oral challenge could also be performed.

Results: The study included 129 children, 63 were randomized to SIF, 66 to HKBBST. The use of HKBBST milk did not alter the proportion of CMA but decreased the proportion of positive SPT to cow's milk (1.7 vs 12.5%, $P=0.03$), and the incidence of digestive (39 vs 63%, $P=0.01$) and respiratory potentially allergic AEs (7 vs 21%, $P=0.03$) at 12 months, and that of respiratory PAAEs at 24 months (13 vs 35%, $P=0.01$).

Conclusions: HKBBST decreased the incidence of PAAEs in children with family history of atopy, during the first months of life and after the formula was stopped. Oral tolerance to cow's milk in infants at high risk of atopy may therefore be improved using not hydrolyzed fermented formulae.

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Keywords: fermented infant formula; *Bifidobacterium breve*; *Streptococcus thermophilus*; prebiotic; cow's milk allergy; family history of atopy

Introduction

Strategies currently proposed to decrease the incidence of cow's milk allergy (CMA) in at-risk children focus on the elimination of allergens: breastfeeding, delayed food diversification, elimination of allergenic foods and the use of formulae with hydrolyzed proteins. All these strategies are still controversial and their actual efficacy remains to be

confirmed (Osborn and Sinn, 2006; von Berg *et al.*, 2007; Greer *et al.*, 2008; Host *et al.*, 2008).

Based on the impact of intestinal microbiota modifications on food allergies, new dietetic strategies are currently being proposed, including the addition of prebiotics and probiotics to hydrolyzed infant formulae to prevent atopic diseases (Wold, 1998; Isolauri *et al.*, 2000; Kalliomaki *et al.*, 2001; Rosenfeldt *et al.*, 2003; Viljanen *et al.*, 2005; Giovannini *et al.*, 2007; Kukkonen *et al.*, 2007; Betsi *et al.*, 2008; Boehm and Moro, 2008). Nonetheless, their efficacy and tolerance has still not been proven (FAO/WHO, 2002; AFSSA, 2003; Agostoni *et al.*, 2004, 2007; Osborn and Sinn, 2007; Hol *et al.*, 2008; Kopp *et al.*, 2008; Lee *et al.*, 2008).

Preclinical studies identified *Bifidobacterium breve* C50 and *Streptococcus thermophilus* 065 as good candidates for the

Correspondence: Dr C Dupont, Université Paris Descartes, Service d'Explorations Fonctionnelles Digestives Pédiatriques, Hôpital Necker-Enfants Malades, 149, rue de Sèvres 75015 Paris, France
E-mail: christophe.dupont@nck.aphp.fr

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improvement of oral tolerance in children at high risk of atopy (Agostoni *et al.*, 2007). Milk fermented with these two bacteria and heated to kill ferments has shown to modify intestinal microbiota in animals and humans (Romond *et al.*, 1997, 1998; Thibault *et al.*, 2004), to strengthen intestinal barrier in mice (Menard *et al.*, 2006) and to prevent the increase of thymus size in non-breastfed, healthy term infants (Indrio *et al.*, 2007). Moreover, the metabolites of these ferments exert immunomodulatory properties, partly through dendritic cells (Menard *et al.*, 2004; Hoarau *et al.*, 2006). An infant formula made of milk fermented with *B. breve* C50 and *S. thermophilus* 065 and heated to kill ferments (heat-killed *Bifidobacterium breve* C50 and *Streptococcus thermophilus* 065 (HKBBST)) was therefore developed.

We report here the results of a controlled clinical trial of the efficacy of this not hydrolyzed fermented infant formula containing HKBBST on the incidence of CMA, sensitization to cow's milk (CM) and potentially allergic adverse events (PAAEs) during the first 24 months of life in children at high risk of atopy. In view of the early appearance of these events (Kvenshagen *et al.*, 2008), a particular focus was made on the first 12 months of life.

Methods

Objectives

The primary objective of this multicenter, randomized, double-blind, controlled study was to determine the effect of HKBBST milk on the incidence of CMA and CM sensitization in children at high risk of atopy.

Secondary objectives were to determine the effect of HKBBST milk on (i) the incidence of sensitization and/or allergy to other allergens than CM and (ii) the incidence of allergic symptoms during the study period.

