Safety of a New Amino Acid Formula in Infants Allergic to Cow's Milk and Intolerant to Hydrolysates

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See ''Primum Non Nocere'' by Agostoni on page 381.

ABSTRACT

Objectives: Amino acid–based formulas (AAFs) are recommended for children with cow's-milk allergy (CMA) failing to respond to extensively hydrolysed formulas (eHFs). We evaluated the effects of a new thickened AAF (TAAF, Novalac), containing a pectin-based thickener, and a reference AAF (RAAF, Neocate) on allergy symptoms and safety, through blood biochemistry analysis and growth.

Methods: Infants (ages < 18 months) with CMA symptoms failing to respond to eHFs were randomised in a double-blind manner to receive TAAF or RAAF for 3 months. All of the infants were then fed TAAF for 3 additional months. Paediatric visits occurred at 1, 3, and 6 months. Blood samples were collected at inclusion and 3 months.

Results: Results at 1 month were previously described. The 75 infants with proven CMA and eHF intolerance tolerated their allocated formula. At 3 months, the dominant allergic symptom had disappeared in 76.2% of the infants with TAAF and in 51.5% of the infants with RAAF ($P = 0.026$). The Scoring Atopic Dermatitis Index significantly improved more with TAAF than with RAAF (-27.3 ± 2.3 vs -20.8 ± 2.2, $P = 0.048$). Of the infants, 92.9% had normal stools (soft or formed consistency) with TAAF vs 75.8% with RAAF ($P = 0.051$). More infants in TAAF group had better quality of nighttime sleep ($P = 0.036$) and low frequency of irritability signs ($P < 0.001$). With both formulas, all of the biochemical parameters were within normal ranges. There were no differences between the 2 groups in any of the anthropometric z scores.

Conclusions: The new TAAF was tolerated by all of the infants with CMA and intolerance to eHFs. Anthropometric and clinical data showed that both formulas were safe.

Key Words: amino acid formula, growth, infants with cow's-milk allergy and intolerance to extensively hydrolysed formulas, safety, Scoring Atopic Dermatitis Index

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What Is Known

- Guidelines recommend amino acid–based formulas for children with cow's-milk allergy symptoms persisting on extensively hydrolysed formulas.
- No randomised controlled study was ever conducted in this specific population.
- Data on the impact of amino acid–based formulas on daily family life are scarce.

What Is New

- Tolerance and safety of the thickened amino acid–based formula were shown in this specific population.
- Thickened amino acid–based formula significantly reduced more Scoring Atopic Dermatitis Index and the number of infants with skin dryness than the reference amino acid–based formula.
- Thickened amino acid–based formula provided additional comfort through improvement of stool consistency, less irritability signs, and better nighttime sleep quality in more infants.

Cow's-milk allergy (CMA) manifests by clinical symptoms related to the abnormal immune response of the host after ingestion of milk proteins and affects 2% to 7% of children (1).

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Guidelines for the dietary management of infants with diagnosed CMA recommend the substitution with extensively hydrolysed casein or whey protein formulas for cow's milk (2–4). Some studies have highlighted the allergenicity of hydrolysates in highly allergic children, caused by residual immunologically active protein (5–7). For these patients, dietary treatment with an amino acid–based formula (AAF) is required (2–4). Several studies assessed the impact of AAFs on the growth of healthy infants (8,9), but few open noncontrolled studies reported growth data in infants with the dual condition of CMA and intolerance to extensively hydrolysed formulas (eHFs), that is, allergy symptoms persisting with eHFs (6,10). Regarding hypoallergenicity and clinical efficacy, published results only refer to retrospective observational studies including a limited number of patients (6,7,10,11). Moreover, controlled trials showing efficacy, tolerance, and safety of AAFs were carried out either in children with proven CMA but no intolerance to eHFs (8,9,12–14) or in children only part of whom also had documented intolerance to eHFs (11).

Results at 1 month of a double-blind, placebo-controlled, randomised study comparing a thickened AAF (TAAF) to a reference AAF (RAAF) on hypoallergenicity/tolerance, efficacy, and safety in 75 infants with CMA failing to respond to eHFs were previously published (15). Here, the results obtained in the same population at 3 and 6 months are reported.

In addition, because of the possible relation between allergic diseases and gut microbiota (16) and the presence of fibre in the TAAF that may affect colonic microbiota composition (17), the main changes in faecal microbiota after 3 months of AAF feeding were investigated. Noninvasive faecal markers have a reliable place in gastroenterology by evaluating the intestinal inflammatory responses. Hence, faecal eosinophil-derived neurotoxin (EDN) as intestinal marker of eosinophilic infiltration was also assessed (18).

