

Effect of a partially hydrolyzed whey infant formula at weaning on risk of allergic disease in high-risk children: A randomized controlled trial

Adrian J. Lowe, PhD,^{a,b} Clifford S. Hosking, FRACP,^c Catherine M. Bennett, PhD,^a Katrina J. Allen, PhD,^b Christine Axelrad, RN,^b John B. Carlin, PhD,^{a,b} Michael J. Abramson, PhD,^d Shyamali C. Dharmage, PhD,^a and David J. Hill, FRACP^b Melbourne and Newcastle, Australia

Background: Partially hydrolyzed whey formula (pHWF) has been recommended for infants with a family history of allergic disease at the cessation of exclusive breast-feeding to promote oral tolerance and prevent allergic diseases.

Objective: To determine whether feeding infants pHWF reduces their risk of allergic disease.

Methods: A single-blind (participant) randomized controlled trial was conducted to compare allergic outcomes between infants fed a conventional cow's milk formula, a pHWF, or a soy formula. Before birth, 620 infants with a family history of allergic disease were recruited and randomized to receive the allocated formula at cessation of breast-feeding. Skin prick tests to 6 common allergens (milk, egg, peanut, dust mite, rye grass, and cat dander) were performed at 6, 12, and 24 months. The primary outcome was development of allergic manifestations (eczema and food reactions) measured 18 times in the first 2 years of life.

Results: Follow-up was complete for 93% (575/620) at 2 years and 80% (495/620) at 6 or 7 years of age. There was no evidence that infants allocated to the pHWF (odds ratio, 1.21; 95% CI, 0.81-1.80) or the soy formula (odds ratio, 1.26; 95% CI, 0.84-1.88) were at a lower risk of allergic manifestations in infancy compared with conventional formula. There was also no evidence of reduced risk of skin prick test reactivity or childhood allergic disease.

Conclusion: Despite current dietary guidelines, we found no evidence to support recommending the use of pHWF at weaning for the prevention of allergic disease in high-risk infants.

(*J Allergy Clin Immunol* 2011;■■■■:■■■■-■■■■.)

Key words: Allergy prevention, infant formulas, partially hydrolyzed whey formula, conventional cow's milk formulas, eczema, asthma, allergic rhinitis, randomized control trial

Partially hydrolyzed whey formulas (pHWFs) have been widely recommended to prevent the development of allergic diseases in early childhood.¹⁻⁶ If beneficial, the use of pHWF is an attractive preventive strategy, because pHWFs are relatively inexpensive to manufacture. These formulas contain smaller, less immunogenic milk protein-derived peptides⁷ of reduced allergenicity that potentially enhance induction of tolerance to cow's milk protein.^{8,9}

The widespread support for the use of pHWF appears to be based on the results of a Cochrane review that found "a significant reduction in infant allergy" (p 11) to be associated with prolonged feeding with pHWF compared with feeding with conventional cow's milk formula (CMF).¹⁰ Despite the authors' caution that further studies were required, this meta-analysis has been widely used to underpin many clinical guidelines in Europe, the United States, and Australia.^{1-6,11} A major problem with meta-analyses is that often only published reports are analyzed.¹² These are more likely to be positive studies because of publication bias, leading the review to overestimate the effectiveness of a treatment.¹³ Publication bias may have affected the results of the Cochrane review on the value of pHWF in preventing allergic disease.¹⁰ There is some evidence of asymmetry in the funnel plot¹⁴ generated for the meta-analysis reported within the Cochrane review¹⁰ (Harbord $P = .06$ ¹⁴), with the smaller studies tending to report stronger protective effects of the pHWF than the larger studies. The German Infant Nutritional Intervention Study (GINI),¹⁵ the largest in this field, reported that pHWF reduced the incidence of eczema in early childhood in a per-protocol analysis that excluded children exclusively breast-fed to 4 months of age. However, an intention-to-treat (ITT) analysis failed to show any benefit of pHWF compared with conventional CMF.¹⁶

The primary aim of the current study was to determine whether the use of a pHWF reduced the incidence of allergic manifestations (eczema and food reactions) up to 2 years of age in high-risk infants compared with a conventional CMF. We also report results

From ^athe Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, School of Population Health, University of Melbourne; ^bthe Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne; ^cthe Department of Paediatrics, John Hunter Children's Hospital, Newcastle; and ^dthe Department of Epidemiology and Preventive Medicine, Monash University, Melbourne.

Nestec Ltd, a subsidiary of Nestlé Australia, provided the study formula and staff funding for the first 6 years of the study.

Disclosure of potential conflict of interest: A. J. Lowe has received research support from Dairy Australia. K. J. Allen has received research support from Wyeth and Nutricia. M. J. Abramson has received research support from the National Health and Medical Research Council. D. J. Hill has received research support from Nestlé Australia, SHS International, and Nutricia. The rest of the authors have declared that they have no conflict of interest.

Editor's note: Following acceptance of this manuscript, several inadvertent errors in data transcription were identified. The authors have made the necessary revisions. This process has resulted in a delay between the paper's original acceptance date and its publication date.

Received for publication January 11, 2010; revised May 3, 2010; accepted for publication May 3, 2010.

Reprint requests: David J. Hill, FRACP, Senior Consultant Allergist, Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Victoria 3053, Australia. E-mail: davidjhill@bigpond.com.

