

Received Date : 20-Jul-2015

Revised Date : 09-Nov-2015

Accepted Date : 15-Nov-2015

Article type : Original

This is the peer reviewed version of the following article

Title Page

Title "Cows' milk exclusion diet during infancy: Is there a long term effect on children's eating behaviour and food preferences?"

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Running Title: Cows' milk exclusion and later behaviour

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Abstract page

Cows' milk exclusion diet during infancy: Is there a long-term effect on children's eating behaviour and food preferences?

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pai.12513

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Background: Dietary restriction during infancy may influence later eating behaviour. The aim of this study was to determine if consuming a cows' milk exclusion (CME) diet during infancy affects eating habits in later childhood, once cows' milk has been reintroduced into the diet.

Methods: Children were recruited from two large birth cohort studies in the UK. A small number of participants were recruited from allergy clinic. Two groups were recruited: an experimental group of children who had consumed a CME diet during infancy and a control group, who had consumed an unrestricted diet during infancy. Parents and children completed questionnaires regarding eating behaviour and food preferences.

Results: 101 children of mean age 11.5 years were recruited (28 CME and 73 control). The CME group scored significantly higher on "slowness of eating" and on the combined "avoidant eating behaviour" construct ($p < 0.01$). The number of foods avoided and symptoms were associated with higher levels of avoidant eating behaviour ($p < 0.05$). The CME group rated liking for several dairy foods (butter, cream, chocolate, full fat milk and ice cream) significantly lower than the control group ($p < 0.05$), although there were no significant differences seen for any other category of food.

Conclusion: This study demonstrated that consuming a CME diet during infancy has persistent and long-term effects on eating habits and food preferences. To reduce future negative eating behaviours, children's exclusion diets need to be as varied as possible and reintroduction of cows' milk products closely monitored.

Key words: Cows Milk Allergy, Eating Behaviour, Fussy Eating, Infant Diet.

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Introduction

Cows' milk allergy (CMA) affects between 1.26-2.8% of young children in the United Kingdom (UK) (1,2), although self reported levels of CMA are much higher (3). In the majority of children, CMA will resolve by age two years, when cows' milk products can

successfully be tolerated(1,2), although severe phenotypes exist with persisting CMA into older childhood (4,5). The usual natural history of CMA therefore provides an opportunity to explore the effect of dietary exclusion in infancy on later dietary outcomes. Breast or formula milk is the sole source of nutrition in the first few months of life and remains the major source of nutrition for some time after the introduction of solid food. Cows' milk is therefore a unique allergen, in that it dominates the early nutrition needs of infants, whether via infant formula or transmitted from the maternal diet via breast milk. Its exclusion has arguably more impact on nutrition and eating habits than the exclusion of other food allergens.

We have recently reported that infants and toddlers consuming a cows' milk exclusion (CME) diet have significantly higher scores for fussy eating and feeding difficulties compared to children consuming an unrestricted diet (6). Whether these differences are persistent over time is unknown. Several factors suggest that consuming a CME diet in infancy may affect eating behaviour in later life. Firstly, it is known that innate taste preferences in early infancy (i.e. liking of sweet and rejection of bitter) can be manipulated by exposure to the altered taste of substitute formula used for management of CMA. These formulae have been shown to affect preference for savoury, sour and bitter foods in infancy (7) and up to the age of 4-5 years of age (8). Secondly, it has been shown that the number and type of certain allergic symptoms such as wheeze, colic, vomiting and diarrhoea are associated with negative eating behaviours (6,9,10). Whether the negative eating behaviours persist once the symptoms resolve is unknown. Finally, it is known that a proportion of food allergic children never reintroduce the culprit food allergen into their diet following a negative oral food challenge (11)(12). This has potential to influence dietary intake if the food(s) is ubiquitous and nutrient dense.

The influence of these factors on eating behaviour, growth and nutritional intake is unclear. To date, no research has evaluated if there is a long-term impact of avoiding cows' milk in early infancy on food preferences and eating behaviours in later life. Therefore the overall aim of this study was to determine if following a CME diet during infancy affects eating habits in later childhood, once cows' milk has been reintroduced into the diet.

Methods

Study Design

This was a cross sectional study of 7-13 year old children from the Isle of Wight and Winchester area, UK. The study design is shown in Figure 1.

