

The role of partially hydrolyzed whey formula for the prevention of allergic disease: evidence and gaps

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Hydrolyzed formulae are created by using enzymatic processes to break native proteins into smaller fragments. They may prevent development of allergic diseases by reducing exposure to intact allergens. Partially hydrolyzed whey formula (pHWF) is particularly promising for allergy prevention, as it is cheap to manufacture and palatable. Scientific organizations have recommended the use of hydrolyzed formula in the first 4–6 months of life for the prevention of allergic disease based on a limited number of trials. Three recent developments challenge these recommendations: our growing understanding of the importance of allergen exposure for induction of immune tolerance, recently published evidence that failed to identify a protective effect of pHWF, which the authors and other experts believe will necessitate updating of systematic reviews, and methodological limitations of available trials and systematic reviews on which these recommendations are based. Until more definitive evidence is obtained, the authors recommend continuing to advocate that 'breast is best', and caution against overstating the potential for pHWF to prevent allergic disease.

KEYWORDS: allergic rhinitis • allergy prevention • asthma • conventional cows' milk formulae • eczema • infant formulae • partially hydrolyzed whey formula

Allergic conditions cause a substantial burden of illness worldwide both in children and adults [1,2]. These conditions impact on the individual, their family and the healthcare system. The common manifestations of allergic disease shift with increasing age of the child; in early infancy, eczema and food allergy are the most common symptoms, while asthma and allergic rhinitis become more important as the child ages. There was a rapid rise in the prevalence of allergic conditions in the later part of the 20th century [3–5]. While prevalence of childhood asthma appears to have reached a plateau, or declined in some countries [1], it remains a major cause of hospitalization and healthcare expenditure. The rates of allergic rhinitis and eczema in children continue to increase in many parts of the world [1], and there is also evidence that the rate of other systemic allergic conditions, including anaphylaxis and food allergy, are increasing [6–8]. Despite active research, the reasons for these shifts in prevalence of disease remain unclear.

Research in this area aims at identifying interventions that can prevent children from

developing these conditions in the first place. If early forms of disease can be prevented, there is a potential for sustained long-term benefits and reduced risk of other forms of allergic disease in later life [9]. Despite substantial research efforts, there are currently no interventions that have been clearly demonstrated to prevent children developing these conditions, although it is known that early life exposures (during pregnancy and early infancy) may be critical [10]. The infant diet, including breast or formula feeding and the introduction of solid foods, has attracted particular attention as early life exposures that may influence allergic disease risk and importantly, have the potential to be easily modified.

Currently, international expert guidelines from the USA (American Academy of Pediatrics) [11], Europe (European Academy of Allergy and Clinical Immunology/European Society for Paediatric Gastroenterology, Hepatology and Nutrition) [12] and Australia (Australasian Society of Clinical Immunology and Allergy) [101] recommend a limited number of interventions for prevention of allergic disease. All

guidelines advise to breastfeed for the first 4–6 months of life, and if breastfeeding is not possible, the use of hydrolyzed formula in infants with a family history of allergic disease in a first-degree relative is recommended. Hydrolyzed formulae are created by using enzymatic processes to break native proteins into smaller fragments [13], and the enzymatic digestion may be partial or extensive resulting in larger (partially hydrolyzed) or smaller (extensively hydrolyzed) peptide fragments, respectively. This process can be used for a range of base proteins, including soy and cows' milk-based proteins. Furthermore, specific major protein fractions from cows' milk (casein vs whey) may be used rather than whole cows' milk protein. There are no agreed upon criteria to define partially (as compared with extensively) hydrolyzed formulae, and protein size is generally used to identify a formula as partially hydrolyzed. Whole cows' milk-based formula contains proteins in the range of 14 kD (α -lactalbumin) to 67 kD (bovine serum albumin) [14]. Based on industrial sources, extensively hydrolyzed formula only contain peptides of ≤ 3 kD, and partially hydrolyzed formula contains peptides of < 5 kD [11]. Despite these proposed definitions, both partially and extensively hydrolyzed formulae have been shown to contain a wide range of peptide sizes [14]. It has been estimated that commercially available partially hydrolyzed whey formula (pHWF) contain 18% of peptides over 6 kD, while extensively hydrolyzed formula contain between 1 and 5% over 3.5 kD [15]. Peptides need to be in the range of 10–70 kD (particularly 10–40 kD) to be able act as an allergen [16].

This review focuses on the role of pHWF in the prevention of allergic disease in healthy children, recommending future directions for research in this area. The creation of a pHWF offered great hope in the field of allergy prevention, as it is relatively cheap to manufacture and is quite palatable, both of which are essential for widespread use as a preventative strategy. Although other forms of hydrolyzed formula are available, the cost and poor palatability of these formulae limit their utility as an intervention for allergic disease prevention. It is important to note that pHWF is not recommended as a treatment for allergic disease (specifically cows' milk allergy), as it has been repeatedly demonstrated that a proportion of children with proven cows' milk allergy will react to pHWF. The authors begin this article by outlining the current recommendations concerning hydrolyzed formula, and then the changing theories concerning allergic disease prevention are summarized. Following this, the authors summarize the evidence concerning the effect of pHWF for allergic disease prevention, highlighting the critical issues with this research, and make recommendations as to how this area can be progressed.

Current recommendations

A range of health organizations have made recommendations or comments concerning the use of pHWF specifically, or hydrolyzed formula in general, for the prevention of allergic disease. It should be noted that these recommendations are all limited to children who are potentially at a higher risk of allergic disease due to a family history of these conditions in a first-degree relative. These recommendations are summarized in TABLE 1.

As seen from TABLE 1, the recommendations from these various organizations are currently quite similar. It should be noted that there has been a shift away from very slow and restrictive introduction of foods into the infant diet that was advocated in the past [17] towards the current more liberal set of advice. These scientific organizations have recommended the use of hydrolyzed formula in the first 4–6 months of life, in high-risk children, for the prevention of allergic disease based on a limited number of trials. The majority of these organizations acknowledge the limited evidence supporting the efficacy of hydrolyzed formula for the prevention of allergic disease. Some organizations also make specific statements concerning pHWF. Since publication of these recommendations and statements, recently published evidence [18] has shown that it is required that these statements and recommendations be updated.