Subjects

Healthy male and female children presenting with family history of allergy at one of the two centers (Neonatology Unit, Saint-Vincent de Paul Hospital, Paris, France or Unit of Internal Medicine, Clinical Immunology and Allergology, Nancy University Hospital, Nancy, France) or at one of the 16 private allergologist investigators were included. Children were randomized in sequential ascending order to either standard infant formula (SIF) or HKBBST milk. The randomization list was created by an independent statistician (OPTIMED, Grenoble, France) and confidentially maintained until approval was received for the study to be unblinded for analysis. Randomization occurred after the selection (for example before the end of the fifth month of pregnancy). To control possible breastfeeding effect, women wishing to breastfeed were randomly balanced between groups. Sample size was calculated based on the primary outcome. The mean percentage of at-risk children who effectively develop allergic manifestations is fairly imprecise, between 6 and 20%, the risk being approximately divided by two by probiotics

(Saarinen *et al.*, 1999; Exl and Fritsche, 2001; Kalliomaki *et al.*, 2001). Based on these data, for rejection of two-sided null hypothesis with a type I error of 5% and a type II error of 30%, at least 62 children had to be included per group, for a total of 124 children. Prior to any action under the study, the investigator collected written consent from both parents or from the legal representative. The protocol was approved by the French ethics committee, and the study was conducted in accordance with the Declaration of Helsinki as (revised in 2004), the regulatory provisions applicable in France and Good Clinical Practices. The study was registered at www.clinical-trial.gov under the reference NCT00792090.

Inclusion criteria

Inclusion criteria were less than 6 months of pregnancy and family at risk of atopy: at least two atopic members, including at least one of the parents (for example mother and/or father and/or at least one brother or sister). Children with known or suspected immune deficiency within family were not included. In addition, because actual use of the formula could not be determined in children without known date of weaning, they were excluded from the analyses.

Interventions

Selection included examination by an allergist and interview with a dietician. Medical and diet questionnaires were filled-in and mothers were explained the diet to be followed until the fourth month after birth and were given advice to prevent atopy. Mothers wishing to breastfeed were encouraged to do so up to the sixth month after birth. From the fifth month of pregnancy, the mother was required to use of calcium supplementation and avoid peanut, nut, egg and fermented milk products or prebiotic- or probiotic-containing food (commercially available milk was allowed). Actual inclusion was at child's birth without additional visit. Parents then had to complete a diary collecting data on weaning and use of milk or formula. Every day, from birth to the end of breastfeeding, mothers of exclusively breastfed children had to drink at least 500 ml of the milk their child had been randomized to. The dietician checked diet compliance and tolerance by the mothers. Following visits were at 4 months after birth (± 2 weeks), 12 months (± 2 weeks) and 24 months (± 4 weeks). During visits, the allergist examined the child, filled out a demographic and medical questionnaire, collected adverse events (AEs) and PAAEs, and systematically performed skin prick tests (SPTs). The allergist was free to perform atopy patch test (APT) or specific IgE quantification according to standardized procedures in case of allergy suspicion. The dietician explained the diet to be followed during the following months (gradual diversification) and filled out a dietetic questionnaire. At the end of breastfeeding, children gradually moved to the formula they had been randomized to. Partially breastfed children were given from birth the formula they had been allocated to. The formula had to be used until the age of 1 year. Parents visited the allergist each time a clinical event appeared.

