

Original Article

Tolerability of partially and extensively hydrolysed milk formulas in children with cow's milk allergy

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Background and Objectives: The safety and tolerability of hydrolysed cow's milk protein-based formulas, particularly partially hydrolysed formulas (pHFs), in children with cow's milk allergy (CMA) remain poorly understood. We evaluated the tolerability of hydrolysed cow's milk-based formulas in children with CMA. **Methods and Study Design:** A three-period double-blind crossover evaluation compared the allergic tolerance against three dietary cow's milk-based formulas: extensively hydrolysed cow's milk formula (eHF), pHF, and regular cow's milk formula (rCMF). The primary outcome was the rate of tolerance against a maximum of 20.0 mL of formula. **Results:** Controlled food challenges were performed in 25 children (18 boys; 7 girls) with a median age of 4.25 years (range: 1–9 years) diagnosed with CMA. The median cow's milk-specific immunoglobulin E level was 31.9 UA/mL (range: 1.16–735 UA/mL). The tolerance rate ratios for rCMF were lower than those for pHF (2 vs 16; $p < 0.01$) and eHF (2 vs 22; $p < 0.01$). The allergic symptom scores induced by intake of pHF and eHF were significantly lower than those of rCMF ($p = 0.01$ and $p < 0.01$, respectively), and the pHF and eHF scores were not significantly different. **Conclusions:** Compared to rCMF, the partially and extensively hydrolysed whey and casein formulas evaluated in this study were better tolerated and therefore safer for children with CMA. Although further confirmation from additional centres is needed, our findings suggest the use of pHF in patients with mild CMA. Some children with CMA react to hydrolysed formulas; therefore, food challenge tests in these children should be undertaken with caution.

Key Words: basophil activation, extensively hydrolysed cow's milk formula, cow's milk allergy, food challenge, partially hydrolysed cow's milk formula

INTRODUCTION

Cow's milk allergy (CMA) is one of the most common food allergies that occur in childhood, requiring affected individuals to avoid dairy products in their everyday lives.^{1,2} Infants with CMA cannot consume the conventional cow's milk-based infant formula. Therefore, in cases where breast-feeding is impossible, the only remaining option is hypoallergenic formulas. Extensively hydrolysed cow's milk formulas (eHFs) or amino acid-based formulas are commonly used as hypoallergenic infant formulas.^{1,3}

These products are generally safe and recommended for infants with CMA, although their cost and undesirable flavour are known disadvantages.⁴ Most (80%) patients with CMA outgrow the allergy by the age of 3–4 years; however, cow's milk elimination diet therapy is frequently prolonged, often until the patient reaches school-going age.¹ Because cow's milk is an excellent source of calcium and protein for growing children, it is desirable to withdraw the elimination diet as early as possible. Some cases of malnutrition and inadequate development have

been reported in children who continue avoiding cow's milk.⁵⁻⁷

However, in spite of adopting these cautionary measures, allergic reactions are inevitable in some cases. Moreover, the processes involved in the manufacturing and cooking of dairy foodstuffs have been reported to reduce antigenicity and increase tolerability.⁸ These "partially" hypoallergenic foodstuffs are considered safer than cow's milk itself for individuals who continue to strictly follow the cow's milk elimination diet. A type of cow's milk-based infant formula, partially hydrolysed formula

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(pHF), is widely distributed in many countries. pHFs are not “hypoallergenic formulas”, and accidental ingestion of pHF has been reported to cause adverse allergic symptoms in patients with CMA.⁹ However, preclinical tests have shown that the antigenicity of pHF is substantially lower than that of conventional infant formula.^{10,11} Therefore, pHF could be considered safe for use while weaning the child off the cow’s milk elimination diet therapy. However, the safety and tolerability of hydrolysed cow’s milk-based formulas in children with CMA remain poorly understood. For these purposes, and because of the possible risk of symptom induction, the allergenicity of pHFs needs to be clinically assessed. Therefore, the aim of this study was to evaluate the safety and tolerability of hydrolysed cow’s milk-based formulas in children with CMA.

METHODS

Study design and participants

This three-period double-blind crossover study was designed to compare allergic tolerance against three dietary cow’s milk-based formulas. Double-blind, controlled food challenges were performed in children aged 1–20 years who had a known history of systemic symptoms induced by ingesting small amounts of milk allergens or who had high levels of cow’s milk-specific IgE. The inclusion criteria for individuals with a high level of specific IgE were selected based on the results of a previous trial reporting that individuals fulfilling these conditions have a 95% probability of having CMA.¹² Children with a current diagnosis of severe persistent asthma were excluded from the study. When paediatricians at Fujita Health University encountered children at the paediatric allergy clinics who satisfied the study criteria, they explained the study to the families and asked them to participate.

Ethics and informed consent

The study procedures and potential risks were explained to all participants and their parents, and written informed consent was subsequently obtained. This study conformed to the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of Fujita Health University, Aichi, Japan (No. 12-127).