Changing theories on the causes of allergic disease

In the 1980s and early 1990s, evidence emerged that high exposure rates to house dust mite (HDM) levels was associated with increased risk of sensitization to HDM and asthma [19,20]. There was also evidence that infants who were exposed to intact cows' milk were at greater risk of developing eczema [21] and asthma [22] when compared with children who were breastfed. This and other evidence lead to the concept of 'avoid allergens – prevent allergic disease'. This concept dominated the thinking concerning allergic disease for many decades. In support of this concept, it has been argued that infants are relatively vulnerable to developing sensitization to allergens, either due to increased mucosal permeability [23] or tendency to mount Th2 type responses [24] to allergens, which in turn increases the risk for developing allergic disease. According to this theory, exposure to allergenic dietary proteins (in particular cows' milk, egg and peanut) may promote the development of allergic disease. It was hoped that allergen avoidance (withholding food allergens for a number of months) might have long-term benefits for the child's health and reduce the risk of developing allergic disease.

The 'avoid allergens – prevent disease' concept inspired a large number of allergen avoidance trials for a variety of allergens [25–27]. While some small studies that combine multiple forms of allergen avoidance in infancy to prevent allergic disease have shown promising results [28], larger studies using HDM avoidance as a sole intervention [29–31] have not shown a clear reduction in the incidence of allergic disease. Despite limited evidence [26], maternal avoidance of food allergens during pregnancy and lactation is generally not recommended due to insufficient evidence that it reduces allergic disease risk, and for concern that it may adversely affect the mother and child's nutrition status [26]. However, this issue remains controversial [32]. Given the inconsistency of results for the studies inspired by the 'avoid allergens – prevent allergic disease' paradigm, there has been waning interest in this avenue of research.

In recent years, there has been a dramatic and exciting shift in thinking on how allergic diseases may be prevented. There is increasing dominance in the notion that there may be a window of opportunity, between approximately 4 and 6 months of life, which may be critical in the development of tolerance to

allergens in our environment [10]. This is, in part, based on our growing understanding of the importance of immune regulatory responses [10], and due to a number of important observations:

- Introduction of cereal grains prior to 4 months and after 6 months is associated with increased risk of parent-reported wheat allergy [33];
- In a population-based cross section study, children exposed to cooked egg between 4 and 7 months have the lowest risk of food challenge confirmed egg allergy compared with introduction after 7 months [34];
- Delayed introduction to cows' milk products (after 9 months) has been associated with an increased risk of eczema and recurrent wheeze, whereas delayed introduction of other foods was associated with increased risk of eczema, recurrent wheeze and sensitization to inhalant allergens [35];
- While a randomized controlled trial of brief exposure to cows' milk protein in the first 3 days of life has been shown not to influence risk of allergic disease [36], an observational study has found that early introduction (<14 days) to cows' milk protein, which is probably also related to prolonged exposure, is associated with reduced risk of IgE-mediated cows' milk allergy [37];

- Early introduction of fish [38,39] has been associated with reduced risk of eczema, whereas early introduction of peanut has been associated with reduced risk of peanut allergy [40].

The possibility of reverse causation having generated these results must be considered, as parents of children at the greatest risk of developing allergic diseases, either due to having a strong family history of these conditions or developing early signs of the conditions themselves, may decide to delay the introduction of various foods to the infant diet because of this risk [41]. The aforementioned studies have attempted to address this issue with varying degrees of success.

The potential for early dietary exposure to help induce tolerance is now being assessed in randomized controlled trials for peanut [102] and egg [103] in high-risk children.

It was during the 'allergen avoidance' era that research into hydrolyzed formula gained prominence.

Evidence of pHWF influencing allergic disease risk

Early studies: small & positive

The initial studies evaluating pHWF for allergic disease prevention were relatively small studies (<100 participants) of high-risk infants, randomized to receive pHWF or conventional cows' milk

Table 1. Selected recommendations and statements concerning the role of hydrolyzed formulae for the prevention of allergic disease.

Organization	Recommendation/statement	Ref.
US FDA [†] (May 2011)	"For healthy infants who are not exclusively breastfed and who have a family history of allergy, feeding a 100% whey protein partially hydrolyzed infant formula from birth up to 4 months of age instead of a formula containing intact cow's milk proteins may reduce the risk of developing atopic dermatitis throughout the first year of life and up to 3 years of age. FDA has concluded that the relationship between 100% whey protein partially hydrolyzed infant formulas and the reduced risk of atopic dermatitis is uncertain, because there is very little scientific evidence for the relationship."	[102]
AAP (January 2008)	"In studies of infants at high risk of atopy and who are not exclusively breastfed for 4–6 months, there is modest evidence that the onset of atopic disease may be delayed or prevented by the use of hydrolyzed formulas compared with formula made with intact cow milk protein, particularly for atopic dermatitis."	[11]
ASCIA (2010)	"If infant formula is required in the first months of life before solid foods are introduced, there is some evidence that hydrolyzed formulas may reduce the risk of allergic disease in high-risk infants, with a history of allergy in their parents or siblings In Australia and New Zealand only partially hydrolyzed formulas (usually labeled 'HA' or Hypoallergenic) are recommended for allergy prevention."	[101]
ESPACI and ESPGHAN	"In bottle fed infants with a documented hereditary atopy risk (affected parent or sibling), the exclusive feeding of a formula with a confirmed reduced allergenicity is recommended because it can reduce the incidence of adverse reactions to food, especially to cows' milk protein. There is no conclusive evidence to support the use of formulas with reduced allergenicity for preventive purposes in healthy infants without a family history of allergic disease."	[77]
Section on Pediatrics, EAACI	"A dietary regimen is effective for prevention of allergy to the avoided food, in high-risk infants, particularly in early infancy regarding food allergy (cow's milk allergy) and eczema (atopic or nonatopic). Evidence that such avoidance affects the later allergic manifestations such as asthma and rhinitis is lacking. The most effective dietary regimen is exclusively breastfeeding for at least 4–6 months or, in case of lack of breast milk, formulas with documented reduced allergenicity for at least 4 months, combined with avoidance of solid food and cow's milk for the same period may be considered."	[12]

[†]This statement is an extract from a letter of reply by the US FDA in response to a petition to allow a health claim concerning partially hydrolyzed whey formula, submitted on behalf of Nestlé Nutrition. The FDA approved four wordings of a health claim concerning the use of pHWF [102]. This is the third of these approved provisional health claims.

AAP: American Academy of Pediatrics; ASCIA: Australasian Society of Clinical Immunology and Allergy; EAACI: European Academy of Allergology and Clinical Immunology; ESPACI: European Society for Paediatric Allergology and Clinical Immunology; ESPGHAN: European Society for Paediatric Gastroenterology, Hepatology and Nutrition.

formula (CMF) from birth [42–44]. These studies showed positive results, although evidence was subsequently found to indicate that the results of one of these studies was likely to have been fabricated [45] and has been removed from systematic reviews on this topic [25].