0091-6749/\$36.00

© 2011 American Academy of Allergy, Asthma & Immunology
doi:10.1016/j.jaci.2010.05.006

Abbreviations used

CMF: Cow's milk formula
 GINI: German Infant Nutritional Intervention Study
 ITT: Intention to treat
 MACS: Melbourne Atopy Cohort Study
 OR: Odds ratio
 pHWF: Partially hydrolyzed whey formula
 SPT: Skin prick test

from a third comparison group in which infants received a soy formula.

METHODS**Inclusion and exclusion criteria**

Between 1990 and 1994 expectant mothers attending the Mercy Maternity Hospital, Melbourne, Australia, were invited to participate in a study of the effect of modification of the infant diet on the risk of infant allergy. Mother-baby pairs were enrolled if the unborn child had a first-degree relative with a history of eczema, asthma, allergic rhinitis, or food allergy. Information leaflets and posters outlined the project's aim. Nurse research staff assessed eligibility and enrolled participants. This study was approved by the Mercy Maternity Hospital Ethics Committee, and all mothers provided written informed consent.

Intervention

There were 2 intervention formulas: a soy-based formula (ProSobee; Mead Johnson Nutrition/Bristol Myers, Melbourne, Australia) and a pHWF (NAN HA; Nestlé, Biessenhoffen, Germany). The control formula was a CMF (NAN; Nestlé, Tongala, Australia). In accordance with World Health Organization guidelines,¹⁷ mothers were encouraged to initiate and maintain breast-feeding for at least 6 months. Study formulas were introduced only at cessation, or partial cessation, of breast-feeding or as a breast milk substitute if breast-feeding was not intended.

Trial design

The trial was registered (retrospectively) with the Australian and New Zealand Clinical Trials Registry (ACTRN12609000734268). The trial commenced before the pHWF was available. The first 97 infants were randomized to either the CMF or soy study groups. When the pHWF became available, a new random allocation series was generated with a higher proportion allocated to the pHWF to obtain equal numbers in each formula group. An independent statistician created each of the computer generated allocation schedules. The random allocation list, containing the coded allocations, was available to research staff. Staff were blind to these allocation codes and to the group of allocation at the time of outcome assessment. Mother-baby pairs were allocated to the next sequential number as they were enrolled in the study and were assigned to the formula code allocated to that number. The cans of formula were labeled at an independent location. Parents of participants were informed of the identity of the assigned formula only after the child's second birthday.

Introduction of rice cereal, pureed apple, and pear was recommended from 4 months of age, and vegetables and other fruit from 6 months. Meats were introduced from 8 months, and nonrice cereals from 9 months. Dairy products, egg, fish, peanut, and nuts were avoided until 12 months of age.

Skin prick tests (SPTs) were performed at 6, 12, and 24 months according to a standard technique¹⁸ by 1 of 3 allergy-trained research nurses. Allergen extracts used were cow's milk, egg white, peanut, house dust mite, rye grass, and cat dander (Bayer, Spokane, Wash), and SPTs were read at 15 to 20 minutes.

Definitions

Outcomes up to 2 years of life, as assessed during 18 telephone interviews with parents (every 4 weeks until 64 weeks, then at 78 and 104 weeks), were defined as follows:

- **Eczema:** Doctor-diagnosed eczema or any rash that was treated with topical steroid preparation (excluding rash that only affected the scalp or nappy region).¹⁹
- **Food reaction:** Within 2 hours of ingesting that food, the child developed an acute skin rash (urticaria, angioedema, erythematous, or morbilliform), a flare of pre-existing eczema, signs of anaphylaxis, or vomiting.²⁰
- **Any allergic manifestation:** Presence of eczema or food reaction within the first 2 years of life.
- **Positive SPT:** A wheal of at least 3 mm (mean) diameter with a positive (histamine) control.

Childhood outcomes, based on parent report during telephone interviews conducted when children were age 6 or 7 years, were defined as follows:

- **Current childhood eczema:** Eczema diagnosed by the family physician in the previous 12 months.
- **Current childhood asthma:** Asthma diagnosed by the family physician in the previous 12 months.
- **Persistent childhood asthma:** Asthma diagnosed by the family physician in the previous 12 months on at least 2 occasions at the follow-up at 5, 6, or 7 years.
- **Current childhood allergic rhinitis:** One or more episodes of nasal discharge and/or congestion in the absence of an upper respiratory tract infection in the previous 12 months that either the family physician or parent attributed to allergic rhinitis (hay fever) and that was treated with an antihistamine and/or nasal steroid.²¹

Outcomes

The primary outcome was *any allergic manifestation* (cumulative incidence) up to 2 years of age. Secondary outcomes were the individual incidence of eczema and food reactions, reported in the first 2 years of life, and SPT reactivity at 6, 12, and 24 months. Additional secondary outcomes were the 2-year period prevalence of eczema, asthma, and allergic rhinitis at ages 6 and 7 years.

Sample size

A total of 176 infants per group were required to have 80% power to detect a 15% absolute difference in risk of allergic manifestation between the formula groups, assuming an α level of 0.05 and a 45% baseline risk of allergic disease within the first 2 years of life. Allowing for approximately a 15% dropout rate over the first 2 years of life, a total of 206 children per group were required.