METHODS

Study Design

The methodology of the study was detailed in 2014 (15). Briefly, infants (ages < 18 months) with allergic symptoms persistent under eHF feeding with 1 or several commercial eHFs available in France or Belgium for ≥2 weeks were selected. CMA diagnosis had to be proven by double-blind placebo-controlled food challenge (DBPCFC), positive skin prick test (SPT) (wheal diameter >6 mm), specific immunoglobulin E ([s]IgE) to milk >5 kU/L, or a combination of both positive cutaneous tests and (s)IgE (19,20). Infants were randomised to the TAAF group (Novalac; United Pharmaceuticals, Paris, France) or to the RAAF group (Neocate; Nutricia, Erlangen, Germany). The TAAF had a similar nitrogen content (1.9 g/100 mL) and differed mostly by the presence of a patented thickening mixture including fibres (0.5 g/100 mL), mainly composed of pectin, which thickens at gastric pH. Infants were fed study formulas in a double-blind manner for 3 months. Then the TAAF was used during 3 supplementary months for both groups to collect anthropometric data. Following analysis of the primary outcome, that is, tolerance/hypoallergenicity of the TAAF at 1 month (15), paediatric visits were programmed 3 and 6 months after dietary treatment initiation.

The objectives of the present analysis were the evaluation at 3 and 6 months of the tolerance/hypoallergenicity of the TAAF and the evolution of symptoms characteristic of CMA. Other secondary outcomes included general symptoms associated with CMA and having an impact on daily family life, the safety through growth (evaluated in accordance with World Health Organization [WHO] growth curves) and biological (including plasma amino acids) parameters, as well as intestinal microbiota and faecal EDN.

At inclusion, the dominant allergic symptom was identified for each infant. Anthropometric measurements and all of the symptoms of CMA, including skin, respiratory, and gastrointestinal tract manifestations were recorded by paediatricians at inclusion and each follow-up visit. Severity of eczema, stool consistency, and regurgitations were assessed by Scoring Atopic Dermatitis (SCORAD) Index (21), Bekkali scale (22) and Vandenplas score (23), respectively. General symptoms associated with CMA were also registered (15): sleep quality, daily crying and sleeping time, irritability signs, and crying frequency. Adverse events were recorded. Parents' satisfaction and infants' acceptability of the product (ie, parents' perception of the appreciation of the formula taste by their infant) as well as presence of gas and intestinal bloating were assessed through parents' diaries.

Blood samples were obtained by venipuncture from a subset of infants at inclusion and at 3 months; usual laboratory parameters were analysed at entry and at 3 months, and amino acid plasma concentrations were determined at 3 months.

Faecal Analyses

For each child, a sample of faeces was collected from the diapers within 3 hours after defecation and then stored at −80°C until assayed. Frozen stool samples were thawed at room temperature immediately before analysis. Faecal microbiota was assessed using quantitative real-time polymerase chain reaction (qPCR) as described in 2006 (24). Extraction of total DNA from faecal content was performed using guanidium isothiocyanate and the mechanical bead-beating method. TaqMan (Applied Biosystems, Saint-Aubin, France) qPCR was used to quantify total bacteria populations and the dominant (>1% of faecal bacteria population) bacterial groups, and genera: *Clostridium* cluster IV (*C leptum* group), *Bacteroides/Prevotella* group, and *Bifidobacterium*. qPCR using SYBR-Green (Applied Biosystems) was performed to quantify *Lactobacillus/Leuconostoc/Pediococcus* group, *Clostridium* cluster XIVa (*C coccoides* group), *Clostridium* cluster XI, *Clostridium* cluster I/II, *Staphylococcus*, *Enterococcus*, and *Escherichia coli*. Primers and probes are available upon request. Standard curves were obtained from serial dilutions of a known concentration of plasmid DNA containing a *16S rRNA* gene insert from each species or group. The coefficients of correlation between $\log_{10}$ CFU and *rRNA* gene copy numbers for each species and group were obtained from ribosomal RNA operon copy number database (25), enabling calculation of the number of colony-forming unit per gram of faeces. The detection limits depended on the bacterial groups and ranged between 10^4 and 10^6 CFU/g.

The concentration of faecal EDN was assayed in duplicate using a ''sandwich''-type enzyme ELISA method which uses a polyclonal antibody system (Immundiagnostik, Bensheim, Germany) according to the manufacturer's instructions. The quantitation limit for EDN was 120 ng/g of stools.

Statistical Analysis

The number of subjects to be included was calculated based on the requirement that a hypoallergenic formula must be tolerated by ≥90% of infants with an overt CMA (95% confidence interval [CI]). The number of subjects needed was 29 per group (8). Considering the study design, allowing the CMA confirmation within 2 months after inclusion, 15% of dropouts and inappropriate selections were anticipated, which led to include 35 infants per group. The tolerance/hypoallergenicity was assessed in infants with confirmed CMA and intolerance to eHFs, defined as the tolerance/hypoallergenicity population. The other secondary endpoints were analysed on the full analysis set (FAS) population, defined as infants

from tolerance/hypoallergenicity population with evaluation of the dominant allergic symptom at 1 month. Safety was assessed on the intention-to-treat (ITT) population, defined as infants enrolled who took study formula.