Some of the positive results from these studies were based on outcomes that were potentially inherently biased towards showing a positive effect of pHWF. The outcome of cows' milk allergy is often reported in these studies, but not all children are given the same opportunity to be diagnosed with this condition. Instead of all children receiving a cows' milk challenge at a specific age, children were only investigated for possible milk allergy in these studies if they exhibited possible symptoms. Take for example, two children enrolled in a study at their birth to investigate the effect of infant formula on development of allergic disease, both of whom develop a cows' milk allergy at 2 months of age during exclusive breastfeeding [46]. One child is assigned to receive pHWF while the other is assigned to CMF at the complete or partial cessation of breastfeeding. The child given the CMF will develop a clinical reaction, which will be diagnosed. By contrast, only approximately 30% of children with a confirmed cows' milk allergy will react to the pHWF, and fewer to extensively hydrolyzed formula [47]. Given the natural history of cows' milk allergy to resolve quite rapidly in most cases [48], the child given pHWF may have outgrown their reaction by the time the intervention has been completed, and they are introduced to whole cows' milk protein. This has potentially led to underdetection of cows' milk allergy in the hydrolyzed formula groups, and thus overestimating the protective effect of these interventions for this outcome.

The difference between 'intention-to-treat' and 'per-protocol' analyses

The main ways in which results from clinical trials are presented are either intention-to-treat (ITT) or per-protocol (PP). It is important to understand the conceptual differences in these approaches, as the results of these analyses have different strengths and limitations, and can lead to quite different conclusions. Analysis by the ITT principle has become the favored form of analysis for clinical trials [104]. This ensures that the randomization process is the only factor that influences the composition of the groups, and avoids confounding bias. However, it is well known that ITT analysis is a conservative form of analysis [49], particularly when a substantial number of participants do not receive the allocated intervention, or receive an intervention that they were not meant to. In the case of the studies in this area, many children are breastfed for a substantial period of time, and may never actually be exposed to the allocated formula (nonexposure), while other children are given nonallocated formula during the intervention period (contamination). These effects of contamination and nonexposure will result in a dilution of the observable effect of the intervention if an ITT analysis is used.

By contrast, PP analyses only include those participants who have received the intervention in the way that it was intended.

The aim of a PP is to estimate the true underlying, or 'biological', effect of the intervention. In these analyses, infants may be excluded if they have not been exposed to the allocated formula by a particular age or do not receive a 'substantial' dose of formula. However, there are a number of important trade-offs that occur when moving from an ITT analysis to a PP analysis. Exclusion of nonexposed children in a PP analysis results in reduced sample sizes and thus statistical power. More importantly, PP analyses are not protected by the randomization process and bias may be introduced at various points. In this particular context, infants may be excluded if they 'break from the feeding protocol'. Such exclusions may bias the measured effects, as the reason for the violation of the feeding protocol may actually be due to the child reacting to their assigned formula. The decisions concerning what age exposure must occur by, the number of days of exposure and the total quantity of formula received to be considered an 'adequate dose' are all potential factors that need to be considered when designing a PP analysis. As these are arbitrary decisions, the results may be influenced by signals in the data, thus inflating any apparent effects. For these reasons, PP analyses are best viewed as hypothesis generating, rather than hypothesis testing.

More recently published studies: larger & mixed results

More recent research into the role of pHWF for allergic disease prevention evolved to include larger numbers of infants [18,50–53]. Again, these studies only recruited high-risk infants, but randomization of infants occurred before birth (or soon afterwards). Many of these infants were breastfed and then introduced allocated formula at some point in the first year of life, while others never received the formula at all due to prolonged breastfeeding. This is a key difference between these studies and earlier, smaller studies of the effect of pHWF.

The results of these larger trials comparing pHWF with CMF, when analyzed using the ITT principle, generally showed no or very limited evidence of a protective effect [54] for all allergic disease outcomes. By contrast, the PP analysis shows that pHWF may have some protective effect for eczema in some studies [51], while this effect was not confirmed in others [18]. Specifically, the GINI group reported a reduced risk of eczema (odds ratio [OR] = 0.56; 95% CI = 0.32–0.99) in the first year of life in a PP analysis that only included the 44.7% of children who were exposed to the allocated formula, and were not exposed to another formula, by 4 months of age [51]. When the MACS cohort was analyzed in the same way, there was no evidence of a protective effect (OR = 1.03; 95% CI = 0.55–1.93, 43.7% of children could be included) [54]. Unlike early life eczema, the PP analyses for both MACS and GINI consistently demonstrate no apparent effect of pHWF on the outcomes of childhood asthma (OR for GINI = 1.64; 95% CI = 0.59–4.53 and OR for MACS = 0.73; 95% CI = 0.37–1.45), and allergic rhinitis (OR for GINI = 0.95; 95% CI = 0.55–1.63 and OR for MACS = 0.80; 95% CI = 0.39–1.64). The impact on eczema but not asthma shown in the GINI study has been argued to indicate pHWF does not influence the atopic march [53].

Various decisions need to be made as to which participants are included in PP analysis, and many of these decisions may influence the study results. As such, it is important that the effects shown in PP analysis can be replicated. Unfortunately, replication of PP results has proven difficult in this area. The lack of a clear ITT effect of pHWF for allergic disease prevention, and the inconsistency of PP results, creates difficulty in interpreting the role of pHWF in the prevention of allergic disease.

The largest studies of the effect of pHWF to date have randomized children prior to birth, and introduced the allocated formula at the cessation, or partial cessation of breastfeeding [18,51]. Although this mimics the 'real world' use of these formulae, it creates a situation where randomized children may receive limited, or even no, exposure to study formula within the 'critical window' of 4–6 months in early life [10]. In the GINI study 62% [52], and in MACS 52% [18], of infants received their allocated formula by 4 months of age. As such, any beneficial effect of pHWF is extremely difficult to demonstrate in ITT analysis (as 40–50% of children are not exposed during the key period).

What do systematic reviews tell us?

It is important to consider all available information when considering the effectiveness of an intervention. The highest level of evidence to support an intervention comes from multiple, well conducted, randomized trials providing consistent effects. Systematic reviews aim to identify all studies that have investigated the effect of an intervention, and summarize its effectiveness. This can be achieved statistically using meta-analysis, to come up with a summary, or pooled, estimate of effect.

A number of systematic reviews have assessed the evidence that pHWF may help reduce the incidence of allergic disease in infants [25,55,56]. Like original publications, systematic reviews can also have methodological limitations. Specific limitations of the review by Alexander and Cabana include no duplicate assessment of study eligibility, and extraction of data, lack of use of Medical Subject Headings search terms, lack of search for unpublished 'gray' literature, inclusion of studies with high likelihood of bias due to nonrandomization, nonblinding or both, and inclusion of studies with high rates of loss to follow-up, and the review being funded by an organization with a financial interest in the results of the review [56]. The review by Szajewska and Horvath was methodologically stronger in terms of duplicate selection of studies and checking of data, but included quasi-randomized trials, only assessed the effect of a single pHWF, and again was funded by an organization with a financial interest in this area [55]. For these reasons, the strongest review evidence on this topic comes from the Cochrane review on this topic by Osborn and Sinn [25]. Furthermore, the majority of guideline documents use this review as a key reference to support the recommendations on this issue, which is why the authors have focused on the meta-analysis conducted by this group.