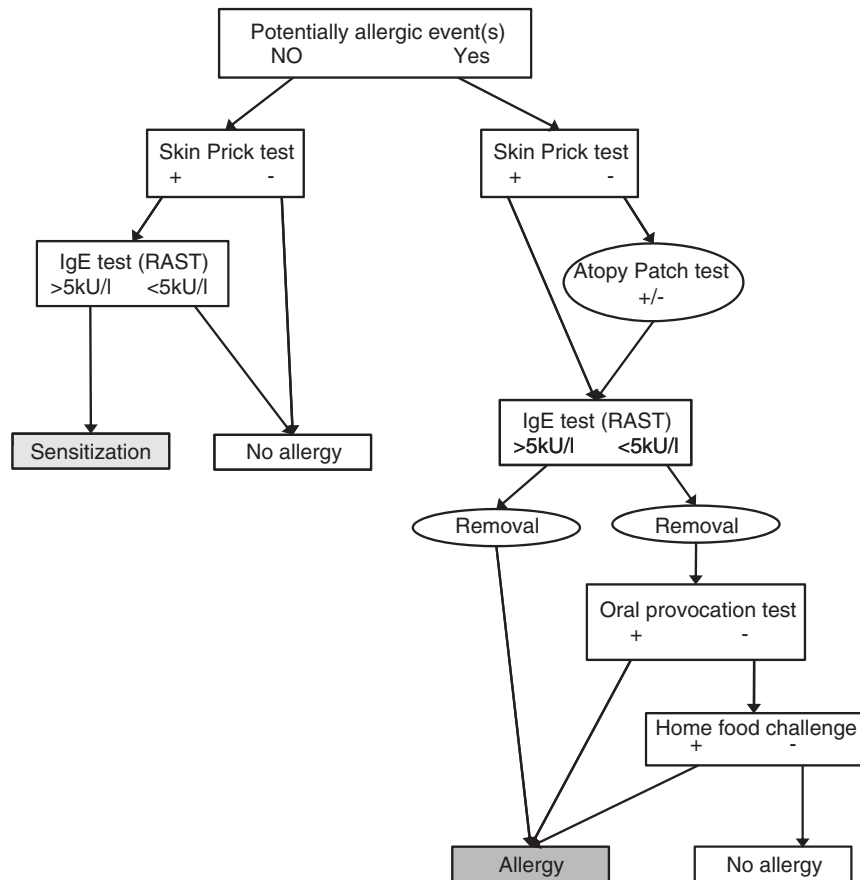


Figure 1 Decision tree of the tests performed during study. IgE quantification and atopy patch tests were performed only for CM and food allergens.

Products under study

Control was a SIF (Gallia, Blédina, France). HKBBST formula had the same nutritional composition as the SIF but was fermented with *B. breve* C50 (4.2×10^9 bacteria per 100g of powder formula) and *S. thermophilus* 065 (3.84×10^7 bacteria per 100g of powder formula) during the manufacturing process and then heated to kill ferments (patented technology, Blédina, France). The appearance, taste and smell of the two products were similar. Allergist, dietitian and mother were blinded to the product allocated during all the study, until the analysis of last result data.

Outcomes measurement

Children were considered allergic to CM and/or to another food allergen when oral food challenge (OFC) was positive. Children were considered sensitized to a food allergen in case of positive skin tests (SPT or APT) or positive specific IgE, independently of allergy-like symptoms.

Data collected and tests performed

Potentially allergic adverse events. OFC being performed after 6 months of age only, physicians were asked to collect AEs

and identify those that have been shown to be associated with the development of allergy during the first months of life (PAAEs) (Vandenplas *et al.*, 2007; Host *et al.*, 2008; Kvenshagen *et al.*, 2008) (Figure 1). PAAEs were grouped into three categories: (i) cutaneous symptoms included atopic dermatitis, urticaria, angioedema, eczema, dry skin when associated to another symptom, rash and pruritus, (ii) digestive symptoms included upper digestive symptoms (vomiting and gastroesophageal reflux), lower digestive symptoms (acute diarrhea, colitis, abdominal pain, constipation, abdominal distension and rectal bleeding) and general symptoms associated (fainting or transient hypotonia and failure to thrive) and (iii) respiratory symptoms included wheezing, wheezing bronchitis and spastic bronchitis. The severity of digestive symptoms was indirectly estimated using the growth curve, with occurrence of a failure to thrive. Each AE has been annotated by the allergist and scored minor, moderate or severe.

Skin prick test. SPTs were standardized and systematically performed at each visit. They were made according to the prick-in-prick method (Dreborg, 1991). SPT to food allergens included CM, hen's egg, codfish, wheat flour, soy flour and

roasted peanut. SPT to aeroallergen extracts included *Dermatophagoides pteronyssinus*, cat and dog dander, mix of grass pollens, birch pollen and *Alternaria alternata* (Allerbio Laboratories, Varennes-en-Argonne, France). A positive control (9% codeine phosphate) and a negative control (solvent, for example, saline) were systematically performed. SPT was considered positive if the mean wheal diameter was $>75\%$ of the positive control wheal or ≥ 3 mm.

Atopy patch test. APTs were standardized and performed using a Finn chamber occluded during 48 h, according to the method described by Kekki *et al.* (1997). APTs were performed with CM, hen's egg, codfish, wheat flour, soy flour and roasted peanut. Reading was made at 72 h.

APT was considered positive according to the International Contact Dermatitis Research Group and the European Task Force on Atopic Dermatitis Consensus Meetings criteria (Turjanmaa *et al.*, 2006).

IgE detection. Blood was sampled when an allergic was suspected. Specific CM or food allergen IgEs were quantified by ImmunoCap (Phadia, Uppsala, Sweden) and considered as positive for scores ≥ 0.35 kUI/l.

Oral food challenge. Children with CMA suspicion were submitted to an OFC to CM after 2 months of an elimination diet and disappearance of the symptoms. If the OFC was negative or doubtful, a second, double-blind OFC was performed by testing CM against placebo (Neocate, Nutricia SHS International, Liverpool, UK).