For quantitative parameters, change from baseline was compared between groups by analysis of covariance (ANCOVA) (or nonparametric ANCOVA in case of nonnormality, assessed by Shapiro-Wilk test) using the baseline value as a covariate. Intragroup changes were analysed using the Student *t* test or Wilcoxon test (nonnormal data). For qualitative parameters, change from baseline within treatment groups was analysed by symmetry test, or by McNemar test for binary variables. The difference between groups for the qualitative parameters' change was analysed using the χ^2, the Fisher, or the Cochran-Mantel-Haenszel (CMH) test. Statistical analyses were conducted using SAS version 9.2. Body mass index (BMI) was calculated for each infant. *z* scores of weight-for-age, length-for-age, weight-for-length, BMI-for-age, and head circumference–for-age were computed based on WHO growth data (26). For microbiota, when species or targeted taxonomic groups were not detected, the arbitrary value of 1.5 $\log_{10}$ colony-forming unit per gram of faecal content was used. Significance was set at $P < 0.05$.

Ethics

The study design was approved by an institutional review board for each country: Ile-de-France III, Paris, France and QFCUH ethics committee, Brussels, Belgium. This study was conducted in accordance with the ethical standards laid down by the Declaration of Helsinki. All of the parents of participating infants provided written informed consent.

RESULTS

Study Population

The characteristics of the study population at entry and at 1 month were described in 2014 (15). Briefly, 86 patients with suspected CMA (ITT population) were included and 75 were diagnosed as allergic to cow's milk and intolerant to eHFs (tolerance/hypoallergenicity population), 42 in the TAAF group, and 33 in the RAAF group (mean age 6.2 ± 4.3 months, 44% boys). They were all assessed by paediatricians at 1 (FAS population) and at 3 months of entering the study. A total of 8 infants dropped out of the study after 3 months because of parents' refusal to continue: 3 in the TAAF group and 5 in the RAAF group, leading respectively to 39 and 28 infants in the TAAF and RAAF groups assessed by paediatricians at 6 months. In addition to CMA, food allergies (egg and/or wheat) were diagnosed in 5 patients based on (s)IgE (27,28) and sensitisation was diagnosed in 30 patients based on either positive (s)IgE or cutaneous tests (soy, egg, wheat, and/or peanut).

Tolerance/Hypoallergenicity

No infant from the tolerance/hypoallergenicity population dropped out for intolerance during the 6-month study, including those who switched from RAAF to TAAF at 3 months.

Efficacy

At 3 months, complete resolution of the dominant allergic symptom was seen in a significantly higher number of subjects in the TAAF group (76.2%) than in the RAAF group (51.5%, $P = 0.026$, χ^2 test). Noticeably, in the 5 infants in the RAAF group for whom the dominant allergic symptom was persistent at 3 months, a change was seen at 6 months under TAAF, with resolution in 4 and improvement in 1. At 3 months, SCORAD Index, eczema, and skin dryness significantly improved in both the groups (Table 1, Fig. 1, Table S1 [*http://links.lww.com/MPG/A458*]). The SCORAD index decreased significantly more in the TAAF group than in the reference group, and skin dryness was resolved in significantly more patients ($P = 0.019$, χ^2 test). Rhinitis and wheezing significantly improved in the TAAF group (Fig. 1, Table S1 [*http://links.lww.com/MPG/A458*]). Regurgitation scores significantly decreased in both the groups (Table 1), and regurgitations disappeared completely in 66.7% of infants with the TAAF and 44.0% with the RAAF. Percentage of infants with soft/formed stools significantly increased in both the groups, from 47.6% to 92.9% and from 51.5% to 75.8% in the TAAF and RAAF groups, respectively ($P < 0.001$ and $P = 0.032$, McNemar test); more infants had normal or improved stools with TAAF than with RAAF (Table 1). Moreover, all of the 7 infants from the RAAF group with hard or liquid stools at 3 months exhibited soft or formed stools with the TAAF at 6 months.

At 3 months, general symptoms associated with CMA showed significant improvement with the TAAF such as frequency of crying, irritability, and sleeping time and quality, and, with the RAAF only quality of daytime sleep (Table 1, Fig. 2, Table S2 [*http://links.lww.com/MPG/A459*]). In both the groups, daily crying time significantly decreased (Table 1). Significant differences between the 2 groups were observed for frequency of irritability and quality of nighttime sleep, with the TAAF being more effective (respectively $P < 0.001$ and $P = 0.036$, χ^2 test and Fisher test).