The systematic review and meta-analysis conducted within the Cochrane review on this topic estimated that prolonged feeding with pHWF resulted in a 27% (95% CI: 10–41%) reduced incidence of 'any allergic disease' in infancy when compared

with feeding with intact CMF [25]. The Cochrane review did not include the recently published results from the MACS trial [18], and inclusion of the data from the MACS trial probably results in a reduced estimate of the effect of pHWF. It should be noted that the MACS trial was previously omitted from the Cochrane reviewer on this topic [25], based on an incorrect assumption made concerning an interim report supplied by the organization that initially funded the project, without directly contacting the study investigators to confirm these details [18]. We have informed the authors of the Cochrane review on this topic, and requested that they consider this study when they next update their review.

The authors have recently raised the possibility that publication bias may have impacted on the systematic review of evidence concerning pHWF for allergic disease prevention [18]. Publication bias arises when small studies showing null, or unfavorable, results are less likely to be published than small studies that show strong effects [57]. This can result in overestimating the effect of an intervention when the results of multiple trials are combined. Publication bias occurs for a range of reasons, but is largely due to it being more difficult to publish negative study results. The creation of clinical trial registries has helped address this issue, as it is now compulsory (for future publication) to have clinical trials registered in a publicly accessible format, where the design and outcomes to be assessed are prospectively documented [58]. This means that groups undertaking systematic reviews are able to identify unpublished studies and include the results in the synthesis of evidence. Unfortunately, the majority of the trials conducted on pHWF for allergic disease prevention were initiated and published before 2005, when the trial registries were initiated [58]. As such, it is not possible to be confident that all trials on this topic have been identified.

The importance of assessment of potential publication bias in systematic reviews has been recognized for some time, although not all reviews have formally taken this into consideration. Unfortunately Osborn and Sinn did not assess potential publication bias [25]. When a funnel plot is generated from the published meta-analysis by Osborn and Sinn (FIGURE 1), there is evidence of asymmetry; that is, there is an absence of smaller studies (denoted by larger estimates of variance) that show null or nonprotective effects (denoted by estimates of effect that are closer or above 1) [25]. This pattern of results is suggestive of publication bias [59]. Alexander and Cabana examined potential publication bias, and also reported asymmetry in the funnel plot of studies addressing this topic [56]. Szajewska and Horvath considered publication bias, but elected not to formally test for it based on limited number of studies available [55].

Asymmetry in a funnel plot is a possible indicator of publication bias, but this pattern of results may also arise from other causes. Specifically, there may be methodological differences between larger studies and smaller studies that reduce the impact of pHWF. It is plausible that the effect of hydrolyzed formula may be different in children who have been breastfed prior to initiation of formula. Human breast milk contains a host of immunologically active components [29]. These components of breast milk may independently help induce allergen tolerance while preventing sensitization

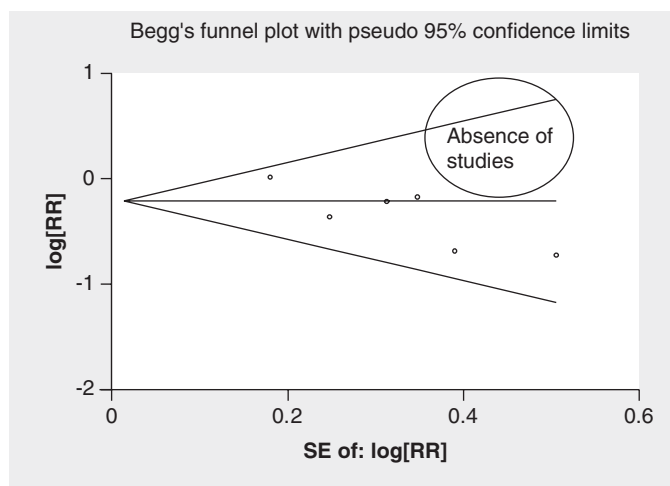


Figure 1. Funnel plot of the Cochrane meta-analysis 14.1. Prolonged feeding: Partially hydrolyzed whey formula versus cow's milk formula; outcome 1: any allergy [26]. This shows a funnel plot of a meta-analysis from the Cochrane review of this topic [26]. The x-axis represents the SE of the estimate for each study (largely determined by the study size): larger studies have smaller SEs, resulting in more precise estimates of the true effect of the intervention, and are plotted further to the left on the funnel plot. The y-axis represents the estimated effect from each study: a value of 0 indicates no effect (RR of 1) while studies with an effect estimate smaller than 1 indicate that partially hydrolyzed whey formula is protective for the outcome of 'any allergy'. In the absence of publication bias it is expected that the funnel plot will show symmetry around the pooled estimate of effect (a value of -0.32, which corresponds to the odds ratio of 0.73). As can be seen, there is distinct asymmetry in this plot, with an absence of small studies in the area indicated by the circle (Harbord $p = 0.06$) [60]. RR: Relative risk; SE: Standard error.

and allergic disease [30]. Feeding CMF following a period of breast-feeding may be less likely to induce allergic disease than feeding CMF from birth. As such, the observable benefit associated with feeding pHWF may be reduced when compared with feeding CMF following breastfeeding. This effect may help in explaining some of the variability in the study results: the smaller studies have only randomized infants who are exclusively formula feed (without prior breastfeeding) while larger studies, which randomize infants prior to birth, compare children who were generally breastfed and then introduced to the study formula at some point in the first year of life, and often after the first 4 months of life.

Limitations of studies on hydrolyzed formula

All studies of pHWF to date have important limitations. As previously noted, it is difficult to successfully blind parents and research staff to the nature of hydrolyzed formula due to differences in taste and smell [60]. This is a greater issue for trials of extensively hydrolyzed formula, where these differences are more marked. This potential for unblinding may lead to bias influencing the study findings. There has also been a lack of assessment of severity of allergic disease – the vast majority of allergic disease in infancy and early childhood is relatively mild disease that causes limited impact on the child and the family. The more severe cases

of allergic disease are less common, but induce a much greater burden [61]. It has been suggested that only mild cases of disease are potentially being prevented or delayed [60]. Furthermore, it has been suggested that hydrolyzed formulae do not prevent allergic disease, but simply delay the diagnosis of these symptoms [60]. Arguing against this possibility is the finding from the GINI study that the cumulative incidence of eczema from birth to 6 years was lower in infants feed pHWF (aRR: 0.79; 95% CI: 0.64–0.97 from ITT analysis). However, it should be noted that although prevalence of eczema from 4–6 years was lower in the PP analysis (aRR: 0.53; 95% CI: 0.34–0.84), this finding was not confirmed in the ITT analysis (aRR: 0.83; 95% CI: 0.61–1.12).