Statistical methods

The primary analysis followed the ITT principle and compared the risk of *any allergic manifestation* between the allocated formula groups by using simple proportions and χ^2 tests. The estimated associations are presented as odds ratios (OR) with 95% CIs, with the CMF as the reference group.

Secondary analyses were also performed for the outcomes of sensitization to cow's milk and any allergen (assessed separately at 6, 12, and 24 months, and also combined by using logistic regression models, estimated by the generalized estimating equations approach) and childhood asthma, allergic rhinitis, and eczema at ages 6 and 7 years (again by using the generalized estimating equations approach).

A number of per-protocol analyses were performed. First, infants were excluded if they were exclusively breast-fed beyond 4 months of life. The 4-month cut-off period was selected to allow direct comparison of results with the GINI study.^{15,16} Second, a per-protocol analysis was performed including only those infants who had received some of the allocated formula by 4 months of age.

To determine whether the effect of the formula on risk of allergic disease varied between those with a family history of eczema (either the mother or father) and those without (neither the mother nor father),¹⁵ a stratified analysis was performed. Interaction effects were assessed by using Wald tests.

Adjusted associations were also estimated, to allow for any confounding due to chance imbalances at baseline. Adjustment was made for infant sex,

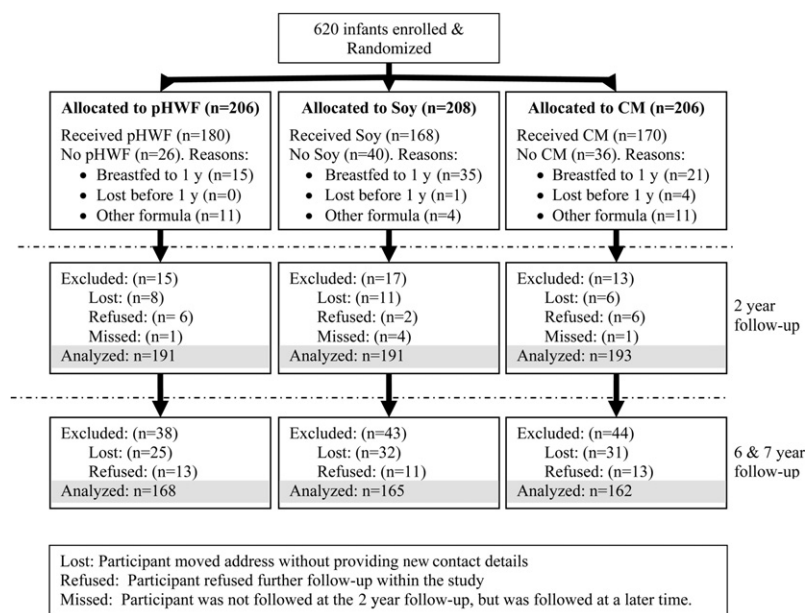


FIG 1. Flow chart of participation in the MACS. CM, Cow's milk formula.

parental smoking during pregnancy, and family history of allergic disease in all models. All statistical analysis was performed by A.J.L. using Stata statistical software (release 9.2; Stata Corp, College Station, Tex).

RESULTS

A total of 620 infants were recruited (Fig 1). Infants allocated to the CMF and pHWF groups were similar on baseline risk factors (see this article's Table E1 in the Online Repository at www.jacionline.org). Infants allocated to the soy formula had a higher proportion of parents with food allergy and siblings with allergic disease (Table E1). There were no differences between the groups in terms of duration of exclusive breast-feeding or age of introduction of solids (Table E1).

Approximately 50% of infants received some of the allocated formula by 4 months of age; 16.5% of infants never received their allocated formula because of either continuing breast-feeding (13.6%; $n = 78/575$) or using a nonallocated formula (2.9%; $n = 17/575$). There were no differences in rates of exposure to the allocated formula between the groups (Fig 2). The majority of mothers fully adhered to the study formula feeding protocol (breast-feeding and then weaning onto allocated formula with no other formula exposures) during the first 6 months of the child's life (91.2%, 86.9%, and 87.4% for the CMF, pHWF and soy groups, respectively) despite only 63% of children having been exposed to the allocated formula by this age. The rates of adherence declined by 12 months of age (75.7%, 69.1%, and 76.4%, respectively).

There were 575 (92.7%) infants followed until 24 months of age (Fig 1); 25 children were lost to follow-up (shifted residence without informing the study), 14 refused ongoing participation, and 6 children did not complete the 2-year follow-up but subsequently rejoined the study (Fig 1).

Primary outcome

Neither the pHWF nor the soy formula reduced the risk of allergic manifestations in the first 2 years of life (Table I).

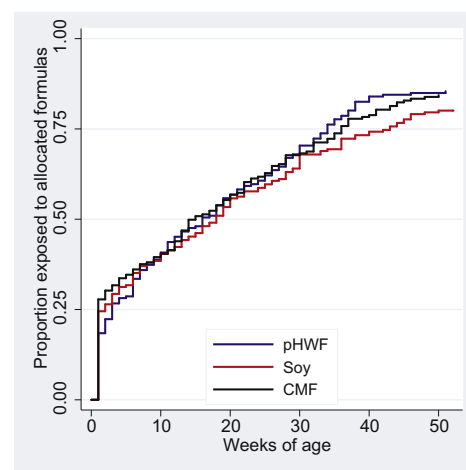


FIG 2. Proportion of infants exposed to the allocated formula from the time of birth (0 weeks) until 52 weeks of age.