Growth Data

At 3 months, infants' growth was similar between the 2 groups with no significant differences for weight, length, weight-for-length, BMI, and head circumference *z* scores. Compared with baseline, growth during 6 months showed significant improvement of weight-for-age *z* score in the group initially fed the TAAF (mean ± SD 0.3 ± 0.6, Fig. 3). In the same group, length, weight-for-length, BMI, and head circumference *z* scores increased by 0.1 (±0.8), 0.1 (±0.8), 0.4 (±0.9), and 0.3 (±0.8), respectively, during the 6-month study. As infants of the RAAF group switched to TAAF during the last 3 months, mean changes of anthropometric data between 3 and 6 months were adjusted with baseline values as covariate. Based on these analyses, at 6 months, no significant differences were noted between groups. Between 3 and 6 months, the mean weight-for-age *z* score significantly increased by 0.1 ± 0.3 in the RAAF group ($P = 0.028$, Wilcoxon test).

Safety

The most common adverse events were gastrointestinal tract affections and infections and were not related to the study product. Incidence of adverse events was not different between groups. A total of 4 serious adverse effects were recorded between 1 and 3 months: 3 in the TAAF group (gastroenteritis, pneumonia, and gastrooesophageal reflux) and 1 in the RAAF group (gastroenteritis); 3 were recorded between 3 and 6 months (malaise, gastroenteritis, and pneumonia). None were related to the study formula and none led to study drop out. In 2014, Dupont et al (15) reported 2 nonserious adverse events that led to study termination within the first month in the RAAF group. Parents' satisfaction with the allocated formula was high in both the groups (90.9% vs 79.0% at 1 month and 90.0% vs 91.7% at 3 months for TAAF and RAAF groups, respectively). Infants' acceptability of the allocated formula was judged as very good or good by more parents in the TAAF group than in the RAAF group at 1 and 3 months (76.5% vs 58.6% at 1 month and 91.4% vs 61.9% at 3 months, not significant CMH

TABLE 1. Change from baseline in SCORAD index scores, regurgitations scores, stool consistency, daily crying, and sleeping time at 3 months

	TAAF, N = 42	RAAF, N = 33	Total, N = 75
SCORAD Index			
N	25	27	52
Mean ± SD	−27.3 ± 2.3*	−20.8 ± 2.2*	−23.9 ± 20.9
Median (minimum, maximum)	−26.5 (−59.0, 5.9)	−25.4 (−72.5, 20.7)	−25.9 (−72.5, 20.7)
P value vs baseline	<0.001†	<0.001†	<0.001†
P value between groups			0.048§
Regurgitation score			
N	27	25	52
Mean ± SD	−1.9 ± 1.7	−1.7 ± 1.9	−1.8 ± 1.7
Median (minimum, maximum)	−1 (−6, 0)	−1 (−6, 2)	−1 (−6, 2)
P value vs baseline	<0.001‡	<0.001†	<0.001‡
P value between groups			0.159
Stool consistency, N (%)			
N	42	33	75
Aggravated** or not formed	3 (7.1)	8 (24.2)	11 (14.7)
Improved or formed	39 (92.9)	25 (75.8)	64 (85.3)
P value between groups			0.051‖
Daily crying time, min			
N	36	28	64
Mean (SD)	−126.4 ± 195.2	−45.7 ± 102.2	−91.1 ± 165.2
Median (minimum, maximum)	−45.0 (−780.0, 75.0)	−17.5 (−300.0, 140.0)	−42.5 (−780.0, 140.0)
P value vs baseline	<0.001‡	0.025†	<0.001‡
P value between groups			0.827¶
Daily sleeping time, min			
N	39	30	69
Mean ± SD	67.2 ± 157.0	35.0 ± 143.8	53.2 ± 151.2
Median (minimum, maximum)	60.0 (−240.0, 420.0)	0.0 (−360.0, 420.0)	30.0 (−360.0, 420.0)
P value vs baseline	0.011†	0.193†	0.005†
P value between groups			0.623§

ANCOVA = analysis of covariance; N = number of subjects; RAAF = reference amino acid–based formula; SCORAD = Scoring Atopic Dermatitis Index; SD = standard deviation; TAAF = thickened amino acid–based formula.

* Adjusted means.

** Stool consistency change from soft/formed to liquid/hard.

† Student *t* test.

‡ Wilcoxon test.

§ ANCOVA.

‖ Fisher test.

¶ ANCOVA based on ranks.