How to move forward

As the authors have outlined earlier, there are a number of key issues with how to interpret the results of the trials assessing the effect of pHWF for allergic disease prevention. A primary challenge is how to design studies that allow the ITT analyses to accurately estimate biological effect of pHWF. Since ITT analyses require studies that randomize children prior to birth to analyze all children, even if they do not receive the allocated formula within a specific time, a true biological effect of pHWF may be missed. There are three options to deal with this issue.

Option 1: an external group specifies a PP analysis plan, obtains data from original study authors, and analyzes pooled data within a meta-analysis

This is an attractive option, as it utilizes data that have already been generated, so should allow for a summary estimate of the biological effect of pHWF in a short-time frame. While the authors support this concept in principle, there are a number of issues that would arise. Knowledge of study results (based on previously published PP results) may influence the PP analysis plan, leading to bias, as the thinking in this area has already been impacted on by the available body of evidence. Furthermore, independence of this group would be critical, as they would need to be free from potential financial vested interests.

Option 2: conduct new studies that only recruit mothers who do not intend to breastfeed

By only recruiting mothers who cannot, or chose not to, breast-feed, a clear assessment of the biological effect of pHWF could be obtained, without the potential influence of combined breast-feeding. The limitations of such a study would be substantial. Identifying sufficient number of mothers who do not intend to breastfeed would be challenging, and ethically, the researchers would need to demonstrate that mothers are not being coerced into formula feeding. Identifying this group of mothers is further complicated by maternity settings in westernized countries where any form of feeding apart from breastfeeding is actively discouraged. Furthermore, such a study does not represent how these formulae are being used in the community – in most westernized settings, where allergic diseases are a major concern, mothers generally breastfeed for some duration, and then may move onto an infant formula (or some form of combined feeding) at some point

in the first year of life. As such, not only would such a trial be very difficult to conduct, the results would be of limited generalizability.

Option 3: recruit mothers prior to birth, but only randomize infants at the point when formula is to be introduced, and only if this is within the prespecified time window (e.g., first 4–6 months of life).

This is probably the best way to address the issues that have been raised in this area of research. This design would require only randomized infants, who are exposed to the study formula by a particular age, to be included in the final analysis. Children who are exclusively breastfed beyond the critical time window are never randomized, so do not need to be included in the ITT analysis. Block randomization, based on age of introduction to formula, could be used to ensure that the groups are equivalent on this potentially important factor. ‘Over-recruitment’ of mother/baby pairs would be needed to allow for a substantial proportion of infants to be excluded from the final analysis. This design would require rapid randomization and provision of study formula to mothers at the point they decide to cease exclusive breastfeeding.

Other issues

There are also a number of broader issues that have not been fully explored in this area. No randomized controlled trial has assessed the effect of pHWF in low-risk children. All clinical trials of pHWF have recruited children at increased risk of allergic disease, primarily based on a history of allergic disease in a first-degree relative. While it is possible that high-risk infants may be the most probable to receive benefit from this formula, and a demonstrated benefit in this group would be important, it has been suggested that the use of pHWF may be more effective in children with a less severe family history of allergic disease [51]. This suggestion is yet to be confirmed. Given the rapid rise in the prevalence of allergic diseases in westernized countries, many children who are at ‘low risk’ do in fact develop these conditions. The advantage of studying high-risk children is that the prevalence of allergic disease is greater in these children, which enhances statistical power, and families with a history of allergic disease may be more willing to accept interventions to prevent these conditions.

There has been limited assessment of extensively hydrolyzed formula for allergic disease prevention. Few studies that have examined extensively hydrolyzed casein formula (eHCF) have suggested that this form of formula may be more effective than pHWF [50,52]. The issues with this formula will relate to cost and acceptability as a preventative strategy, due to taste and odor.

There is a critical need for studies in this area to better define and validate allergic disease outcomes in early life, and in particular, the definition of eczema. The available evidence has consistently shown an absence of effect of pHWF on the development of asthma [18,53]. Where there are conflicting findings relates to the outcome of early life eczema. At this time, a wide variety of diagnostic criteria for eczema have been used, including variants on the Hanifin and Rajka [62], the UK working party [63] and other criteria [64,65]. This makes comparability of study results highly questionable. Furthermore, none of these definitions have been

validated in children younger than 12 months of age [66]. Concern has been raised that many of the definitions used have a low sensitivity (miss cases) or include elements (particularly persistence of symptoms) that are not appropriate to define incidence of eczema in very young children [67]. A uniformly agreed definition will need to be identified to help move research in this area forward. A limitation of the published studies is that it is generally assumed that all eczema is allergic in nature. We [68] and others [69] have found that a large proportion of eczema in infancy is nonatopic in nature.

It may also be necessary to conduct further *in vitro* and animal-based studies to clarify the mechanisms of action for hydrolyzed formula, and whether there are differences between partially and extensively hydrolyzed formulae. It is currently unclear which cows’ milk protein component (whey versus casein), or length of peptide, may have the greatest beneficial effect on infant immune function. One of the most compelling suggested mechanisms is that the small proportion of intact proteins in pHWF is sufficient to induce oral tolerance. The study by Fritsché *et al.* demonstrated, in rats, feeding partially hydrolyzed CMF from day 1 induces antigen specific (anti- β -lactoglobulin) suppression of IgE and IgG antibodies in a dose-dependant manner [70]. Furthermore, intact cows’ milk protein formula showed a similar effect, whereas extensively hydrolyzed proteins did not. How this translates to humans is uncertain, particularly given the suggestion from some studies that extensively hydrolyzed casein formula may have a greater impact on reducing the incidence of allergic disease than pHWF [50,52,71].

The effect of hydrolyzed formula may be influenced by the period of exclusivity of this intervention. There is concern that ‘contamination’ by exposure to whole cows’ milk protein will dilute the effect of hydrolyzed formula feeding. At this time, the available literature does not allow us to directly assess how long this period of exclusivity needs to be, or even how relevant this contamination effect is for allergic disease prevention. Unfortunately, many of the published trials do not report how long the period of exclusivity was maintained.