Formulas

We used three formulas: a pHF (E-akachan®, Morinaga Milk Industry Co., Ltd., Tokyo, Japan), an eHF (MA-

mi®, Morinaga Milk Industry Co., Ltd., Tokyo, Japan), and a regular cow’s milk formula (rCMF; Hagukumi®, Morinaga Milk Industry Co., Ltd., Tokyo, Japan). The terms pHF and eHF denote cow’s milk formulas prepared via enzymatic hydrolysis of cow’s milk protein, which decreases the antigenicity of casein and whey proteins. The hydrolysate for eHF was treated with ultrafiltration. The pHF and rCMF contained 1.52 g protein per 100 mL, and the eHF 1.76 g protein per 100 mL. The molecular weight profiles of the formulas are shown in Table 1.

Food challenges

All food challenges were performed in a randomised, double-blind manner in a hospital ward over a period of 3 days, with 1-week intervals between the challenges. These intervals were used to avoid carry-over effects. Parents of children receiving antihistamines were requested to withhold medications for 72 hours before and during the challenge. A total volume of 20 mL of the pHF, eHF, or rCMF was administered every 30 minutes in 5–7 increments. The challenge was discontinued if objective allergic symptoms, such as urticaria, cough, or wheezing, occurred or if subjective allergic symptoms, such as abdominal pain, occurred and a paediatric allergist determined that the symptoms were induced by the formula. Clinical symptoms occurring within 2 hours of administering the highest dose were defined as allergic reactions. Participants were observed for 2 hours after the final dose and then discharged. Participants with positive results from the food challenge at any testing dose remained under observation until after the associated symptoms had resolved.

Randomisation and blinding

A technician who was not directly involved in the challenges conducted the randomisation and prepared the formulas for the food challenges. For each challenge, the technician selected a formula from the box that contained powder sticks of the three formulas and prepared a 100-mL solution of the formula using the same procedure each time. The practitioners administering the food challenge picked up the formula and performed the challenge. The practitioners and participants did not know which formulas were used on which days until all food challenges were completed.

Table 1. Molecular weight profiles[†] of the study formulas

Molecular weight	Partially hydrolysed formula	Extensively hydrolysed formula	Regular cow’s milk formula
	E-akachan®	MA-mi®	Hagukumi®
	%	%	%
<500	54.1	65.2	46.7
500–1000	21.0	22.6	14.6
1000–1200	7.5	6.7	4.9
1200–2000	9.2	4.4	7.4
2000–3500	5.4	1.1	5.5
>3500	2.9	Trace amounts [‡]	21.0

[†]Measured with high-performance liquid chromatography. Defatted formula samples were applied to a high-performance liquid chromatography system (LC-20AD, Shimadzu, Tokyo, Japan) with a poly-hydroxyethyl aspartamide column (PolyLC, Columbia, MD, USA).

[‡]The hydrolysate for extensively hydrolysed formula (MA-mi®) was treated with ultrafiltration.

Symptom score and threshold dose

The severity of allergic symptoms during the food challenges was scored according to the system reported by Astier et al.¹³ The symptom panel was divided into five categories based on the affected organs (Table 2). A paediatric allergist evaluated the symptoms of the participants after intake of formulas and scored them on a scale between 0 and 5. The threshold dose was the highest dose in the food challenge that did not elicit an adverse reaction.

Blood sampling

Blood samples were collected from participants within 2 weeks before the first food challenge. These samples were used to evaluate the cow's milk-specific IgE levels and the basophil activations.

Measurement of serum cow's milk-specific IgE levels

The serum samples were used for the evaluation of cow's milk-specific IgE levels. ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) measurements were carried out according to the manufacturer's instructions. The levels of cow's milk-specific IgE were assessed in all participants, and 0.35 UA/mL was used as the sensitisation cut-off as suggested by the manufacturer.

Basophil activation

Basophil activation was determined using an allergenicity kit (Beckman Coulter Inc., Fullerton, CA, USA) according to the manufacturer's instructions. All assays involved the use of whole fresh blood collected within 24 hours of sampling. Briefly, heparin-anticoagulated peripheral blood samples were incubated at 37°C for 15 minutes with fluorescein isothiocyanate-labelled anti-chemoattractant receptor-homologous molecule on Th2 cells (CRTH2), phycoerythrin-labelled anti-CD203c, and phycoerythrin-cyanine 7-labelled anti-CD3 monoclonal antibodies in the presence of the allergen. Phosphate-buffered saline and anti-IgE antibodies (10 µg/mL) were used as negative and positive controls, respectively. Samples were analysed using a FACSCalibur cell analyser with CellQuest software (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Basophils were identified on the basis of their forward and side-scatter properties and the absence of CD3 expression and presence of CRTH2 expression (Figure 1). For the assessment of allergen-specific basophil activation, 100 µg/mL skim milk (Difco, Becton Dickinson, Madrid, Spain) and 100 µg/mL pHF were used. Based on previously published data, non-responder status was defined as an anti-IgE-induced CD203c expression level of <10%.¹⁴ Data were acquired