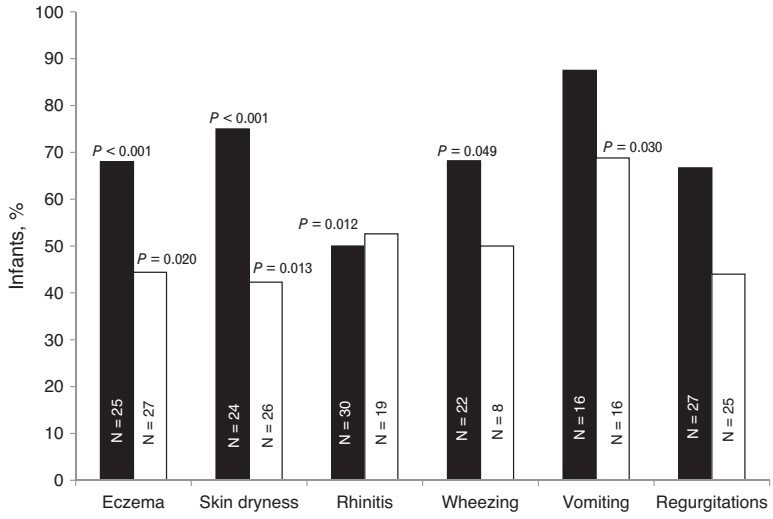

FIGURE 1. Proportion of infants with resolution of allergic symptoms at 3 months. All of the *P* values are versus baseline. Black column: TAAF; white column: RAAF. N = number of subjects; RAAF = reference amino acid–based formula; TAAF = thickened amino acid–based formula.

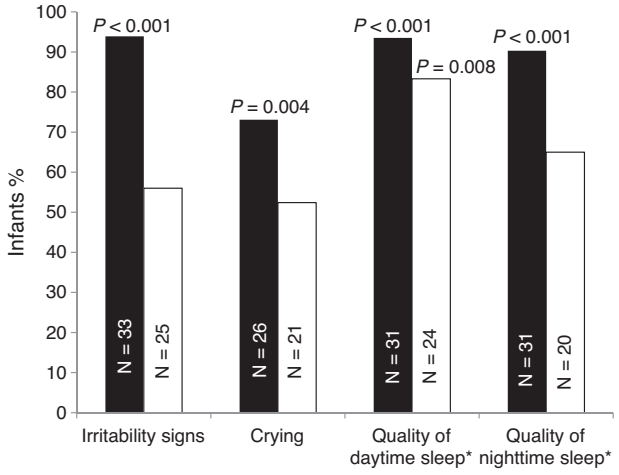

FIGURE 2. Proportion of infants with resolution (∗improvement) of general symptoms at 3 months. All of the *P* values are versus baseline. Black column: TAAF; white column: RAAF. N = number of subjects; RAAF = reference amino acid–based formula; TAAF = thickened amino acid–based formula.

test). No differences between groups were noted concerning the presence of gas and intestinal bloating at 1 and 3 months.

From inclusion to 3 months, there was a significant fall in mean plasma eosinophils concentrations in both the groups: from 0.49 (±0.55) to 0.28 (±0.22) 10^9/L in the TAAF group (n = 32, $P = 0.017$) and from 0.53 (±0.47) to 0.22 (±0.19) 10^9/L in the RAAF group (n = 29, $P < 0.001$, Wilcoxon test), with no significant difference between the groups. All of the other mean biochemical parameters, IgG, IgA, IgM, serum ferritin, and complete blood count were within normal range values at 3 months. In the subset of infants (25 in the TAAF group and 22 in the RAAF group) with plasma amino acid evaluation at 3 months, there were no differences between both the groups in plasma essential amino acid concentrations except for valine, higher in the RAAF group ($P = 0.049$, Wilcoxon test) (Fig. S1, *http://links.lww.com/MPG/A460*).

Faecal Analysis Data

At 3 months, infants of both the groups were colonized at high levels by bacterial groups usually encountered in the dominant microbiota of infants, that is, *Bacteroides/Prevotella*, *C coccoides* group, and *Bifidobacterium* (median $>10^9$ CFU/g of faeces, Fig. S2 [*http://links.lww.com/MPG/A461*]). A total of 2 infants, however, in the RAAF group were not colonized by bifidobacteria. Concerning the subdominant microbiota (median levels comprised between $10^{5.5}$ and 10^9 CFU/g of faeces), >90% of the infants were colonized by *E coli*, *Enterococcus*, and *Clostridium* cluster I/II and cluster XI. By contrast, colonization occurred less frequently with the *C leptum* group, *Lactobacillus/Leuconostoc* group, and *Staphylococcus* (53% to 83% of the infants depending on the bacterial groups and the formula group). After 3 months of AAF feeding, the evolution in the total bacteria counts was significantly different between TAAF and RAAF groups ($P = 0.021$), with a stable bacterial count in the TAAF group and a slight increase in the RAAF group. Despite no significant differences between groups for any genera, some different trends in the evolution were observed. Bifidobacteria decreased in both the groups, but the decrease was moderate in the TAAF group, -0.21 $\log_{10}$ CFU/g (±0.45), and higher in the RAAF group, -1.15 $\log_{10}$ CFU/g (±0.52) (adjusted means). When expressed as percentage of total bacteria, this trend was more marked, with bifidobacteria remaining stable in the TAAF group, 1.7% (±8.7), and decreasing in the RAAF group, −20.3% (±10.1). Similar trend was observed for the *Lactobacillus/Leuconostoc* group: -0.43 $\log_{10}$ CFU/g (±0.51) in the TAAF group versus -1.01 $\log_{10}$ CFU/g (±0.59) in the RAAF group. Likewise, there were increases in percentages of *Bacteroides/Prevotella* and *C coccoides* groups in the TAAF group (Fig. S3, *http://links.lww.com/MPG/A462*). These modifications tended to modify the balance of the microbiota, with a trend to a higher abundance of the bifidobacteria, *Bacteroides/Prevotella*, and *C coccoides* groups in the TAAF group compared with the RAAF group at 3 months.