There does not appear to be any evidence of harm caused to children by feeding them pHWF. As such, does it really matter what recommendation is made concerning the use of pHWF for allergic disease prevention? There are very few concrete recommendations that we can currently make for how parents may help reduce the risk that their child develops allergic disease [72], which makes it tempting to retain the recommendations to use pHWF if there remains some possibility that this formula reduces this risk. Nevertheless, this desire to provide concrete recommendations should be balanced with not overstating the claims of the beneficial effects of pHWF. If the proposed benefits of pHWF are overstated to parents, this may have negative impacts on the duration of breastfeeding. The evidence is unclear if breastfeeding reduces risk of allergic disease [73–75], but it is clear that breastfeeding is beneficial for a range of nonallergic disease outcomes [76], and should be promoted as a public health priority.

Conclusion

The use of pHWF for allergic disease prevention is controversial with a long and chequered history. Only if pHWF is proven to

be protective against the development of clinical disease should it be recommended. The evidence concerning the effect of these formulae is mixed, and there are a number of important methodological limitations described here that have not been fully taken into consideration in formulating the existing guidelines. Given these significant methodological limitations, and recently published evidence [18], updating of systematic reviews and existing guidelines on the use of pHWF for allergic disease prevention is warranted and timely. Until more definitive evidence is obtained, overstating the potential for pHWF to prevent allergic disease should be avoided, and we recommend continuing to advocate that 'breast is best'.

Expert commentary

The increased prevalence of childhood allergic diseases over recent decades in western societies, together with the health burden they create, has prompted vigorous efforts to identify strategies to prevent the development of these conditions. The use of various infant formulae has been the key among these prevention strategies, including partially hydrolyzed whey. Despite over 30 years of research on this topic, the use of these formula remains controversial, and the best advice we can provide mothers is to maintain breastfeeding for the first 4–6 months of the child's life.

Five-year view

Given the long history of controversy concerning the role of pHWF for the prevention of allergic disease, it appears likely that

this will remain the case for the next 5 years. Formula studies have major logistical issues, and are open to a range of potential bias including difficulty in blinding the distinct smell of each formula. New developments to improve the quality of formula trials are required to obtain definitive evidence on their role in preventing allergic disease. If this is not possible, alternate new approaches for prevention should be explored such as early introduction of allergen. In the next 5 years, trial-based evidence relating to the effect of allergen exposure in early life (4–6 months of age) will emerge and elucidate the role of early life exposure to intact allergens on the development of allergic disease.

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Key issues

- Hydrolyzed formulae are manufactured by using enzymatic processes to break native proteins into smaller fragments.
- Various scientific organizations recommend that infants with a family history of allergic disease in a first degree relative who are unable to be breastfed in the first 4–6 months of life be introduced to hydrolyzed formula (as opposed to standard cows' milk formulae) to reduce the infants risk of developing allergic diseases, particularly eczema.
- The main form of hydrolyzed formula used for allergic disease prevention is partially hydrolyzed whey, due to it being both palatable and cheap to manufacture.
- These recommendations have recently been challenged by a shifting understanding of how immune tolerance to allergens is achieved, recognition of methodological issues with literature in this area, and recently published evidence that failed to identify a protective effect.
- Updating and reappraisal of systematic reviews and guidelines in this area is required.
- Until more definitive evidence is obtained, the public health message that 'breast is best' should continue to be promoted, and overstating the potential for partially hydrolyzed whey formula to prevent allergic disease should be avoided.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

- 1 Asher MI, Montefort S, Björkstén B *et al.*; ISAAC Phase Three Study Group. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 368(9537), 733–743 (2006).
- 2 Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Respiratory Health Survey (ECRHS). *Eur. Respir. J.* 9(4), 687–695 (1996).
- 3 Robertson CF, Heycock E, Bishop J, Nolan T, Olinsky A, Phelan PD. Prevalence of asthma in Melbourne schoolchildren: changes over 26 years. *BMJ* 302(6785), 1116–1118 (1991).
- 4 Robertson CF, Roberts MF, Kappers JH. Asthma prevalence in Melbourne schoolchildren: have we reached the peak? *Med. J. Aust.* 180(6), 273–276 (2004).
- 5 Devenny A, Wassall H, Ninan T, Omran M, Khan SD, Russell G. Respiratory symptoms and atopy in children in Aberdeen: questionnaire studies of a defined school population repeated over 35 years. *BMJ* 329(7464), 489–490 (2004).
- 6 Gupta R, Sheikh A, Strachan DP, Anderson HR. Time trends in allergic disorders in the UK. *Thorax* 62(1), 91–96 (2007).