Criteria considered for positive OFC to CM were onset, reappearance or worsening eczema lesions, urticaria, angioedema, anaphylactic shock, hemodynamic modification, fainting, cough, dyspnea, wheezing, diarrhea, abdominal pain, vomiting and constipation. According to Garcia-Ara *et al.* (2001), who previously showed a positive predictive value of CM allergy $>95\%$ for children <12 months when specific IgE level are >5 kU/l. OFC was not performed if SPT to CM was positive and specific IgEs were >5 kUI/l. For ethical considerations, oral challenge was not performed in case of previous history of anaphylactic shock, laryngeal edema, severe asthma or any life-threatening immunological complaint. As food allergy manifestations with digestive symptoms can be >72 h, OFC was sometimes followed by home food challenge. OFC was performed as early as possible but never before the age of 6 months.

Statistical analyses

Tests were two-sided and the level of significance (α) was set at 0.05. χ^2 and Fisher's exact tests were used for qualitative variables (test: positive or negative/symptom observed: yes or no): skin or IgE testing, cutaneous, digestive, general and respiratory symptoms. Results presented here are from Per-Protocol populations, which showed no substantial

difference as compared with ITT populations. Statistical analyses were performed using SAS, version 8.2 (SAS Institute, Cary, NC, USA).

Results

From January 2004 to April 2006, 136 mothers were selected and 129 children were included and randomly allocated to SIF ($N=63$, of which 55 completed the study) or HKBBST milk ($N=66$, of which 53 completed the study) (Figure 2). Characteristics of the children at inclusion are described in Table 1 and no statistically significant difference was observed between groups. Use of other milk or formula during the study was low and did not differ between groups.

Sensitization and CMA

At 4 months, a trend was observed toward a lower proportion of positive SPT to CM in the HKBBST group than in the standard group (1.6 vs 7.9%; $P=0.06$) (Table 2). However, no significant difference was found between groups concerning sensitization to CM, other foods or aeroallergens. OFC was not performed before the age of 6 months, so that CMA could not be diagnosed at 4 months.

At 12 months, although CMA incidence was not different between groups, the proportion of positive SPT to CM was significantly decreased in the HKBBST group as compared to the standard group (1.7 vs 12.5%; $P=0.03$) (Table 2). When specific IgEs for foods other than CM were quantified, children in the HKBBST group showed a higher proportion of negative biological test than children in the standard group (90.9 vs 33.3%; $P=0.01$). APT to CM, CM-specific IgEs, SPT and APT to other foods and sensitization to aeroallergens were not different between groups.

The incidence of CMA remained not different between groups at 24 months but the proportions of positive SPT to CM and children with detectable anti-milk IgE tended to be decreased in the HKBBST group as compared to the standard group (Table 2). No difference was observed between groups with regards to sensitization to other foods.

Potentially allergic adverse events. During the study, 43 of the 55 children (78.2%) had at least 1 PAAE in the standard group; they were 37 of the 53 children of the HKBBST group (69.8%; $P=0.32$). Compared to SIF, HKBBST milk significantly decreased the proportion of children with at least one digestive PAAE at 4 months (42.9 vs 26.2%; $P=0.047$) (Figure 3). At 12 months, children of the HKBBST group showed a significantly lower proportion of respiratory PAAEs (21.4 vs 6.8%; $P=0.031$) and digestive PAAEs (62.5 vs 39.0%; $P=0.012$), as compared to standard group. At 24 months, the proportion of digestive PAAEs remained decreased in the HKBBST group, as compared to the placebo group, but this did not reach significance ($P=0.08$). However, the decreased proportion of respiratory PAAE observed at 12 months in the HKBBST group remained significant at 24 months (34.6 vs