EDN values ranged from <120 to 3475 ng/g at inclusion and from <120 to 3324 ng/g at 3 months, showing a high interindividual variability (n = 38). The trend (median, range) was similar with a decrease for both the groups: −196 ng/g (−1954 to 2026) in the TAAF group and −166 ng/g (−2469 to 2832) in the RAAF group, with no significant difference between the groups.

DISCUSSION

This study demonstrates the efficacy and safety in the long term of both AAFs in infants with proven CMA and intolerance to eHFs. All of the infants tolerated their allocated AAF for 3 months, and the TAAF was also tolerated by all of the infants who completed the 6-month study, including the infants who switched from RAAF to TAAF at 3 months. As reported in 2014, at 1 month, a complete resolution of the major CMA symptom occurred in 61.9% and 51.5% of infants in TAAF and RAAF groups, respectively (15). Results presented here confirm that at 3 months, both AAF formulas improved the major CMA symptom, the percentage of resolution being significantly higher with the TAAF.

One could argue that a DBPCFC, considered as the criterion standard (3) for CMA diagnosis, was not performed in all of the patients. Only a minority of subjects (26.7%) had no challenge and diagnosis based on (s)IgE assay and SPT values above validated cutoff levels for active CMA (19). This disposition in the protocol was chosen to favour the enrolment process of families dealing with an already complicated medical history (previous failure with 1 or more eHFs). In fact, the primary endpoint of this study, the tolerance/hypoallergenicity of the TAAF at 1 month in >90% (with 95% CI) of infants with both CMA and intolerance to eHFs, requires a sample size of ≥29 subjects with no reaction (2). This minimum number of subjects was reached in the TAAF group, even in the subgroup of subjects with CMA proven by a DBPCFC (15), and 100% of them tolerated the TAAF for ≥3 months. In addition, the percentage of the major CMA symptom resolution did not statistically differ between the infants with CMA proven by (s)IgE/SPT and those with CMA proven by a DBPCFC at 1 and 3 months; in the latter, this percentage remained significantly higher in the TAAF group compared with the RAAF group at 3 months.

In case of CMA, recommendations are first dietary treatment with eHFs to eliminate cow's-milk protein in the diet (1,2,4). eHFs have been successfully used to treat most of the infants with cow's-milk allergy. Some infants, however, are sensitive to these formulas, so their CMA symptoms persist with eHFs. AAFs are the recommended choice for these infants. Several studies reported hypoallergenicity and tolerance of AAF in infants with proven CMA (8,14,29) or in infants intolerant to eHFs (6,10), but no randomised controlled trial had ever been carried out in infants with CMA and allergy symptoms persisting with eHFs (3).

Beyond testing the tolerance of the AAFs, the aim was to quantify their efficacy in a large cohort of infants with both CMA and intolerance to eHFs. As CMA is characterized by a multitude of symptoms, including fussiness, irritability, emesis, poor feeding, and diarrhoea at presentation (1,4,30), paediatricians had to