- 7 Jørgensen IM, Jensen VB, Bülow S, Dahm TL, Prahl P, Juel K. Asthma mortality in the Danish child population: risk factors and causes of asthma death. *Pediatr. Pulmonol.* 36(2), 142–147 (2003).
- 8 Foucard T, Graff-Lonnevig V. Asthma mortality rate in Swedish children and young adults 1973–88. *Allergy* 49(8), 616–619 (1994).
- 9 Burgess JA, Lowe AJ, Matheson MC, Varigos G, Abramson MJ, Dharmage SC. Does eczema lead to asthma? *J. Asthma* 46(5), 429–436 (2009).
- 10 Prescott SL, Smith P, Tang M *et al.* The importance of early complementary feeding in the development of oral tolerance: Concerns and controversies. *Pediatr. Allergy Immunol.* 19(5), 375–380 (2008).
- **Key paper outlining the concept that there may be a window of opportunity for exposure to allergens that will help induce immune tolerance.**
- 11 Greer FR, Sicherer SH, Burks AW; American Academy of Pediatrics Committee on Nutrition; American Academy of Pediatrics Section on Allergy and Immunology. 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* 121(1), 183–191 (2008).
- 12 Høst A, Halken S, Muraro A *et al.* Dietary prevention of allergic diseases in infants and small children. *Pediatr. Allergy Immunol.* 19(1), 1–4 (2008).
- 13 Muraro A, Dreborg S, Halken S *et al.* Dietary prevention of allergic diseases in infants and small children. Part I: immunologic background and criteria for hypoallergenicity. *Pediatr. Allergy Immunol.* 15(2), 103–111 (2004).
- 14 Oldæus G, Björkstén B, Einarsson R *et al.* Antigenicity and allergenicity of cow milk hydrolysates intended for infant feeding. *Pediatr. Allergy Immunol.* 2(4), 156–164 (1991).
- 15 Bindels JG, Boerma JA. Hydrolysed cow's milk formulae. *Pediatr. Allergy Immunol.* 5(3), 189–190 (1994).
- 16 Aas K. What makes an allergen an allergen. *Allergy* 33(1), 3–14 (1978).
- 17 American Academy of Pediatrics. Committee on Nutrition. Hypoallergenic infant formulas. *Pediatrics* 106(2), 346–349 (2000).
- 18 Lowe AJ, Hosking CS, Bennett CM *et al.* Effect of a partially hydrolyzed whey infant formula at weaning on risk of allergic disease in high-risk children: a randomized controlled trial. *J. Allergy Clin. Immunol.* 128(2), 360–365.e4 (2011).
- **Recent publication on the second largest trial of partially hydrolyzed whey formula for allergy prevention in high-risk infants.**
- 19 Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. *N. Engl. J. Med.* 323(8), 502–507 (1990).
- 20 Holt PG, Vines J, Britten D. Suppression of IgE responses by antigen inhalation: failure of tolerance mechanism(s) in newborn rats. *Immunology* 63(4), 591–593 (1988).
- 21 Grulee CG, Sanford HN. The influence of breast and artificial feeding on infantile eczema. *J. Pediatr.* 9, 223–225 (1936).
- 22 Oddy W. Breastfeeding and childhood asthma. *Essence* 35, 1–5 (1999).
- 23 Kalach N, Rocchiccioli F, de Boissieu D, Benhamou PH, Dupont C. Intestinal permeability in children: variation with age and reliability in the diagnosis of cow's milk allergy. *Acta Paediatr.* 90(5), 499–504 (2001).
- 24 Prescott SL, Macaubas C, Smallacombe T *et al.* Reciprocal age-related patterns of allergen-specific T-cell immunity in normal vs. atopic infants. *Clin. Exp. Allergy* 28(Suppl. 5), 39–44; discussion 50 (1998).
- 25 Osborn DA, Sinn J. Formulas containing hydrolysed protein for prevention of allergy and food intolerance in infants. *Cochrane Database Syst. Rev.* 4, CD003664 (2009).
- **Cochrane systematic review of the effect of hydrolyzed formula for allergic disease prevention.**
- 26 Kramer MS, Kakuma R. Maternal dietary antigen avoidance during pregnancy or lactation, or both, for preventing or treating atopic disease in the child. *Cochrane Database Syst. Rev.* 4, CD000133 (2006).
- 27 Mührshahi S, Marks GB, Criss S, Tovey ER, Vanlaar CH, Peat JK; CAPS Team. Effectiveness of an intervention to reduce house dust mite allergen levels in children's beds. *Allergy* 58(8), 784–789 (2003).
- 28 Arshad SH, Bateman B, Sadeghnejad A, Gant C, Matthews SM. Prevention of allergic disease during childhood by allergen avoidance: the Isle of Wight prevention study. *J. Allergy Clin. Immunol.* 119(2), 307–313 (2007).
- 29 Marks GB, Mührshahi S, Kemp AS *et al.* Prevention of asthma during the first 5 years of life: a randomized controlled trial. *J. Allergy Clin. Immunol.* 118(1), 53–61 (2006).
- 30 Woodcock A, Lowe LA, Murray CS *et al.*; NAC Manchester Asthma and Allergy Study Group. Early life environmental control: effect on symptoms, sensitization, and lung function at age 3 years. *Am. J. Respir. Crit. Care Med.* 170(4), 433–439 (2004).
- 31 Koopman LP, van Strien RT, Kerkhof M *et al.*; Prevention and Incidence of Asthma and Mite Allergy (PIAMA) Study. Placebo-controlled trial of house dust mite-impermeable mattress covers: effect on symptoms in early childhood. *Am. J. Respir. Crit. Care Med.* 166(3), 307–313 (2002).
- 32 Sicherer SH, Wood RA, Stablein D *et al.* Maternal consumption of peanut during pregnancy is associated with peanut sensitization in atopic infants. *J. Allergy Clin. Immunol.* 126(6), 1191–1197 (2010).
- 33 Poole JA, Barriga K, Leung DY *et al.* Timing of initial exposure to cereal grains and the risk of wheat allergy. *Pediatrics* 117(6), 2175–2182 (2006).
- 34 Koplin JJ, Osborne NJ, Wake M *et al.* Can early introduction of egg prevent egg allergy in infants? A population-based study. *J. Allergy Clin. Immunol.* 126(4), 807–813 (2010).
- 35 Snijders BE, Thijs C, van Ree R, van den Brandt PA. Age at first introduction of cow milk products and other food products in relation to infant atopic manifestations in the first 2 years of life: the KOALA Birth Cohort Study. *Pediatrics* 122(1), e115–e122 (2008).
- 36 de Jong MH, Scharp-van der Linden VT, Aalberse RC, Oosting J, Tijssen JG, de Groot CJ. Randomised controlled trial of brief neonatal exposure to cows' milk on the development of atopy. *Arch. Dis. Child.* 79(2), 126–130 (1998).
- 37 Katz Y, Rajuan N, Goldberg MR *et al.* Early exposure to cow's milk protein is protective against IgE-mediated cow's milk protein allergy. *J. Allergy Clin. Immunol.* 126(1), 77.e1–82.e1 (2010).
- 38 Hesselmar B, Saalman R, Rudin A, Adlerberth I, Wold A. Early fish introduction is associated with less eczema, but not sensitization, in infants. *Acta Paediatr.* 99(12), 1861–1867 (2010).
- 39 Alm B, Aberg N, Erdes L *et al.* Early introduction of fish decreases the risk of