Table 2. Symptom score used by Astier et al¹³ to evaluate clinical reactions in this study

Symptom score	Symptoms
0	No symptoms
1	Abdominal pain that resolved without medical treatment, rhinoconjunctivitis or urticaria with <10 papulas, or rash
2	One organ involved, abdominal pain requiring treatment, generalised urticaria, non-laryngeal angioedema, or mild asthma (cough)
3	Two organs involved
4	Three organs involved, laryngeal oedema, hypotension, or asthma requiring treatment
5	Cardiac and respiratory symptoms requiring hospitalisation in the intensive care unit

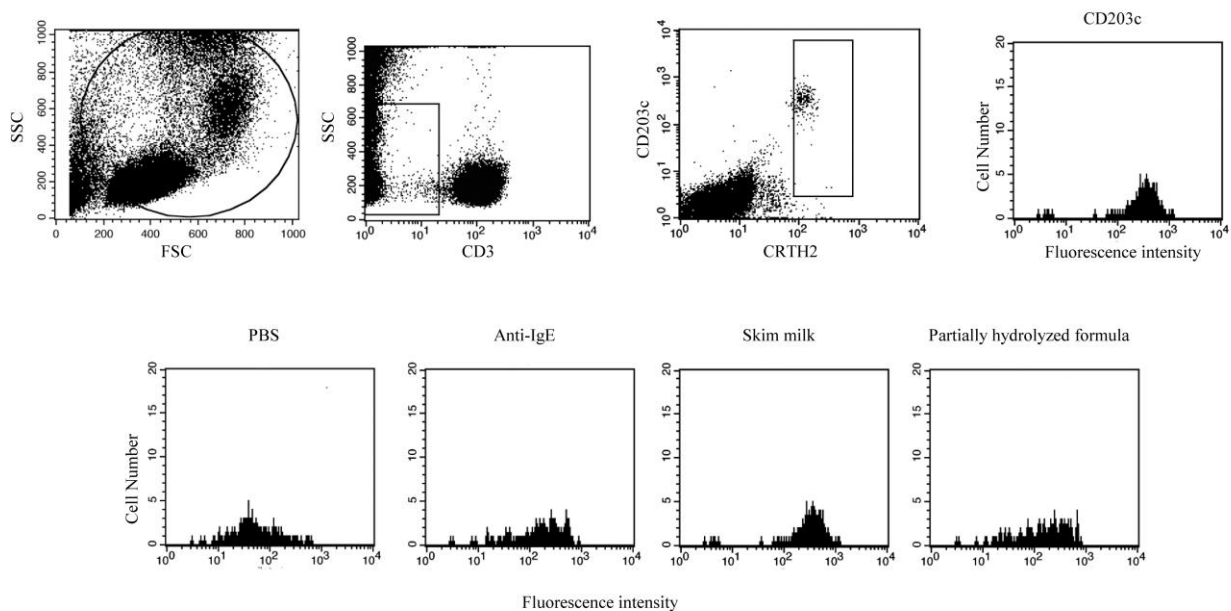


Figure 1. Example of optimal basophil gating for the CD203c protocol. Basophils were detected on the basis of the forward and side-scatter characteristics, negative CD3 expression, and positive CRTH2 expression. CD203c expression was then measured in these gated cells. SSC: side-scatter characteristics; pHF: partially hydrolysed cow's milk-based formula; eHF: extensively hydrolysed cow's milk-based formula; rCMF: regular cow's milk formula.

Table 3. Characteristics of participants with cow's milk protein allergy

ID no.	Age (years, months)	Sex	Total IgE (IU/mL)	Cow's milk-specific IgE (U _A /mL)	CD203c + basophil (%)	
					Skim milk	Partially hydrolysed formula
1	2y0m	M	527	169	NR	NR
2	5y5m	M	251	38.7	78.9	89.4
3	5y6m	F	120	11.9	4.11	7.20
4	3y3m	M	6020	78.6	55.3	26.1
5	4y3m	M	3220	224	79.5	60.4
6	4y1m	M	475	45.4	3.23	7.49
7	6y3m	M	508	3.34	52.1	3.22
8	4y0m	F	279	11.3	NR	4.02
9	4y8m	M	402	14.5	62.4	13.6
10	1y11m	M	1610	56.6	6.67	4.39
11	4y3m	M	139	3.49	NR	NR
12	3y2m	F	168	9.49	21.1	4.88
13	8y8m	F	79.4	3.11	40.8	2.47
14	5y3m	F	607	99.7	24.1	28.5
15	8y8m	F	1520	84.3	61.1	31.1
16	5y10m	F	1676	735	6.00	7.45
17	2y1m	M	369	13.1	39.9	19.3
18	4y1m	M	785	31.9	77.9	15.7
19	1y8m	M	83.7	1.16	43.5	5.14
20	9y7m	M	4401	43.8	91.1	16.6
21	7y11m	M	201	6.41	69.0	23.5
22	3y10m	M	880	106	5.21	4.94
23	4y10m	M	143	9.59	NA	NA
24	3y0m	M	219	5.87	64.0	2.75
25	2y10m	M	4148	476	NR	NR

F: Female; IgE: immunoglobulin E; M: male

for 500 basophils, and samples with <200 cells were excluded.