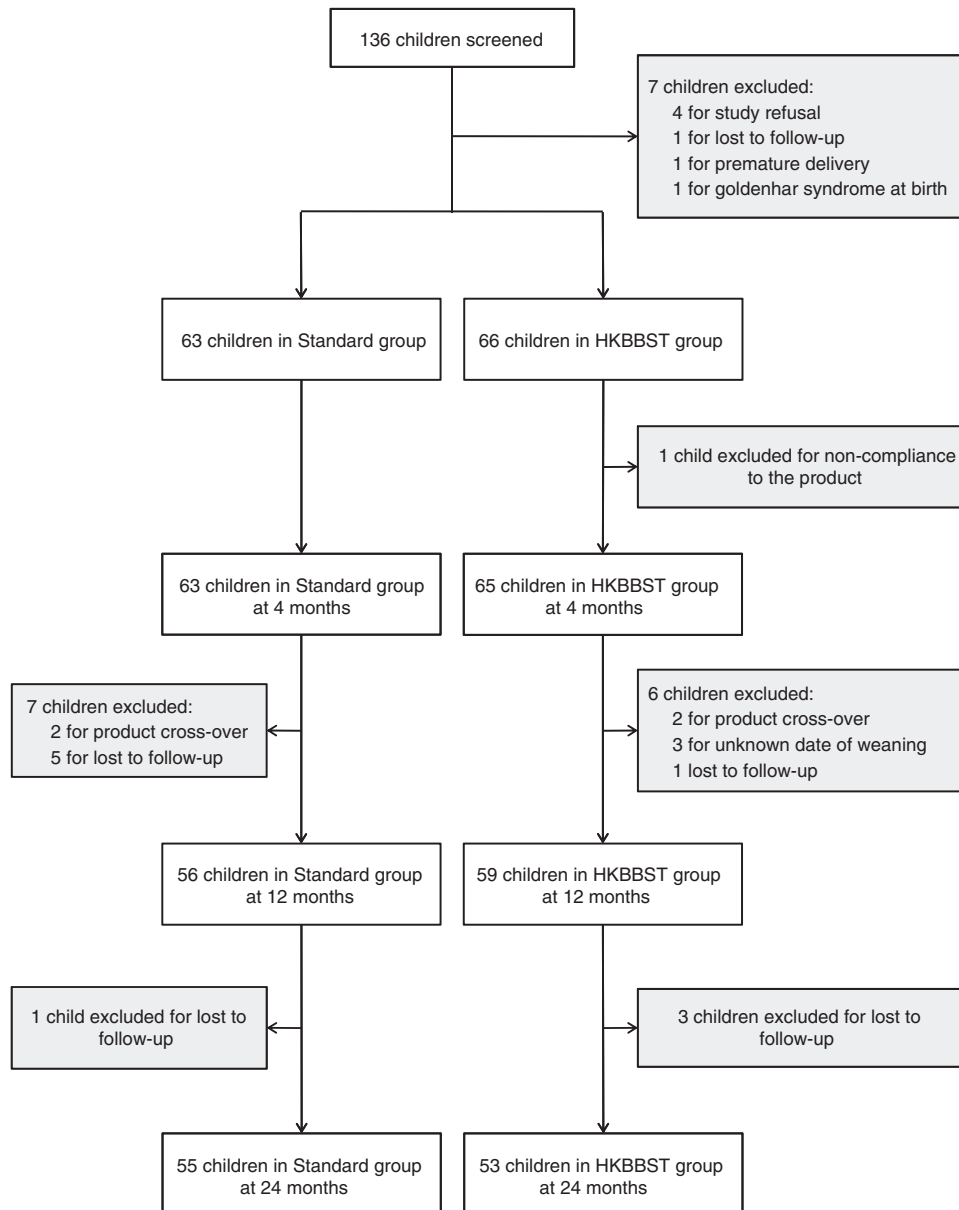


Figure 2 Flow diagram of the children participating in the study.

13.2%; $P=0.010$). No significant difference was found with regards to general intensity of PAAEs (Table 3).

Discussion

The present study did not show decreased incidence of CMA in children using HKBBST milk. However, HKBBST milk decreased the proportion of positive SPT to CM, the incidence of digestive and respiratory PAAEs, and the proportion of positive IgE tests against other foods than CM over the first 12 months of life. In addition, the impact

of HKBBST persisted until the 24th month of life with decreased incidence of respiratory PAAE. As such, this is the first study showing efficacy of a fermented infant formula with non-hydrolyzed milk proteins on respiratory and digestive allergic manifestations and biological signs of sensitization to food during the first year of life.

The present study was performed on a subsample of the general population (for example children at high risk of atopy) but the proportion of CMA was lower than expected. Nonetheless, the rapid and careful follow-up by the dietician and the allergist of children presenting positive biological test (for example SPT, APT or IgE) or PAAEs may have resulted