Secondary outcomes in the first 2 years

There was no evidence of differences between the groups on any secondary clinical outcome (Table I) or SPT reactivity (Table II). Using a 2-mm mean wheal diameter to define a positive SPT did not change the overall pattern of results or the conclusion that there was no evidence of a difference between the groups. There were 19 (3.2%) children with large SPT (≥ 6 mm) wheals to cow's milk, consistent with IgE-mediated cow's milk allergy,²² and these children were evenly distributed between the groups (CMF, 6/197; pHWF, 4/196; and soy, 9/201).

Secondary outcomes at ages 6 and 7 years

Between 6 and 7 years of age, 80% (495/620) of children had a telephone interview. There were no differences between the groups in the rates of childhood eczema, asthma, or allergic rhinitis (Table I).

TABLE I. Unadjusted associations between allocated formula and risk of allergic disease

Outcome	Conventional formula (CMF)	Hydrolyzed formula (pHWF)		Soy formula	
	% (n/N)	% (n/N)	Crude OR (95% CI)	% (n/N)	Crude OR (95% CI)
Any allergic manifestation: 0-1 y (228/575)	37.3 (72/193)	37.7 (72/191)	1.02 (0.67-1.54)	44.0 (84/191)	1.32 (0.88-1.98)
Any allergic manifestation: 0-2 y (300/575)	48.7 (94/193)	53.4 (102/191)	1.21 (0.81-1.80)	54.5 (104/191)	1.26 (0.84-1.88)
Secondary outcomes					
Eczema within first 2 y	43.0 (83/193)	48.7 (93/191)	1.26 (0.84-1.88)	46.1 (88/191)	1.13 (0.76-1.69)
Food reactions within first 2 y					
Any food (92/575)	13.5 (26/193)	15.2 (29/191)	1.15 (0.65-2.04)	19.4 (37/191)	1.54 (0.89-2.67)
Cow's milk protein (17/575)	3.1 (6/193)	1.6 (3/191)	0.50 (0.12-2.02)	4.2 (8/191)	1.36 (0.46-4.00)
Cow's milk with + SPT to cow's milk (3/575)	0 (0/193)	0.5 (1/191)	NE	1 (2/191)	NE
Peanut with + SPT to peanut (1/575)	0.5 (1/193)	0 (0/191)	NE	0 (0/191)	NE
Egg with + SPT to egg (8/575)	1.0 (2/193)	0.5 (1/191)	0.50 (0.04-5.59)	2.6 (5/191)	2.57 (0.49-13.40)
Childhood outcomes (period prevalence at 6-7 y)					
Eczema (157/493)	31.5 (51/162)	33.5 (56/167)	1.08 (0.69-1.68)	30.5 (50/164)	0.95 (0.60-1.48)
Asthma (148/495)	32.1 (52/162)	28.0 (47/168)	0.91 (0.57-1.45)	29.7 (49/165)	0.97 (0.61-1.54)
Rhinitis (117/495)	22.2 (36/162)	22.0 (37/168)	0.94 (0.56-1.58)	26.7 (44/165)	1.27 (0.77-2.10)
Persistent asthma (120/494)	25.5 (41/161)	24.2 (40/165)	0.88 (0.53-1.46)	24.2 (40/165)	0.94 (0.57-1.55)

NE, OR not estimable.

TABLE II. Unadjusted associations between allocated formula and risk of positive SPT

Outcome	Conventional formula (CMF)	Hydrolyzed formula (pHWF)		Soy formula	
	% (n/N)	% (n/N)	Crude OR (95% CI)	% (n/N)	Crude OR (95% CI)
Positive SPT (any allergen) at					
6 mo (95/552)	16.9 (30/177)	18.3 (35/191)	1.10 (0.64-1.88)	16.3 (30/184)	0.95 (0.55-1.66)
12 mo (146/544)	29.2 (52/178)	25.0 (47/188)	0.81 (0.51-1.28)	26.4 (47/178)	0.87 (0.55-1.38)
2 y (136/449)	31.6 (50/158)	26.0 (38/146)	0.76 (0.46-1.25)	33.1 (48/145)	1.07 (0.66-1.73)
Repeated measures*	—	—	0.90 (0.61-1.33)	—	0.98 (0.67-1.44)
Positive SPT to cow's milk at					
6 mo (23/552)	5.1 (9/177)	4.2 (8/191)	0.82 (0.31-2.16)	3.3 (6/184)	0.63 (0.22-1.81)
12 mo (32/544)	5.1 (9/178)	5.9 (11/188)	1.17 (0.47-2.89)	6.7 (12/178)	1.36 (0.56-3.31)
2 y (16/449)	3.8 (6/158)	1.4 (2/146)	0.35 (0.07-1.77)	5.5 (8/145)	1.48 (0.50-4.37)
Repeated measures*	—	—	0.89 (0.40-1.99)	—	1.01 (0.44-2.30)

*These estimates are based on repeated measures (combining results from the 6, 12, and 24 month SPT using the generalized estimating equations approach), meaning it is not possible to report simple proportions.