Statistical analysis

According to a previous study using pHF,¹⁵ power calculation in this study was based on the aim of detecting a difference of 50% between groups in the percentage of tolerance. Assuming a two-tailed alpha of 0.016, a sample size of 22 participants for each formula was needed to achieve a power of at least 80%.

The primary outcome was the rate of tolerance against 20.0 mL of formula. Tolerance was defined as ingestion of the entire amount of 20.0 mL of formula without the development of allergic symptoms. Rates were analysed using the Fisher's exact test. Threshold doses and allergic symptom scores were compared using the Friedman test. We used the Bonferroni correction to account for multiple comparisons (significance at *p* values of <0.016). The results for non-normal continuous variables are presented as medians and interquartile ranges. If a child presented with an allergic reaction after the first intake, the threshold was considered to be 0.0 mL. Basophil activation test results were compared using Wilcoxon matched-pair signed rank tests. Differences with *p* values of <0.05 were considered significant. The correlation between IgE, basophil activation, and threshold of food challenge test was analysed using the Spearman correlation test (significance at *p* values of <0.05). All statistical analyses were performed using GraphPad Prism 6.01 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Food challenge outcomes

In total, 25 children were enrolled in this study, including 18 boys and 7 girls with a median age of 4.25 years (range: 1–9 years) and a median milk-specific IgE level of 31.9 UA/mL (range: 1.16–735 UA/mL). Specific data of each child is shown in Table 3. Of the 25 enrolled children, 24 received one formula of each of the three formulas on the three test days, separated by 1-week intervals. One child refused to drink the formula during the first food challenge and was excluded from the analysis.

The details of food challenge tests are shown in Table 4. The tolerance rate ratios for the rCMF were lower than those for the pHF (2 vs 16; *p*<0.01) and eHF (2 vs 22; *p*<0.01). However, this rate was not significantly different between the eHF and pHF (*p*=0.07). The threshold doses of the pHF (median: 20.0 mL; 95% confidence interval [95% CI]: 11.0–17.9) and eHF (median: 20.0 mL; 95% CI: 17.2–20.5) were significantly higher than those of the rCMF (median: 2.90 mL; 95% CI: 2.50–7.34; *p*<0.01 and *p*<0.01, respectively; Figure 2a). Additionally, we found no significant difference between the thresholds for the pHF and eHF. The allergic symptom scores for the pHF (median: 0.00; 95% CI: 0.218–1.17) and eHF (median: 0.00; 95% CI: -0.0753–0.423) were significantly lower than those of rCMF (median: 2.00; 95% CI: 1.34–2.14; *p*=0.01 and *p*<0.01, respectively; Figure 2b), and the pHF and eHF scores were not significantly different. One participant was required to receive an intramuscular adrenaline injection against symptoms induced by ingestion of regular cow's milk formula. None of the symptoms in-

Table 4. Food Challenge Results

ID no.	Partially hydrolysed formula				Extensively hydrolysed formula				Regular cow's milk formula			
	TH (mL)	Sxs	Tx	Sx score	TH (mL)	Sxs	Tx	Sx score	TH (mL)	Sxs	Tx	Sx score
1	0.5	U, CO		2	20			0	0.35	U	AH	1
2	8.5	CO		2	20			0	8.5	U, CO, WH	B, AH	2
3	20			0	20			0	1.5	CO	B, AH	2
4	20			0	20			0	8.5	U	AH	1
5	4	U		1	20			0	4	U, CO	B, AH	3
6	4	U, CO		3	20			0	1.8	CO, WH	B, AH	3
7	20			0	20			0	20			0
8	20			0	20			0	4	U, CO, WH	B, AH	2
9	20			0	20			0	20			0
10	20			0	20			0	1.8	U	AH	1
11	20			0	20			0	9	U, CO, WH	B, AH	2
12	4	U	AH	1	20			0		U	AH	1
13	20			0	20			0		U	AH	1
14	1.8	U	AH	1	4	U, CO	B, AH	2		U	AH	1
15	20			0	20			0	9	U, CO, WH	B, AH, S, AD	4
16	1.8	U, CO, WH, AB	B, AH	3	9	CO, WH	B, AH	2	0.8	AB, U, CO	B, AH	3
17	1.8	CO, AB	B, AH	3	20			0		U, CO		2
18	20			0	20			0	0.8	CO	B, AH	2
19	20			0	20			0	0.8	U	AH	1
20	20			0	20			0	4	U		2
21	20			0	20			0	4	U, CO, AB	B, AH	2
22	20			0	20			0	9	U, CO, WH	B, AH	2
23	20			0	20			0	1.8	AB		1
24	20			0	20			0	8.5	U	AH	1
Median	20.0			0.00	20.0			0.00	4.00			2.00

AB: abdominal pain; AD: intramuscular adrenaline; AH: antihistamine drug; B: bronchodilator; CO: cough; S: systemic steroid; Sx: symptom; TH: threshold; Tx: treatment; U: urticaria; WH: wheeze.