Table 1 Characteristics of the children included in the study

	Total (N = 129)	Standard (N = 63)	HKBBST (N = 66)	P-value
Gestational age (weeks)	39.1 ± 1.2	39.0 ± 1.2	39.3 ± 1.2	0.18
Birth weight (kg)	3.39 ± 0.39	3.32 ± 0.35	3.46 ± 0.42	0.053
Birth height (cm)	50.1 ± 2.1	49.8 ± 2.1	50.4 ± 2.1	0.13
Female gender	58 (45.0)	31 (49.2)	27 (40.9)	0.34
<i>Family history of allergy</i>				
Mother	95 (73.6)	44 (69.8)	51 (77.3)	0.34
At least one of mother's offspring	116 (89.9)	56 (88.9)	60 (90.9)	0.7
Father	105 (81.4)	55 (87.3)	50 (75.8)	0.09
At least one of father's offspring	111 (86.0)	54 (85.7)	57 (86.4)	0.91
<i>Duration of breastfeeding (days)</i>				
No breast feeding	47 (36.4)	21 (33.3)	26 (39.4)	
0-4 months	46 (35.7)	25 (39.7)	21 (31.8)	
>4 months	36 (27.9)	17 (26.9)	19 (28.8)	
Time (days) for infant formula to become the main food	126.2 ± 73.2	116.5 ± 71.8	135.1 ± 74.2	0.24

Abbreviation: HKBBST, heat-killed *Bifidobacterium breve* C50 and *Streptococcus thermophilus* 065.

Results are presented as mean $\pm$ s.d. or number (percentage).

in a decreased incidence and/or severity of CMA and sensitizations cannot be ruled out.

With regard to biological tests and PAAEs, the trend observed at 4 months toward a lower proportion of positive SPT to CM became significant at 12 months and lasted until the 24th month of life, well after HKBBST had been stopped. In addition, the proportion of children with digestive PAAEs at 4 months was almost twice as low in the HKBBST group as in the standard group, which persisted until at least the 12th month. With regards to respiratory PAAEs, a trend toward lower incidence could be observed at 4 months that increased during the study to be significant at 12 and 24 months. This study is the first to show the early and persisting protective effect of a fermented infant formula without live bacteria, and containing intact, not hydrolyzed CM proteins, on biological tests and PAAEs in infants with family history of atopy. Previous preclinical and clinical studies suggest that this may be related to the positive effect of HKBBST milk on intestinal flora and thymus size in healthy infants (Thibault *et al.*, 2004; Indrio *et al.*, 2007).

The particularity of HKBBST milk resides in its original formulation. It is neither a hypoallergenic formulation, in which casein would have been hydrolyzed, nor a formulation exclusively containing probiotics or prebiotics. In addition, HKBBST formula was the same formula as the SIF used as comparator in the present study, but fermented with *B. breve* C50 and *S. thermophilus* 065 and heated to kill ferments. Both milks were not hydrolyzed and contained intact CM proteins, which was confirmed by gel electrophoresis and liquid chromatography (data not shown). Therefore, the effect we describe can be attributed to the

Table 2 Allergy and/or sensitization to cow's milk, other foods and aeroallergens at 4, 12 and 24 months

	4 months				12 months				24 months			
	Total (N = 128)	Standard (N = 63)	HKBBST (N = 65)	P-value	Total (N = 115)	Standard (N = 56)	HKBBST (N = 59)	P-value	Total (N = 108)	Standard (N = 55)	HKBBST (N = 53)	P-value
Cow's milk allergy ^a	NA	NA	NA	NA	15 (31)	9 (15)	6 (16)	0.64	28 (30)	15 (16)	13 (14)	1.0
Sensitization to cow's milk ^b	23 (125)	14 (63)	9 (62)	0.11	32 (115)	18 (56)	14 (59)	0.31	34 (108)	20 (55)	14 (53)	0.27
Skin prick test	6 (125)	5 (63)	1 (62)	0.06	8 (115)	7 (56)	1 (59)	0.03	8 (108)	7 (55)	1 (53)	0.06
Atopy patch test	21 (45)	12 (22)	9 (23)	0.53	27 (52)	15 (24)	12 (28)	0.36	28 (53)	17 (26)	11 (27)	0.16
IgE ≥ 0.35 kU/l	3 (31)	1 (15)	2 (16)	1.0	9 (46)	7 (22)	2 (24)	0.13	11 (45)	8 (22)	3 (23)	0.07
Sensitization to other foods ^b	29 (126)	13 (63)	16 (63)	0.20	44 (115)	21 (56)	23 (59)	NS	45 (108)	22 (55)	23 (53)	0.72
Skin prick test	8 (126)	3 (63)	5 (63)	0.19	24 (115)	11 (56)	13 (59)	0.75	25 (108)	12 (55)	13 (53)	0.74
Atopy patch test	26 (46)	13 (23)	13 (23)	0.98	35 (54)	19 (26)	16 (28)	0.48	35 (53)	20 (26)	15 (27)	0.14
IgE ≥ 0.35 kU/l	0 (5)	0 (1)	0 (4)	0.36	3 (14)	2 (3)	1 (11)	0.01	5 (17)	3 (6)	2 (11)	0.28
Sensitization to aeroallergens (SPT) ^b	2 (125)	1 (63)	1 (62)	0.24	7 (115)	4 (56)	3 (59)	0.71	13 (108)	6 (55)	7 (53)	0.71

Abbreviations: HKBBST, heat-killed *Bifidobacterium breve* C50 and *Streptococcus thermophilus* 065; NA, not applicable; SPT, skin prick test.