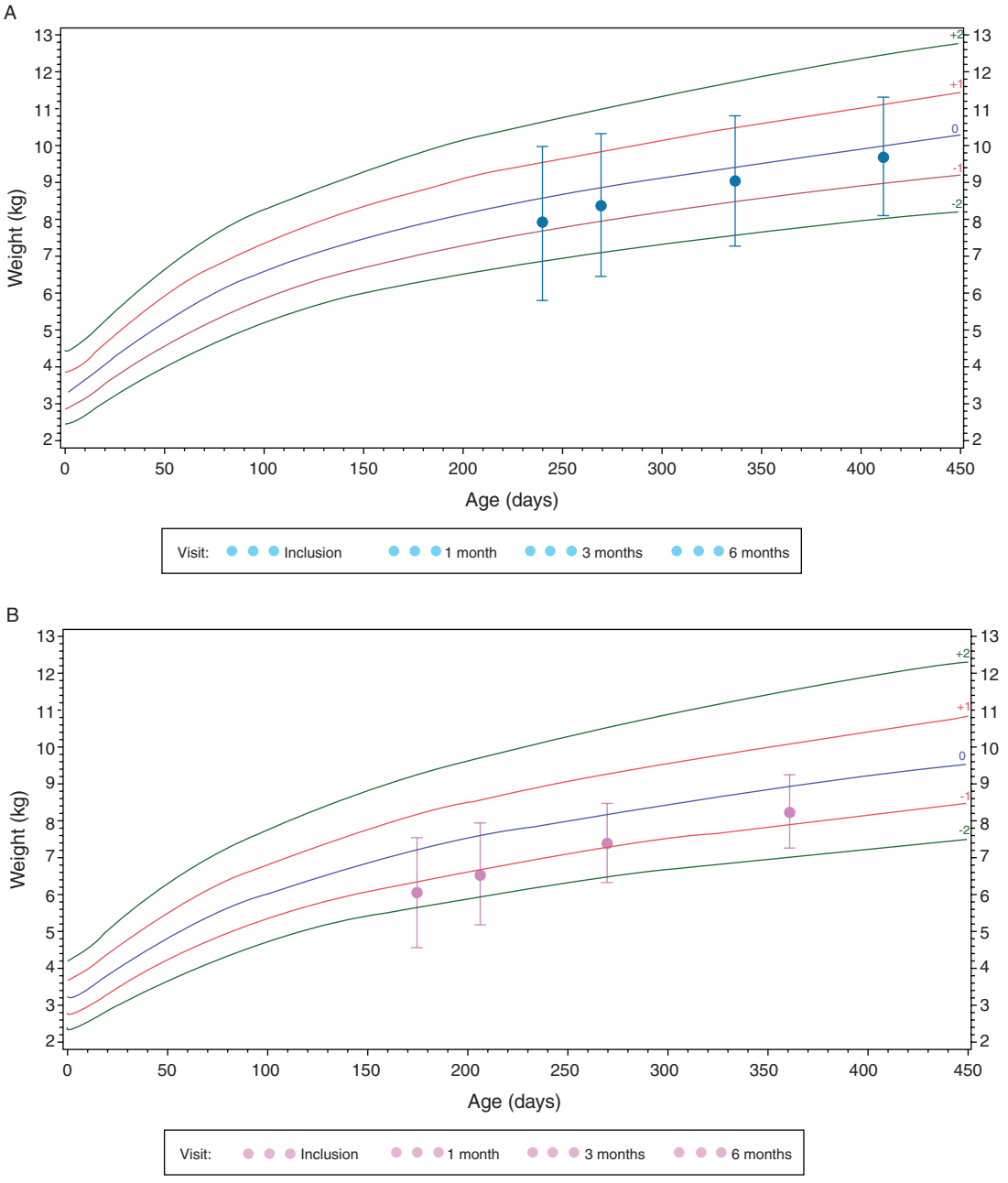

FIGURE 3. Mean weight (±SD) at each visit shown in comparison with the WHO growth standards for boys (A) and girls (B) initially fed TAAF. TAAF = thickened amino acid–based formula; WHO = World Health Organization.

determine in this study the dominant allergic symptom for each subject at inclusion and to assess its evolution at each follow-up visit. This dominant allergic symptom, however, did not reflect the complete clinical situation of each patient: for the subjects in which this symptom was not improved after 1 month, other allergic symptoms were resolved or improved. In addition, in 1995, Hill et al (31) evaluated children with multiple food intolerance, including CMA, and requiring an AAF feeding. Their allergic symptoms were scored during an eHFs challenge and compared with an AAF, as placebo; the score was most of the time lower with the AAF than with the eHF but never equal to zero. Recently, another tool, the Cow's Milk-related Symptom Score (CoMiSS), was proposed to allow the assessment and quantification of the evolution of CMA symptoms during a therapeutic intervention (32). Several trials, in which children with CMA on an eviction diet were followed, reported the evolution of CoMiSS values; after 1 month of dietary treatment, these values significantly decreased but were not equal to zero (33–35). Finally, the percentages of resolution of the dominant allergic symptom did not differ at 1 month between the TAAF and the RAAF, the latter having been considered for decades as the reference for severe CMA treatment (6,7).