This study had two groups: a CME group and a control group. Children were eligible for inclusion in the CME group if they had consumed a substitute formula and/or a CME diet in the first year of life for ≥ 3 months. Children excluding other foods, in addition to cows' milk were also eligible for inclusion. Participants were primarily recruited from two birth cohort studies; the Food Allergy and Intolerance Research (FAIR) and Prevalence of Infant Food Allergy (PIFA) studies. The FAIR study recruited infants born in 2001/2002 from the Isle of Wight (1). The PIFA study recruited infants born in 2006-2008 from the Winchester area (13). A small number of participants were recruited from NHS allergy clinics from the Isle of Wight.

The control group, also recruited from the FAIR and PIFA studies, was composed of children who had consumed an unrestricted diet during infancy. Children with current food allergy or any medical conditions requiring a special diet were excluded.

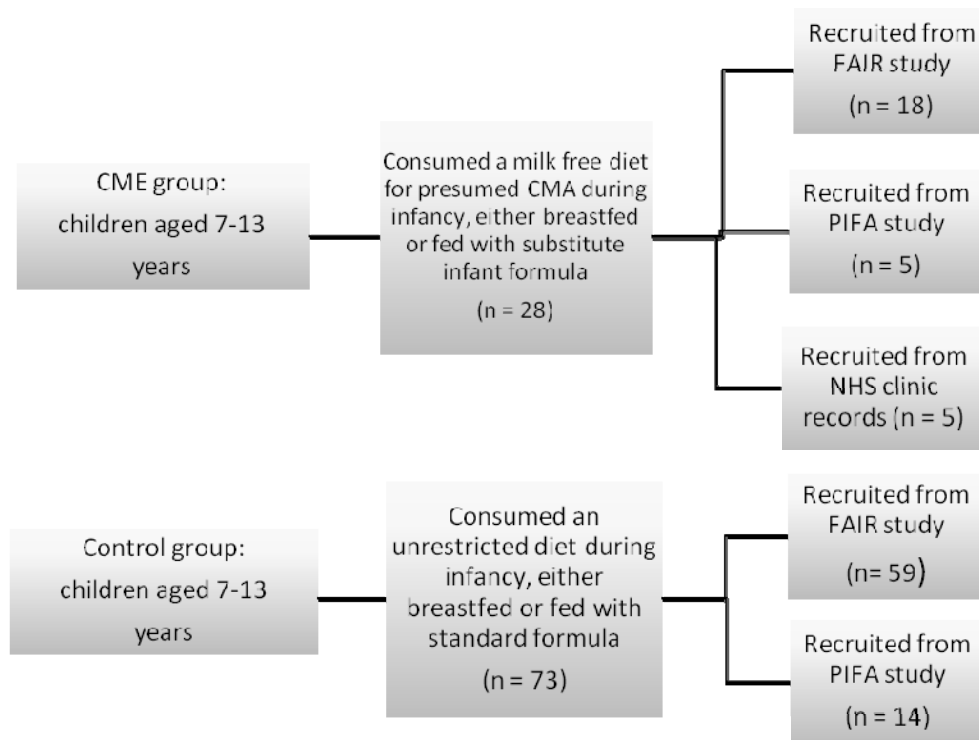


Figure 1. Study design and numbers recruited.

Data collection

Data collection took place between July 2013 and April 2015.

The Child Eating Behaviour Questionnaire (CEBQ) (14) was completed by parents. It consists of four subscales that measure one positive aspect (“enjoyment of food”) and three negative aspects of eating behaviour (“fussy eating”, “food responsiveness” and “slowness in eating”). Items are measured using a five-point scale.

Children’s food preferences were assessed using a questionnaire consisting of 115 common food and drink items categorised into nine groups (15). Children were asked to choose between six options to indicate “how much you like each food”.

Extensive information about social demographics, infant feeding, family and allergy history was available from the original birth cohort dataset. For participants recruited from NHS allergy clinics, this information was extracted from medical notes.

The study was approved by Berkshire NHS ethics committee (reference 13/SC/0194).

Data analysis

Questionnaires were scored according to published guidelines by the original authors. Data was analysed using SPSS software (IBM, version 20). Descriptive statistics were calculated. Differences between the CME and control groups were compared using Mann Whitney or chi square tests. Analysis of Covariance (ANCOVA) tests were undertaken to compare groups whilst controlling for covariates. Spearman rho correlations were performed. The significance level was set at 0.05 for all analyses. A power calculation for a two tailed outcome, at 80% power and at a significance level of 0.05 indicated that 129 participants were required for this study. Sample size calculations were made on the basis of a detecting a 15% difference in food preference category scores with a ratio of 1:2 CME group: control group.