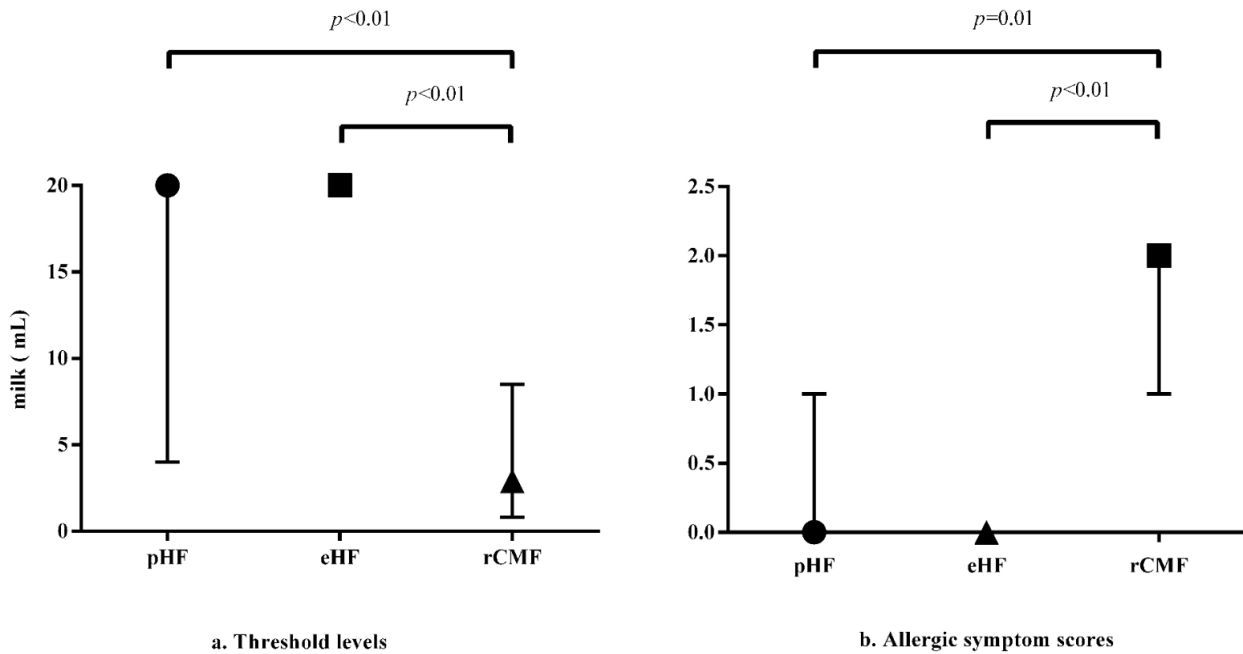


Figure 2. Food challenge results. The median values and interquartile ranges for (a) the threshold levels and (b) the allergic symptom scores are shown. Comparisons of the threshold levels and symptom scores between the formulas were analysed using the Friedman test. pHF: partially hydrolysed cow's milk-based formula; eHF: extensively hydrolysed cow's milk-based formula; rCMF: regular cow's milk formula.

duced by the pHF or eHF required an intramuscular adrenaline injection. Although the symptom scores for two participants were higher after pHF ingestion than scores after rCMF ingestion, the thresholds for the pHF were higher than those for the rCMF in both children. Two children could ingest all doses of the three formulas without symptoms.

Basophil responsiveness to in vitro stimulation with skimmed milk and pHF

Basophil activation after in vitro stimulation with pHF (median: 10.5%; 95% CI: 8.50–28.9) was significantly lower than that after stimulation with skimmed milk (median: 47.8%; 95% CI: 30.6–58.0; $p < 0.01$; Figure 3). Six children were defined as non-responders.