Adjusted analysis

Adjustment for sex, parental smoking, and family history of allergic disease did not alter the associations between the allocated group and the risk of any allergic manifestation in the first 2 years of life or any of the secondary outcomes and did not change the interpretation of the results (see this article's [Table E2](#) in the Online Repository at www.jacionline.org).

Interactions with family history of eczema

There was no evidence that pHWF protected against the development of allergic manifestation in those children with or without a family history of eczema (see this article's [Table E3](#) in the Online Repository at www.jacionline.org; all *P* values for all interaction terms >.15).

Per-protocol analysis

None of the per-protocol analyses produced substantially different findings from the ITT analysis. Limiting the analysis to children whose parents were compliant with the study feeding protocol did not alter the study conclusions (primary outcome OR, 1.20; 95% CI, 0.75-1.93, for pHWF [*n* = 132 and 146]). Excluding infants exclusively breast-fed for more than 4 months

did not alter the results (OR, 1.22; 95% CI, 0.72-2.04, for pHWF [*n* = 110 and 121]). Similarly, including only infants who had consumed some of the allocated formula (OR, 1.16; 95% CI, 0.66-2.02 [*n* = 97 and 102]) or consumed the allocated formula for at least 2 weeks during the first 4 months of life (OR, 1.10; 95% CI, 0.59-2.04 [*n* = 82 and 80]) did not alter the results. Similar results were obtained when the analysis was limited to children who consumed the allocated formula for at least 4 (OR, 1.06; 95% CI, 0.55-2.03 [*n* = 73 and 73]) and 8 weeks (OR, 1.00; 95% CI, 0.48-2.09 [*n* = 62 and 55]) in the first 4 months of life. Limiting the analysis to children who consumed at least 100 mL per day for each of these durations produced similar results. Finally, limiting the analysis to children who were exposed to the allocated formula within the first 2 weeks of life again did not produce any evidence of benefit (OR, 0.91; 95% CI, 0.41-2.01 [*n* = 43 and 58]), although the reduced numbers limited the precision of this comparison. Similarly, interpretation for all secondary outcomes did not change in any of these analyses (data not shown).

DISCUSSION

This randomized controlled trial failed to show any beneficial effect of the pHWF for the prevention of any allergic disease

outcome up to 7 years of age in high-risk children compared with a conventional cow's milk-based formula.

This is the second largest trial to randomize individual infants to receive either pHWF or a conventional cow's milk formula. An ITT analysis of the largest study, the GINI study,^{15,16,23} also failed to demonstrate a clear benefit of pHWF over conventional formula for the outcomes of allergic manifestations and eczema up to 12 months, and childhood eczema, asthma, or allergic rhinitis (2-year period prevalence at 6 years).²³ Although 1 report from GINI showed some benefit for the cumulative prevalence of allergic manifestations up to 3 years of age (relative risk, 0.77; 95% CI, 0.61-0.98),²³ a previous analysis of the same outcome within GINI did not (population odds ratio, 0.94; 95% CI, 0.73-1.20).¹⁶ Most of the reported benefits of pHWF in GINI are from a per-protocol analysis in which children were excluded if they had not received the allocated formula within the first 4 months of life. It is well accepted that the main conclusions of a randomized controlled trial should be based on an ITT analysis because per-protocol analysis can bias the findings.²⁴

The Cochrane review and meta-analysis of 6 studies of this topic¹⁰ suggested a benefit of pHWF for the prevention of "any allergic manifestation in infancy" compared with conventional CMF (pooled OR, 0.73; 95% CI, 0.59-0.90). However, when our results are added to this pooled estimate, there is no longer evidence of a protective effect of pHWF for "any allergic disease in infancy" (pooled OR, 0.91; 95% CI, 0.79-1.05). The ITT analysis of the 2 largest studies in this area (GINI²³ and Melbourne Atopy Cohort Study [MACS]) failed to show any benefit of pHWF compared with conventional CMF, whereas studies with far fewer participants showed stronger protective effects of the pHWF.²⁵⁻²⁷ These conflicting results suggest that publication bias may have had an impact on this Cochrane review.¹⁰

Alternatively, the effect of pHWF may be influenced by previous breast-feeding. The studies that demonstrated a strong protective effect of pHWF were of a small number of children who received pHWF *without receiving any breastfeeding*.²⁵⁻²⁸ By contrast, studies including MACS that randomized a larger number of infants to pHWF *at weaning* from breast milk showed a much weaker effect.^{23,29} The negative findings of our study, and others that randomized before birth, may be a result of infants having less formula than those starting pHWF from birth. In addition, the effect of pHWF may be modified by previous breast-feeding; human breast milk contains a number of important immunologically active components³⁰ that may both modify induction of allergen tolerance³¹ and have an effect on the impact of pHWF on the risk of allergic disease. These possibilities might explain why the per-protocol (exposure within the first 4 months) analysis of the GINI study showed stronger evidence of a protective effect than the ITT, because the per-protocol analysis would have selected out those who were predominantly breast-fed. To resolve this issue conclusively, studies that randomize large numbers of infants to specific infant formula and preclude previous breast-feeding are required. However, this may be logistically difficult and potentially unethical given the current evidence on benefits of breast-feeding.

The importance of the first 4 months of life for dietary interventions to prevent allergic disease has been emphasized in the literature^{32,33} because this is believed to be the critical time of oral immune tolerance development. However, we were unable to show an impact of the pHWF even when we limited our analysis to those children exposed within this time frame. Similarly, including only infants with a significant exposure to the allocated

formula within the first 4 months of life did not reveal a protective effect of pHWF.