- eczema in infants. *Arch. Dis. Child.* 94(1), 11–15 (2009).
- 40 Du Toit G, Katz Y, Sasieni P *et al.* Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J. Allergy Clin. Immunol.* 122(5), 984–991 (2008).
- 41 Lowe AJ, Carlin JB, Bennett CM *et al.* Atopic disease and breast-feeding—cause or consequence? *J. Allergy Clin. Immunol.* 117(3), 682–687 (2006).
- 42 Vandenplas Y, Hauser B, Van den Borre C, Sacre L, Dab I. Effect of a whey hydrolysate prophylaxis of atopic disease. *Ann. Allergy* 68(5), 419–424 (1992).
- 43 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.* 414, 1–21 (1996).
- 44 Chandra RK, Singh G, Shridhara B. Effect of feeding whey hydrolysate, soy and conventional cow milk formulas on incidence of atopic disease in high risk infants. *Ann. Allergy* 63(2), 102–106 (1989).
- 45 Sterken E. The impact of scientific misconduct on child health. *Public Health Nutr.* 9(2), 273 (2006).
- 46 Saarinen KM, Savilahti E. Infant feeding patterns affect the subsequent immunological features in cow's milk allergy. *Clin. Exp. Allergy* 30(3), 400–406 (2000).
- 47 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* 32(1), 74–79 (2002).
- 48 Høst A. Frequency of cow's milk allergy in childhood. *Ann. Allergy Asthma Immunol.* 89(6 Suppl. 1), 33–37 (2002).
- 49 Fisher L, Dixon DO, Herson J *et al.* Intention to treat in clinical trials. In: *Statistical Issues in Drug Research and Development*. Peace KE (Ed.). Marcel Dekker, NY, USA (1990).
- 50 Halken S, Hansen KS, Jacobsen HP *et al.* Comparison of a partially hydrolyzed infant formula with two extensively hydrolyzed formulas for allergy prevention: a prospective, randomized study. *Pediatr. Allergy Immunol.* 11(3), 149–161 (2000).
- 51 von Berg A, Koletzko S, Grühl A *et al.*; German Infant Nutritional Intervention Study Group. 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.* 111(3), 533–540 (2003).
- **First of three papers from the GINI study, the largest study of three hydrolyzed formulae for allergic disease prevention**
- 52 von Berg A, Koletzko S, Filipiak-Pittroff B *et al.*; German Infant Nutritional Intervention Study Group. 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.* 119(3), 718–725 (2007).
- 53 von Berg A, Filipiak-Pittroff B, Krämer U *et al.*; GINIplus study group. 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.* 121(6), 1442–1447 (2008).
- 54 Hill DJ, Lowe AJ, Hosking CS *et al.* Reply. *J. Allergy Clin. Immunol.* 129, 262 e2–263 e2 (2012).
- 55 Szajewska H, Horvath A. Meta-analysis of the evidence for a partially hydrolyzed 100% whey formula for the prevention of allergic diseases. *Curr. Med. Res. Opin.* 26(2), 423–437 (2010).
- 56 Alexander DD, Cabana MD. Partially hydrolyzed 100% whey protein infant formula and reduced risk of atopic dermatitis: a meta-analysis. *J. Pediatr. Gastroenterol. Nutr.* 50(4), 422–430 (2010).
- 57 Hopewell S, Loudon K, Clarke MJ, Oxman AD, Dickersin K. Publication bias in clinical trials due to statistical significance or direction of trial results. *Cochrane Database Syst. Rev.* 1, MR000006 (2009).
- 58 De Angelis C, Drazen JM, Frizelle FA *et al.*; International Committee of Medical Journal Editors. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. *N. Engl. J. Med.* 351(12), 1250–1251 (2004).
- 59 Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315(7109), 629–634 (1997).
- 60 Brand PL, Vlieg-Boerstra BJ, Dubois AE. Dietary prevention of allergic disease in children: are current recommendations really based on good evidence? *Pediatr. Allergy Immunol.* 18(6), 475–479 (2007).
- 61 Su JC, Kemp AS, Varigos GA, Nolan TM. Atopic eczema: its impact on the family and financial cost. *Arch. Dis. Child.* 76(2), 159–162 (1997).
- 62 Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta. Derm. Venereol.* 92, 44–47 (1980).
- 63 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.* 131(3), 406–416 (1994).
- 64 Wolkerstorfer A, de Waard van der Spek FB, Glazenburg EJ, Mulder PG, Oranje AP. Scoring the severity of atopic dermatitis: three item severity score as a rough system for daily practice and as a pre-screening tool for studies. *Acta Derm. Venereol.* 79(5), 356–359 (1999).
- 65 Oranje AP. Development of childhood eczema and its classification. *Pediatr. Allergy Immunol.* 6(Suppl. 7), 31–35 (1995).
- 66 Simpson EL, Keck LE, Chalmers JR, Williams HC. How should an incident case of atopic dermatitis be defined? A systematic review of primary prevention studies. *J. Allergy Clin. Immunol.* 130(1), 137–144 (2012).
- 67 Foley P, Zuo Y, Plunkett A, Marks R. The frequency of common skin conditions in preschool-age children in Australia: atopic dermatitis. *Arch. Dermatol.* 137(3), 293–300 (2001).
- 68 Lowe AJ, Hosking CS, Bennett CM *et al.* Skin prick test can identify eczematous infants at risk of asthma and allergic rhinitis. *Clin. Exp. Allergy* 37(11), 1624–1631 (2007).
- 69 Kusel MM, Holt PG, de Klerk N, Sly PD. Support for 2 variants of eczema. *J. Allergy Clin. Immunol.* 116(5), 1067–1072 (2005).
- 70 Fritsché R, Pahud JJ, Pecquet S, Pfeifer A. Induction of systemic immunologic tolerance to β -lactoglobulin by oral administration of a whey protein hydrolysate. *J. Allergy Clin. Immunol.* 100(2), 266–273 (1997).
- 71 Oldaeus G, Anjou K, Björkstén B, Moran JR, Kjellman NI. Extensively and partially hydrolysed infant formulas for allergy prophylaxis. *Arch. Dis. Child.* 77(1), 4–10 (1997).
- 72 Prescott SL, Tang ML; Australasian Society of Clinical Immunology and Allergy. The Australasian Society of Clinical Immunology and Allergy position statement: Summary of allergy prevention

- in children. *Med. J. Aust.* 182(9), 464–467 (2005).
- 73 Oddy WH, Holt PG, Sly PD *et al.* Association between breast feeding and asthma in 6-year old children: findings of a prospective birth cohort study. *BMJ* 319(7213), 815–819 (1999).
- 74 Matheson MC, Erbas B, Balasuriya A *et al.* Breast-feeding and atopic disease: a cohort study from childhood to middle age. *J. Allergy Clin. Immunol.* 120(5), 1051–1057 (2007).
- 75 Kramer MS, Matush L, Vanilovich I *et al.* Effect of prolonged and exclusive breast feeding on risk of allergy and asthma: cluster randomised trial. *BMJ* 335(7624), 815 (2007).
- 76 Ip S, Chung M, Raman G *et al.* Breastfeeding and maternal and infant health outcomes in developed countries. *Evid. Rep. Technol. Assess. (Full Rep.)* 153, 1–186 (2007).
- 77 Høst A, Koletzko B, Dreborg S *et al.* Dietary products used in infants for treatment and prevention of food allergy. Joint Statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on Hypoallergenic Formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. *Arch. Dis. Child.* 81(1), 80–84 (1999).
- Websites**
- 101 ASCIA. ASCIA Infant Feeding Advice. www.allergy.org.au/images/stories/aer/infobulletins/2010pdf/ASCIA_Infant_Feeding_Advice_2010.pdf
- 102 Schneeman BO. 100% whey-protein partially hydrolyzed infant formula and reduced risk of atopic dermatitis. www.fda.gov/Food/LabelingNutrition/LabelClaims/QualifiedHealthClaims/ucm256731.htm
- 103 The LEAP study: learning early about peanut allergy. www.leapstudy.co.uk/index.html
- 104 The CONSORT group. www.consort-statement.org
- 105 Palmer D. Early regular egg exposure during infancy to prevent egg allergy: a randomised controlled trial. Australian New Zealand Clinical Trials Registry. www.anzctr.org.au/trial_view.aspx?ID=335455

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