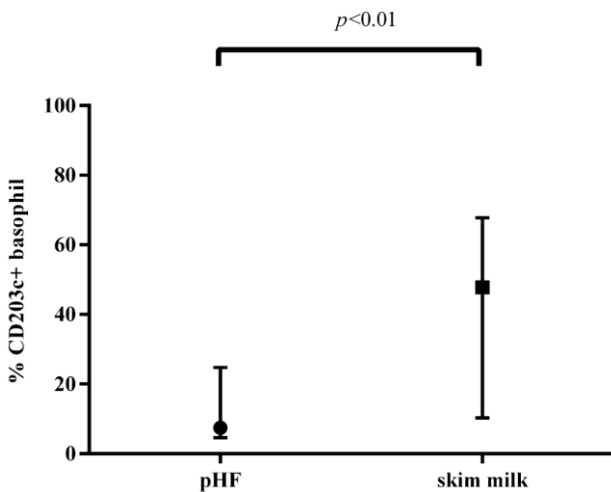


Figure 3. Allergen-specific basophil activation results. Basophil activation by a partially hydrolysed cow's milk-based formula was significantly lower than that by skim milk (median values: 10.5 vs 47.8, $p < 0.01$). Statistical comparisons were performed using the Wilcoxon matched pairs signed rank test.

Correlation between IgE or basophil activation, and threshold of food challenge test

The cow's milk-specific IgE level was significantly correlated with the threshold of food challenge using pHF ($r = -0.431$, $p = 0.040$) and eHF ($r = -0.438$, $p = 0.036$), and was not significantly correlated with the threshold of food challenge using rCMF ($r = 0.048$, $p = 0.827$). Basophil activation after in vitro stimulation with pHF was not significantly correlated with the threshold of food challenge using pHF ($r = -0.370$, $p = 0.099$), eHF ($r = -0.171$, $p = 0.459$) and rCMF ($r = 0.095$, $p = 0.682$).

DISCUSSION

The results of the present study demonstrate that children with CMA can ingest greater amounts of pHF and eHF than they can ingest rCMF. Fourteen participants (63%) with confirmed CMA drank 20 mL of the pHF without developing any allergic symptoms. Furthermore, the results show that, compared to rCMF intake, pHF and eHF intake induce milder allergic symptoms. We found no significant differences between the pHF and eHF in the rate of tolerance or induction of allergic symptoms.

Several reports demonstrated that approximately 27.3–100.0% children with CMA react to partially hydrolysed formula.^{15–18} The median age of participants in these reports ranged from 2–4 years old, which is younger than the median age of children in the present study. There is no absolute demarcation between the eHF and pHF in terms of the degree of hydrolysis; eHFs generally contain amino acids and peptides with molecular weights $< 3,000$ Da, while pHFs generally contain larger peptides with molecular weights of approximately 5,000 Da.¹⁹ Various hydrolysed cow's milk-based formulas are available, and differences in their hydrolysed components might elicit different allergic reactions.²⁰ Kido et al¹⁵ reported that 40 of 55 children (72.7%) with CMA could ingest the pHF used in the present study without any adverse reaction in

an open food challenge. The participants (median age, 17 months; interquartile range, 8–37 months) with CMA in that report could not tolerate 150 mL of rCMF, but compared with the participants in this study, who could not tolerate 20 mL of rCMF, they might have had milder allergic symptoms after rCMF intake. Furthermore, the age of participants in that study was younger than the median age of participants in the present study. As most young children with CMA tend to outgrow CMA,¹ their CMA might be milder than that of older children. Therefore, compared to the results of Kido et al,¹⁵ the rates of tolerance of the pHF in the present study were lower. Furthermore, these past tolerability reports for hydrolysed formulas have not shown a significant difference in the endurable amount of formula or the extent of allergic symptoms.

The basophil activation test has attracted attention for its utility in assessing immediate allergic responses, including food allergy, as well as for diagnosing desensitisation.^{14,21} A previous study on a mouse model showed that, compared to the rCMF, the pHF used in the present study induced a lower level of basophil activation.¹⁰ However, there have been no reported measurements of basophil activation in response to the pHF in patients with CMA. Some reports have previously shown that pHF had a lower response in the skin prick test compared to that of regular cow's milk formula among children with CMA.^{18,22} We found that basophil activation against the pHF in children with CMA was low; this was the same tendency as that observed in mice in the previous study. However, the basophil activation against the pHF was not significantly correlated with the threshold of food challenge using pHF. Furthermore, we did not evaluate basophil activation against eHF. We plan to compare the reactivity against various formulas using basophil activation, skin prick test and Enzyme-Linked Immunosorbent Assay in a future study.

We used the scoring system reported by Astier et al.¹³ Although this scoring system has been reported among several studies,^{23,24} no validated scoring system to quantify the severity of a clinical response induced by intake of allergen. Other scoring systems should also be performed to quantify the responses induced by hydrolysed formulas. A recent study evaluated the results of food challenge test using both the scoring system by Astier et al and the scoring system developed by van der Zee et al^{25,26}; that study showed that, as symptoms increased, the severe end of the range was reached more quickly with the Astier et al. scoring system than with the van der Zee et al. system. In the present study, although pHF and eHF intake induce milder allergic symptoms, other systems should evaluate the result of the food challenge with hydrolysed formulas.