In contrast with the GINI study,¹⁵ we did not observe any difference in the effect of a pHWF between children with or without a family history of eczema. It is highly unlikely that pHWF has a differential effect on the basis of family history of eczema.

Our study has a number of important strengths. We have studied the effect of a pHWF on high-risk children until they were 7 years of age, when the diagnosis of asthma³⁴ and allergic rhinitis is clearer. Skin prick tests were performed on 3 occasions to cow's milk as well as 5 other common allergens. This allowed the assessment of a specific effect of pHWF on the risk of cow's milk sensitization as well as on atopic diseases. The rate of follow-up during early life was exceptional. The sample size in this study was sufficient to detect important differences between the formulas in allergy prevention. It was not designed to demonstrate equivalence.

The design of our study has some weaknesses. The allocation sequence was available to the research staff throughout the study. Therefore, the research staff would have known the *coded* group of allocation for the next participant to be enrolled. Despite this, examination of the enrollment into the study indicates that it was time-consecutive, and the staff members undertaking the distribution of formulas were blind to the formula codes. Thus selection bias and ascertainment bias were unlikely to influence the results of this study. In addition, we relied in part on parent-reported outcomes that have not been validated. However, none of the current definitions of eczema^{35,36} have been validated in children under the age of 2 years, although a standardized assessment at the time of SPTs within this study may have improved the measurement of this outcome. We have demonstrated good agreement between the International Study of Asthma and Allergies in Childhood definitions of eczema, asthma, and hay fever and those used in this study at age 6 to 7 years (all κ values $\geq .74$; unpublished data A. Lowe, December 2007).

This study tested the effect of a pHWF at weaning on the incidence of allergic manifestations. It does not provide information concerning the impact of exclusive feeding with pHWF nor other forms of partially or extensively hydrolyzed or amino acid-based formula.

Conclusion

There was no evidence that introducing pHWF at the cessation of breast-feeding reduced the risk of allergic manifestations, including eczema, asthma, and allergic rhinitis, in this study of high-risk infants. Our findings do not support the recommendation that pHWF should be used after breast-feeding as a preventive strategy for infants at high risk of allergic diseases.

We thank Dr John Thorburn, FRACP, for assistance in patient recruitment and administrative assistance and the Mercy Maternity Hospital Department of Obstetrics for participant recruitment. We thank Anne Balloch for assistance with data management and all of the MACS children and parents for their participation and ongoing support for this study.

Clinical implications: The authors found no evidence to support the use of pHWF at weaning for the prevention of allergic disease in infants with a family history of allergic disease.

REFERENCES

- Host A, Halken S. Primary prevention of food allergy in infants who are at risk. *Curr Opin Allergy Clin Immunol* 2005;5:255-9.