The wide array of symptoms studied allowed a large documentation of symptom evolution. Regarding cutaneous symptoms, mean SCORAD index values observed in infants with CMA at

baseline ranged from 19.4 ± 16.1 (29) to 21 (95% CI 16–26) (5). Niggemann et al (13) reported a median SCORAD index of 18.5. All of these trials showed decreases of SCORAD index with AAFs. The present results confirm the efficacy of AAFs to reduce eczema severity in infants with CMA and intolerance to eHFs, the TAAF reducing significantly more the SCORAD index than the RAAF. The major difference between both AAFs and that may explain the observed difference is the presence of a pectin-based thickener in the TAAF. To the best of our knowledge, no clinical study has reported a significant effect of a pectin-based complex on skin health improvement in allergic infants. An earlier study found that a prebiotic mix, containing in particular acidic pectin oligosaccharides, had an effect on eczema prevention in infants with no atopy risk but no impact on eczema severity (36). In addition, results on the effect of prebiotic and probiotic blends on SCORAD evolution in infants and young children with atopic dermatitis are inconsistent (37,38). Given these contradictory results, the possible role of pectin on skin symptoms is unclear, requiring further investigation.

Previous studies suggest that pectin could play an integral role in improving stool consistency (34,35), and this was shown by a significant decrease in the number of liquid and hard stools in the TAAF group. Pectin is a dietary fibre known for having an impact on faecal weight (39). Other mechanisms could be considered such as enhanced colonic fluid absorption through the production of short-chain fatty acids (40) by colon microbiota (41), which may explain the positive effects of a pectin-based diet on diarrhoea resolution (42).

Very little data are available on the effect of AAFs on general symptoms related to CMA and affecting daily family life. Vanderhoof et al (7) reported the evolution of crying time and duration of sleep patterns in such patients. Fifteen days after AAF initiation, crying time was significantly reduced, but no change in the sleep duration was observed. The present results confirm the impact of AAF on reduced crying time but also show a significant increase in daily sleeping time. In addition, the TAAF showed a better improvement of irritability signs and quality of night sleep, compared with the RAAF.

Compared with healthy infants, allergic infants may have impaired growth, which is partially linked to improper food substitutions following allergen elimination (43). Moreover, CMA may also increase energy requirements because of inflammation (ie, skin or gastrointestinal) and disrupted sleep, and reduce the absorption of major nutrients (ie, CMA-induced enteropathy) (44). Mean weight and length z scores of the infants included in this study were < -0.5 at inclusion, showing poor weight and length gains in these infants, as already evidenced by other clinical studies (5,10,34,35,45,46). Previous clinical studies assessed the impact of AAF on the growth of healthy infants (8,9), or infants with CMA (5,13,29), but only 2 open noncontrolled studies reported growth data in infants with CMA and intolerance to eHFs (6,10). They all showed improvement of anthropometric data with AAF. In the present study, growth of infants fed the TAAF was similar to that of infants fed the RAAF, confirming the nutritional safety of this formula.

Essential amino acid plasma concentrations did not differ between the groups, except for valine, which was closer to breast-fed concentrations in the TAAF group, and slightly higher in the RAAF group compared with the TAAF group, however not clinically significant. They were all in the range of the concentrations measured in 6-month healthy infants (47) and similar to those in breast-fed infants (48).

Three months after AAF initiation, plasma eosinophils significantly decreased in both the groups. Same results were reported with AAF feeding but in older children (11,49). EDN is a faecal marker of intestinal immune stimulation related to allergic inflammatory responses, especially eosinophilic infiltration (18). Kalach et al (18) showed that infants with intestinal symptoms during a cow's milk challenge had higher faecal EDN levels than those with other symptoms or those tolerating cow's milk, suggesting an eosinophilic degranulation in the intestine. Our study confirms the high level of faecal EDN in this population. Three months after AAF initiation, children from both the groups showed a decrease of their EDN values, which may reflect a decrease of their intestinal inflammation.

Microbiota composition is in both groups in accordance with previous studies performed in infants at a similar age (50). The intervariability observed between infants is likely because of the diversity in age at sampling and in the perinatal determinants of the studied infants which are known to affect the bacterial colonization (50). No significant differences were observed between both the formulas throughout the study. Although the number of samples may not have been sufficient to detect a statistical difference, in the TAAF group, however, bifidobacteria percentages remained stable throughout the study whereas a slight decrease was observed in the RAAF group. Such observation was reported by Thompson-Chagoyan et al (51) who showed in CMA infants followed for 6 months on an elimination diet a significant increase in percentages in lactobacilli and a significant decrease in bifidobacteria. In TAAF, pectin, which is known to increase bifidobacteria counts (41), may have counterbalanced the effect of an elimination diet on decreasing bifidobacteria percentages.

This study presents several limitations. First, the number of faecal samples collected was low compared with the number of subjects included and may explain the absence of statistical differences observed between the groups. Secondly, our population may not have been homogenous enough regarding the CMA symptomatology to detect a possible impact of the dietary treatment on EDN values. In fact, this marker being associated with eosinophils intestinal infiltration, it may be more relevant in patients with gastrointestinal symptoms (18).

In conclusion, we have shown that the TAAF is hypoallergenic, efficient to alleviate symptoms, nutritionally adequate, and able to support growth during long-term feeding in these infants.

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