Five participants showed moderate allergic symptoms after ingesting the pHF, and two participants showed symptoms after eHF ingestion. The median milk-specific IgE level of these participants was 72.6 UA/mL, which was higher than that of other participants. The cow's milk-specific IgE level was significantly correlated with the threshold of food challenge using pHF and eHF. Therefore, food challenge tests in infants with high milk-specific IgE levels should be conducted with caution. A

lower-allergenic pHF should be selected and ingested initially under the observation of a doctor.

This study had some limitations. First, the total formula amounts for the food challenge were low (20 mL). Therefore, we are unable to rule out the possibility that some children develop allergic symptoms when they ingest more than that amount. However, we enrolled children with a history of systemic symptoms induced by small amounts of milk allergen ingestion. The Adverse Reactions to Food Committee of the American Academy of Allergy, Asthma & Immunology stated that the most sensitive patients may react at the first 10–100 mg dose of the challenge food, and in these patients, low-dose challenges should be considered.²⁷ Although some children could ingest much more formula than the maximum administered amount, food challenges using large amounts of formula pose a risk of inducing anaphylaxis, including anaphylactic shock. To evaluate the threshold of formulas, each ingestion dose of should be small. For example, a previous pHF food challenge trial among 10 participants with CMA showed that half of the participants reacted after the first ingestion of 15 mL of pHF.¹⁸ Secondly, the sample size of this study was small. Power calculation was based on the aim of being able to detect a difference of 50% between groups in the percentage of tolerance. Assuming a two-tailed alpha of 0.016, a sample size of 22 participants was needed to achieve a power of at least 80%. Differences in symptom scores might be found by studying larger cohorts.

In conclusion, compared to rCMF, the partially and extensively hydrolysed whey and casein formulas evaluated in this study are better tolerated and safer for children with CMA. Although further confirmation from additional centres is needed, our findings support the use of pHF in patients with mild CMA. Some children with CMA react to hydrolysed formulas; therefore, food challenge tests in these children should be undertaken with caution.

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AUTHOR DISCLOSURES

TM and HI are employees of Morinaga Milk Industry Co., Ltd., who supplied the hydrolysed formulas used for the food challenge tests. They only analysed formulas of the present study. Neither played any role in conducting the trial, analysing the data, writing the manuscript, or in the decision to submit the manuscript for publication. The other authors declare no conflict of interests associated with this manuscript.

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REFERENCES

1. Fiocchi A, Brozek J, Schunemann H, Bahna SL, von Berg A, Beyer K et al. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guidelines. *Pediatr Allergy Immunol.* 2010;21(Suppl 21):1-125. doi: 10.1111/j.1399-3038.2010.01068.x.
2. Indinnimeo L, Baldini L, De Vittori V, Zicari AM, De Castro G, Tancredi G, Lais G, Duse M. Duration of a cow-milk exclusion diet worsens parents' perception of quality of