2. Prescott SL, Tang MLK. Australasian Society of Clinical Immunology and Allergy position statement: summary of allergy prevention in children. *Med J Aust* 2005;182:464-7.
3. Miniello VL, Francavilla R, Brunetti L, Franco C, Lauria B, Lieggi MS, et al. Primary allergy prevention: partially or extensively hydrolyzed infant formulas? *Minerva Pediatr* 2008;60:1437-43.
4. Hays T, Wood RA. A systematic review of the role of hydrolyzed infant formulas in allergy prevention. *Arch Pediatr Adolesc Med* 2005;159:810-6.
5. Heine RG, Tang ML. Dietary approaches to the prevention of food allergy. *Curr Opin Clin Nutr Metab Care* 2008;11:320-8.
6. Pali-Scholl I, Renz H, Jensen-Jarolim E. Update on allergies in pregnancy, lactation, and early childhood. *J Allergy Clin Immunol* 2009;123:1012-21.
7. Mäkinen-Kiljunen S, Sorva R. Bovine beta-lactoglobulin levels in hydrolysed protein formulas for infant feeding. *Clin Exp Allergy* 1993;23:287-91.
8. Fritsche R, Pahud JJ, Pecquet S, Pfeifer A. Induction of systemic immunologic tolerance to beta-lactoglobulin by oral administration of a whey protein hydrolysate. *J Allergy Clin Immunol* 1997;100:266-73.
9. Pecquet S, Bovetto L, Maynard F, Fritsche R. Peptides obtained by tryptic hydrolysis of bovine beta-lactoglobulin induce specific oral tolerance in mice. *J Allergy Clin Immunol* 2000;105:514-21.
10. Osborn DA, Sinn J. Formulas containing hydrolysed protein for prevention of allergy and food intolerance in infants. *Cochrane Database Syst Rev* 2006;4.
11. Grimshaw KE, Allen K, Edwards CA, Beyer K, Boulay A, van der Aa LB, et al. Infant feeding and allergy prevention: a review of current knowledge and recommendations: a EuroPrevall state of the art paper. *Allergy* 2009;64:1407-16.
12. Nieto A, Mazon A, Pamies R, Bruno L, Navarro M, Montanes A. Sublingual immunotherapy for allergic respiratory diseases: an evaluation of meta-analyses. *J Allergy Clin Immunol* 2009;124:157-61, e1-32.
13. Turner EH, Matthews AM, Linardatos E, Tell RA, Rosenthal R. Selective publication of antidepressant trials and its influence on apparent efficacy. *N Engl J Med* 2008;358:252-60.
14. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629-34.
15. von Berg A, Koletzko S, Grubl A, Filipiak-Pittroff B, Wichmann HE, Bauer CP, et al. The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. *J Allergy Clin Immunol* 2003;111:533-40.
16. von Berg A, Koletzko S, Filipiak-Pittroff B, Laubereau B, Grubl A, Wichmann HE, et al. Certain hydrolyzed formulas reduce the incidence of atopic dermatitis but not that of asthma: three-year results of the German Infant Nutritional Intervention Study. *J Allergy Clin Immunol* 2007;119:718-25.
17. WHO. Complementary feeding: report of the global consultation: summary of guiding principles. Geneva: World Health Organization; 2002.
18. Aas K, Belin L. Standardization of diagnostic work in allergy. *Int Arch Allergy Appl Immunol* 1973;45:57-60.
19. Lowe AJ, Abramson MJ, Hosking CS, Carlin JB, Bennett CM, Dharmage SC, et al. The temporal sequence of allergic sensitization and onset of infantile eczema. *Clin Exp Allergy* 2007;37:536-42.
20. Hill DJ, Firer MA, Shelton MJ, Hosking CS. Manifestations of milk allergy in infancy: clinical and immunologic findings. *J Pediatr* 1986;109:270-6.
21. Lowe AJ, Hosking CS, Bennett CM, Carlin JB, Abramson MJ, Hill DJ, et al. Skin prick test can identify eczematous infants at risk of asthma and allergic rhinitis. *Clin Exp Allergy* 2007;37:1624-31.
22. Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. *Clin Exp Allergy* 2000;30:1540-6.
23. von Berg A, Filipiak-Pittroff B, Kramer U, Link E, Bollrath C, Brockow I, et al. Preventive effect of hydrolyzed infant formulas persists until age 6 years: long-term results from the German Infant Nutritional Intervention Study (GINI). *J Allergy Clin Immunol* 2008;121:1442-7.
24. Piantadosi S. Clinical trials: a methodologic perspective. New York: Wiley-Interscience; 1997.
25. Marini A, Agosti M, Motta G, Mosca F. Effects of a dietary and environmental prevention programme on the incidence of allergic symptoms in high atopic risk infants: three years' follow-up. *Acta Paediatr Suppl* 1996;414:1-21.
26. Vandenplas Y, Hauser B, Van den Borre C, Clybourn C, Mahler T, Hachimi-Idrissi S, et al. The long-term effect of a partial whey hydrolysate formula on the prophylaxis of atopic disease. *Eur J Pediatr* 1995;154:488-94.
27. Lam BCC, Yeung CY. The effect of breast milk, infant formula and hypoallergenic formula on incidence of atopic manifestation in high risk infants. 6th Annual Scientific Meeting of Hong Kong College of Physicians and Hong Kong College of Paediatricians, Hong Kong 1992 (October).
28. de Seta L, Sian P, Cirillo G, DiGruttola M, Cimaduomo L, Coletta S. The prevention of allergic diseases with a hypoallergenic formula: a follow-up at 24 months: the preliminary results. *Pediatr Med Chir* 1994;16:251-4.
29. Willems R, Duchateau J, Magrez P, Denis R, Casimir G. Influence of hypoallergenic milk formula on the incidence of early allergic manifestations in infants predisposed to atopic diseases. *Ann Allergy* 1993;71:147-50.
30. Field CJ. The immunological components of human milk and their effect on immune development in infants. *J Nutr* 2005;135:1-4.
31. Verhasselt V, Milcent V, Cazareth J, Kanda A, Fleury S, Dombrowicz D, et al. Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. *Nat Med* 2008;14:170-5.
32. Host A, Halken S, Muraro A, Dreborg S, Niggemann B, Aalberse R, et al. Dietary prevention of allergic diseases in infants and small children. *Pediatr Allergy Immunol* 2008;19:1-4.
33. 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.
34. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995;332:133-8.
35. Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980;92:44-7.
36. Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. Working Party's diagnostic criteria for atopic dermatitis, III: independent hospital validation. *Br J Dermatol* 1994;131:406-16.

REFERENCE

- E1. McMillan J, Jones FL. The ANU3_2 scale: a revised occupational status scale for Australia. *J Sociol* 2000;36:64-80.