^aAllergy was confirmed by a positive oral food challenge (OFC) with immediate or delayed reaction; OFC was not performed before 6 months of age. Results are presented as n (number of children tested).

Sensitization to an allergen corresponded to positive skin test(s) to this allergen or positive specific IgE (ImmunoCap Phadia > 0.35 kU/l). SPT was considered positive if the mean wheal diameter was $\geq 75\%$ of the positive control wheal or ≥ 3 mm. Atopy patch test (APT) was considered positive according to the International Contact Dermatitis Research Group and the European Task Force on Atopic Dermatitis Consensus Meetings criteria (Turjanmaa *et al.*, 2006). SPTs were systematically performed and other tests were decided by the investigator.

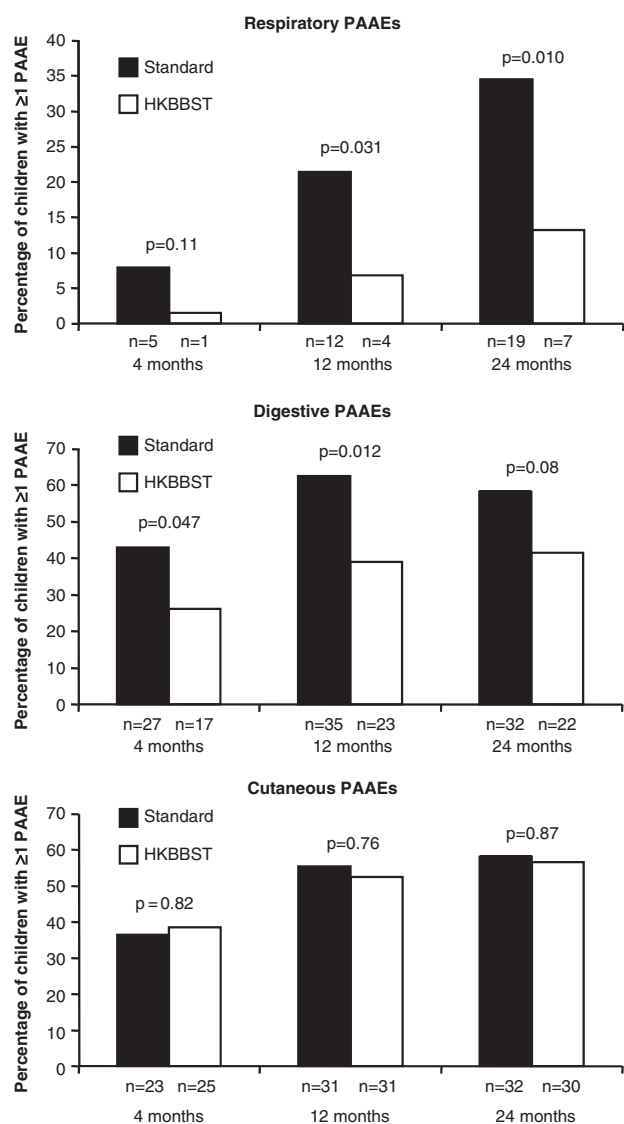


Figure 3 Percentage of children with respiratory, digestive or cutaneous PAAEs in standard and HKBBST groups at 4, 12 and 24 months.

fermentation with *B. breve* C50 and *S. thermophilus* 065 followed by heating. As suggested by the study by Sashihara *et al.* (2006) showing in a murine model of allergy that proteoglycans derived from different heat-killed strains of lactic bacteria modulate cytokines production, this effect may be due to thermoresistant peptidoglycans contained in the cell wall of the ferments used. The role of peptidoglycans or of other components of the cell wall is further supported by the fact that *B. breve* C50 can activate the Toll-like receptor (TLR)2 (Hoarau *et al.*, 2006), the main ligands of which are components of the cell wall of Gram-positive bacteria.