- life in children with food allergies. *BMC Pediatr.* 2013;13:203. doi: 10.1186/1471-2431-13-203.
3. Koletzko S, Niggemann B, Arato A, Dias JA, Heuschkel R, Husby S et al. Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI Committee practical guidelines. *J Pediatr Gastroenterol Nutr.* 2012;55:221-9. doi: 10.1097/MPG.0b013e31825c9482.
 4. Pedrosa M, Pascual CY, Larco JI, Esteban MM. Palatability of hydrolysates and Other substitution formulas for cow's milk-allergic children: a comparative study of taste, smell, and texture evaluated by healthy volunteers. *J Investig Allergol Clin Immunol.* 2006;16:351-6.
 5. Black RE, Williams SM, Jones IE, Goulding A. Children who avoid drinking cow milk have low dietary calcium intakes and poor bone health. *Am J Clin Nutr.* 2002;76:675-80.
 6. Isolauri E, Sutas Y, Salo MK, Isosomppi R, Kaila M. Elimination diet in cow's milk allergy: risk for impaired growth in young children. *J. Pediatr.* 1998;132:1004-9.
 7. Price CE, Rona RJ, Chinn S. Height of primary school children and parents' perceptions of food intolerance. *Br Med J (Clin Res Ed).* 1988;296:1696-9.
 8. Kim JS, Nowak-Wegrzyn A, Sicherer SH, Noone S, Moshier EL, Sampson HA. Dietary baked milk accelerates the resolution of cow's milk allergy in children. *J Allergy Clin Immunol.* 2011;128:125-31 e2. doi: 10.1016/j.jaci.2011.04.036.
 9. Ellis MH, Short JA, Heiner DC. Anaphylaxis after ingestion of a recently introduced hydrolyzed whey protein formula. *J Pediatr.* 1991;118:74-7.
 10. Iwamoto H, Matsubara T, Nakazato Y, Namba K, Takeda Y. Evaluation of the antigenicity of hydrolyzed cow's milk protein formulas using the mouse basophil activation test. *Toxicol Lett.* 2016;242:53-9. doi: 10.1016/j.toxlet.2015.11.024.
 11. van Esch BC, Knipping K, Jeurink P, van der Heide S, Dubois AE, Willemsen LE, Garssen J, Knippels LM. In vivo and in vitro evaluation of the residual allergenicity of partially hydrolysed infant formulas. *Toxicol Lett.* 2011;201:264-9. doi: 10.1016/j.toxlet.2011.01.010.
 12. Komata T, Soderstrom L, Borres MP, Tachimoto H, Ebisawa M. The predictive relationship of food-specific serum IgE concentrations to challenge outcomes for egg and milk varies by patient age. *J Allergy Clin Immunol.* 2007;119:1272-4. doi: 10.1016/j.jaci.2007.01.038.
 13. Astier C, Morisset M, Roitel O, Codreanu F, Jacquenet S, Franck P, et al. Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. *J Allergy Clin Immunol.* 2006;118:250-6. doi: 10.1016/j.jaci.2006.04.053.
 14. Ciepela O, Zwiasek J, Zawadzka-Krajewska A, Kotula I, Kulus M, Demkow U. Basophil activation test based on the expression of CD203c in the diagnostics of cow milk allergy in children. *Eur J Med Res.* 2010;15(Suppl 2):21-6.
 15. Kido J, Nishi N, Sakaguchi M, Matsumoto T. Most cases of cow's milk allergy are able to ingest a partially hydrolyzed formula. *Ann Allergy Asthma Immunol.* 2015;115:33031 e2. doi: 10.1016/j.anaai.2015.07.013.
 16. Giampietro PG, Kjellman NI, Oldaeus G, Wouters-Wesseling W, Businco L. Hypoallergenicity of an extensively hydrolyzed whey formula. *Pediatr Allergy Immunol.* 2001;12:83-6.
 17. Caffarelli C, Plebani A, Poiesi C, Petroccione T, Spattini A, Cavagni G. Determination of allergenicity to three cow's milk hydrolysates and an amino acid-derived formula in children with cow's milk allergy. *Clin Exp Allergy.* 2002;32:74-9.
 18. Egan M, Lee T, Andrade J, Grishina G, Mishoe M, Gimenez G, Sampson HA, Bunyavanich S. Partially hydrolyzed whey formula intolerance in cow's milk allergic patients. *Pediatr Allergy Immunol.* 2017;28:401-5. doi: 10.1111/pai.12718.
 19. Greer FR, Sicherer SH, Burks AW. Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. *Pediatrics.* 2008;121:183-91. doi: 10.1542/peds.2007-3022.
 20. Fritsche R. Animal models in food allergy: assessment of allergenicity and preventive activity of infant formulas. *Toxicol Lett.* 2003;140-141:303-9.
 21. Sato S, Tachimoto H, Shukuya A, Kurosaka N, Yanagida N, Utsunomiya T et al. Basophil activation marker CD203c is useful in the diagnosis of hen's egg and cow's milk allergies in children. *Int Arch Allergy Immunol.* 2010;152(Suppl 1):54-61. doi: 10.1159/000312126.
 22. Oldaeus G, Bradley CK, Bjorksten B, Kjellman NI. Allergenicity screening of "hypoallergenic" milk-based formulas. *J Allergy Clin Immunol.* 1992;90:133-5.
 23. Glaumann S, Nopp A, Johansson SG, Borres MP, Nilsson C. Oral peanut challenge identifies an allergy but the peanut allergen threshold sensitivity is not reproducible. *PLoS One.* 2013;8:e53465. doi: 10.1371/journal.pone.0053465.
 24. Dolle S, Lehmann K, Schwarz D, Weckwert W, Scheler C, George E, Franken P, Worm M. Allergenic activity of different tomato cultivars in tomato allergic subjects. *Clin Exp Allergy.* 2011;41:1643-52. doi: 10.1111/j.1365-2222.2011.03841.x.
 25. van der Zee T, Dubois A, Kerkhof M, van der Heide S, Vlieg-Boerstra B. The eliciting dose of peanut in double-blind, placebo-controlled food challenges decreases with increasing age and specific IgE level in children and young adults. *J Allergy Clin Immunol.* 2011;128:1031-6. doi: 10.1016/j.jaci.2011.07.050.
 26. Pettersson ME, Koppelman GH, Flokstra-de Blok BMJ, Kollen BJ, Dubois AEJ. Prediction of the severity of allergic reactions to foods. *Allergy.* 2018;73:1532-40. doi: 10.1111/all.13423.
 27. Nowak-Wegrzyn A, Assa'ad AH, Bahna SL, Bock SA, Sicherer SH, Teuber SS. Work Group report: oral food challenge testing. *J Allergy Clin Immunol.* 2009;123:S365-83. doi: 10.1016/j.jaci.2009.03.042.