TABLE E1. Comparison of baseline factors between the allocated formula groups

Baseline factor	Hydrolyzed formula (pHWF) (n = 206)	Soy formula (n = 208)	Conventional formula (CMF) (n = 206)
Male infant	50.8%	51.0%	51.9%
Maternal history of allergic disease			
Asthma	42.9%	46.6%	40.3%
Eczema	38.5%	36.1%	42.2%
Hay fever	61.5%	59.1%	61.2%
Food allergy	37.4%	43.8%	34.5%
Paternal history of allergic disease			
Asthma	21.6%	27.1%	28.4%
Eczema	19.1%	18.4%	24.0%
Hay fever	47.5%	48.3%	42.6%
Food allergy	20.6%	28.5%	14.7%
Demographic factors			
Median maternal age (y) (IQR)	31 (29-34)	32 (29-34.5)	31 (28-34)
Median paternal age (y) (IQR)	33 (30-36)	33 (31-36)	32.5 (29.5-36.5)
Median maternal education (y) (IQR)	15 (12-15)	15 (12-15)	15 (11-15)
Median paternal education (y) (IQR)	15 (12-15)	15 (12-15)	15 (11-15)
Median SES of father's occupation (IQR)*	48.6 (34.0-61.9)	43.6 (29.2-62.6)	41.6 (27.2-61.9)
Home environment			
Owner-occupied home	84.0%	81.7%	80.1%
Any gas cooking	75.1%	73.2%	79.7%
Any gas heating	74.1%	66.8%	71.8%
Any pet	70.1%	70.8%	65.7%
Maternal smoking during pregnancy	7.8%	4.8%	10.2%
Paternal smoking during pregnancy	19.0%	16.4%	22.7%
Sibling factors			
No older siblings	43.2%	33.7%	43.7%
Any older sibling with food allergy	35.4%	50.5%	26.2%
Any older sibling with eczema	35.0%	46.6%	33.5%
Any older sibling with asthma	32.5%	45.7%	27.7%
Any older sibling with hay fever	19.5%	26.4%	19.4%
Early diet (wk)			
Median duration of exclusive breast-feeding (IQR)	14 (3-20)	15 (1-21)	13 (1-20)
Median duration of any breast-feeding (IQR)†	42 (22-60)	47 (17-64)	44 (24-60)
Median age of introduction to solid foods (IQR)	20 (18-22)	19 (16-24)	20 (17-22)

IQR, Interquartile range; SES, socioeconomic status.

*SES classified using the Australian National University (ANU)-3 system,^{E1} which ranges from 0 to 100, with higher values indicating higher SES.

†Excludes 37 infants who were not breast-fed.

TABLE E2. Adjusted associations between allocated formula and risk of positive SPT and allergic disease

Outcome	Hydrolyzed formula (pHWF) adjusted OR (95% CI)	Soy formula adjusted OR (95% CI)
Any allergic manifestation		
0-1 y (228/575) [†]	0.97 (0.63-1.48)	1.23 (0.81-1.88)
0-2 y (300/575) [†]	1.22 (0.81-1.85)	1.21 (0.80-1.84)
Secondary outcomes		
Eczema within first 2 y (264/575) [†]	1.24 (0.82-1.88)	1.11 (0.73-1.68)
Positive SPT within first 2 y*		
Any allergen	0.88 (0.59-1.30)	0.92 (0.61-1.38)
Cow's milk	0.79 (0.35-1.77)	0.78 (0.32-1.92)
Any food reaction within first 2 y	0.95 (0.51-1.75)	1.21 (0.67-2.19)
Childhood outcomes (period prevalence) at 6-7 y		
Eczema (157/493)	1.10 (0.70-1.72)	0.90 (0.57-1.42)
Asthma (148/495) [‡]	0.82 (0.50-1.33)	0.82 (0.50-1.34)
Rhinitis (117/495) [§]	0.91 (0.54-1.55)	1.24 (0.74-2.09)

All ORs compared to the conventional CMF group. All models adjusted for infant sex and parental smoking during pregnancy unless otherwise stated. Also adjusted for *parent and sibling food allergy, [†]parent and sibling eczema, [‡]parent and sibling asthma, or [§]parent and sibling allergic rhinitis.

TABLE E3. Unadjusted associations between allocated formula and risk of allergic disease outcomes according to family history of eczema

Outcome	No family history of eczema			Family history of eczema present			P value for interaction
	Conventional formula (CMF)	Hydrolyzed formula (pHWF)		Conventional formula (CMF)	Hydrolyzed formula (pHWF)		
	% (n/N)	% (n/N)	OR (95%CI)	% (n/N)	% (n/N)	OR (95% CI)	
Primary outcome							
Any allergic manifestation: 0-2 y	49.4 (38/77)	49.4 (41/83)	1.00 (0.54-1.86)	47.4 (54/114)	55.7 (59/106)	1.39 (0.82-2.37)	.43
Secondary outcomes							
Eczema in first 2 y	40.3 (31/77)	44.6 (37/83)	1.19 (0.64-2.24)	44.7 (51/114)	50.9 (54/106)	1.28 (0.75-2.18)	.86
Positive SPT within first 2 y							
Cow's milk (3 mm+)*	—	—	0.95 (0.24-3.84)	—	—	0.88 (0.34-2.30)	.93
Any allergen (3 mm+)*	—	—	1.18 (0.63-2.18)	—	—	0.78 (0.47-1.28)	.31
Childhood outcomes at age 6-7 y							
Eczema	16.4 (11/67)	25.4 (18/71)	1.83 (0.79-4.23)	40.9 (38/93)	38.9 (37/95)	0.89 (0.52-1.53)	.16
Asthma	28.4 (19/67)	26.8 (19/71)	1.10 (0.53-2.32)	35.5 (33/93)	28.4 (27/95)	0.76 (0.41-1.38)	.44
Allergic rhinitis	17.9 (12/67)	25.4 (18/71)	1.54 (0.67-3.51)	24.7 (23/93)	20.0 (19/95)	0.73 (0.37-1.44)	.17

*These estimates are based on repeated measures (combining results from the 6, 12, and 24 month SPT using the generalized estimating equations approach), meaning it is not possible to report simple proportions.