Comparison of our results with similar infant formulae (for example prebiotic- or probiotic-containing formulations)

Table 3 Total number and severity of PAAEs at 4, 12 and 24 months

	4 months			12 months			24 months		
	Total	Standard	HKBBST	Total	Standard	HKBBST	Total	Standard	HKBBST
	N = 128	N = 63	N = 65	N = 115	N = 56	N = 59	N = 108	N = 55	N = 53
Number of PAAEs	72 (56.3)	38 (60.3)	34 (52.3)	81 (70.4)	42 (75.0)	39 (66.1)	80 (74.1)	43 (78.2)	37 (69.8)
Intensity of PAAEs									
Minor	35 (48.6)	22 (57.9)	13 (38.2)	33 (40.7)	19 (45.2)	14 (35.9)	30 (37.5)	17 (39.5)	13 (35.1)
Moderate	31 (43.1)	13 (34.2)	18 (52.9)	41 (50.6)	19 (45.2)	22 (56.4)	43 (53.8)	21 (48.8)	22 (59.5)
Severe	6 (8.3)	3 (7.9)	3 (8.8)	7 (8.6)	4 (9.5)	3 (7.7)	7 (8.8)	5 (11.6)	2 (5.4)
Incidence of atopic dermatitis	45 (35.2)	21 (33.3)	24 (36.9)	55 (47.8)	26 (46.4)	29 (49.1)	48 (44.4)	24 (43.6)	24 (45.3)
Severity of atopic dermatitis									
Minor	24 (51.1)	14 (63.6)	10 (40)	38 (63.3)	18 (66.7)	20 (60.6)	51 (63)	25 (61)	26 (65)
Moderate	19 (40.4)	7 (31.8)	12 (48)	18 (30)	8 (29.6)	10 (30.3)	27 (33.3)	15 (36.6)	12 (30)
Severe	4 (8.5)	1 (4.5)	3 (12)	4 (6.7)	1 (3.7)	3 (9.1)	3 (3.7)	2.44 (3)	2 (5)

Abbreviation: PAAEs, potentially allergic adverse events. Results are presented as N (percentage).

is only possible to a very limited extent since very few studies have been published that concerned a not hydrolyzed formula in children under 1 year of age and at increased risk of allergy (Betsi *et al.*, 2008). Nonetheless, our results support the study by Kalliomaki *et al.* (2001), which showed that the use of probiotics could prevent allergy-like events (for example a 50% decrease in the incidence of atopic dermatitis) in children under 1 year. However, the study in question showed no impact on the proportion of food sensitizations and its results failed to be replicated (Kopp *et al.*, 2008). Our results also support the study of Kukkonen *et al.* (2007), which has shown that the use of a preparation containing four different probiotics along with galacto-oligosaccharides prevented eczema, but this was shown in 2-year-old children. With regard to other infant formulae, even if efficacy has still not been proven for soybean protein-based formulae, some formulae with partially or completely hydrolyzed casein (hypoallergenic) have shown good results. However, due to differences in methodologies and results, generalization across studies is difficult and no consensus was found so far concerning their use (Greer *et al.*, 2008).

Comparison with other studies is also difficult because of the very young age of the children at inclusion and the particular methodology we had to design. As the diagnosis of an allergy can hardly be set before 6 months of age, any symptom (for example, PAAEs) or biological data possibly related to allergy had to be collected, whereas most studies mainly collect and analyze atopic dermatitis. PAAEs were clinical symptoms that could correspond to allergic symptoms but for which clear association with allergy could not be made. Established rule states that the allergenic origin of a symptom is confirmed only if it disappears after allergen avoidance and reappears during OFC. For some children who presented with PAAEs disappearing after CM eviction, it was not possible to prove the role of CM because the oral challenge was negative. One can wonder whether these symptoms were, or not, transient symptoms of CM allergy with rapid natural recovery before the age of 6 months. We are fully aware that a positive biological test or PAAEs are not sufficient to set the diagnosis of an allergy. Nonetheless, even if the PAAEs considered are not as solid as OFC, they correspond to symptoms commonly observed in clinical practice, which are most often incapacitating for children and families; their avoidance would largely improve the quality of life of the children and their families. Furthermore, this study demonstrates that studies on the impact of an infant formula on allergy-like events can be conducted within the first year of life.

In conclusion, the present study shows that the HKBBST fermented infant formula, containing intact milk proteins, decreases the proportion of sensitization to CM proteins and the incidence of PAAEs in children at high risk of atopy, as early as the very first months of life and during a long period of time after its cessation. Fermented milks may represent a new dietetic strategy to promote oral tolerance to CM.

Conflict of interest

Cécile Aubert-Jacquín is the Danone employee. Martine Morisset, Denise-Anne Moneret-Vautrin and Christophe Dupont have received honoraria and/or compensation with regards to the study, as an investigator, coordinator or expert, in relation with the time spent on the study. The authors declare no conflict of interest.

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