Results

Description of participants

101 participants were recruited, 28 in the CME group and 73 in the control group. The proportion of participants recruited from the FAIR study, PIFA study and NHS allergy clinics is shown in Figure 1. Demographic characteristics of participants are detailed in Table 1. There was no differences between groups for any of these variables, except maternal and sibling food allergy.

Table 1. Demographic characteristics of participants overall and by dietary exclusion group.

*significant difference between CME and control group $p < 0.05$

	All (n =101)	CME group (n =28)	Control group (n = 73)
Median age in years	11.5	11.33	11.58
(minimum-maximum)	(7.04 – 13.83)	(7.25 – 13.83)	(7.04 – 12.44)
Male (%)	53 (52.5)	12 (42.9)	41 (56.2)
<i>Ethnicity</i>			
White British (%)	98 (97)	28 (100)	70 (95.9)
Other (%)	3 (3)	0 (0.0)	3 (4.1)
<i>Maternal education</i>			
None (%)	2 (2.0)	0 (0.0)	2 (2.7)
GCSE /A-level or equivalent (%)	62 (62.0)	20 (74.0)	42 (57.5)
Graduate / Postgraduate (%)	36 (36.0)	7 (25.9)	29 (39.8)
<i>Paternal education</i>			
None (%)	8 (8.1)	3 (11.1)	5 (6.9)
GCSE /A-level or equivalent (%)	56 (56.6)	17 (62.9)	39 (54.1)
Graduate / Postgraduate (%)	35 (35.3)	7 (25.9)	28 (38.9)
<i>Family history of food allergy</i>			
Maternal (%)*	23 (22.5)	10 (35.7)*	13 (17.8)*
Paternal (%)	16 (15.6)	7 (25.9)	9 (12.3)
Sibling (%)*	18 (17.6)	10 (35.7)*	8 (11.0)*

GCSE = General Certificate of Secondary Education

Infant Feeding

Details of participants' infant feeding history are shown in Table 2. Significantly more of the control group had ever been breastfed compared to the CME group ($p = 0.02$) and they were breastfed for longer ($p = 0.017$). A greater proportion of those in the CME group had been fed with predominantly readymade baby food ($p = 0.018$).

Table 2: Infant feeding characteristics of participants. *significant difference between groups (p < 0.05)

	All (n = 101)	CME group (n = 28)	Control group (n = 73)
<i>Ever breastfed</i>			
Yes (%)*	78 (78.0)	17 (60.7)*	61 (84.7)*
No (%)	22 (22.0)	11 (39.3)	11 (15.3)
<i>Ever given formula milk</i>			
Yes (%)	91 (91.0)	28 (100.0)	63 (87.5)
No (%)	9 (9.0)	0 (0.0)	9 (12.5)
<i>Breastfeeding duration*</i>			
Never (%)	22 (22.0)	11 (39.4)*	11 (15.3)*
< 6 months	45 (45.0)	14 (49.9)*	31(43.0)*
> 6 months (%)	33 (33.0)	3 (10.7)*	30 (41.7)
Age at introduction of solid foods (weeks)	16 (10-26)	16 (11-24)	16 (10-26)
<i>Predominant type of weaning food</i>			
Homemade (%)	50 (50.5)	15 (53.6)	35 (49.3)
Readymade baby food (%)*	5 (5.1)	4 (14.3)*	1 (1.4)*
A mixture of both (%)	44 (44.4)	9 (32.1)	35 (49.3)

Symptoms, dietary exclusion and skin prick test (SPT) status

The CME group reported significantly more symptoms than the control group (p < 0.001). The most commonly reported symptom overall was vomiting, reported by 59.4% of all participants (89.3% of the CME group and 47.9% of the control group). All participants in the control group who were formula fed were fed standard infant formula. Within the CME group the most commonly used formula was soya, used by 50% of the CME participants. In the CME group, substitute formula was initiated at a median age of 11.5 weeks (range 2-40), with a median duration of usage of 67.5 weeks (range 16-205 weeks). Four participants (14.3% of CME group) had a positive SPT to cows' milk. For those with a negative SPT, diagnosis of cows' milk allergy was made on the basis of clinical history and supervised dietary exclusion/reintroduction.

Food allergens excluded by the CME group are displayed in Figure 2.

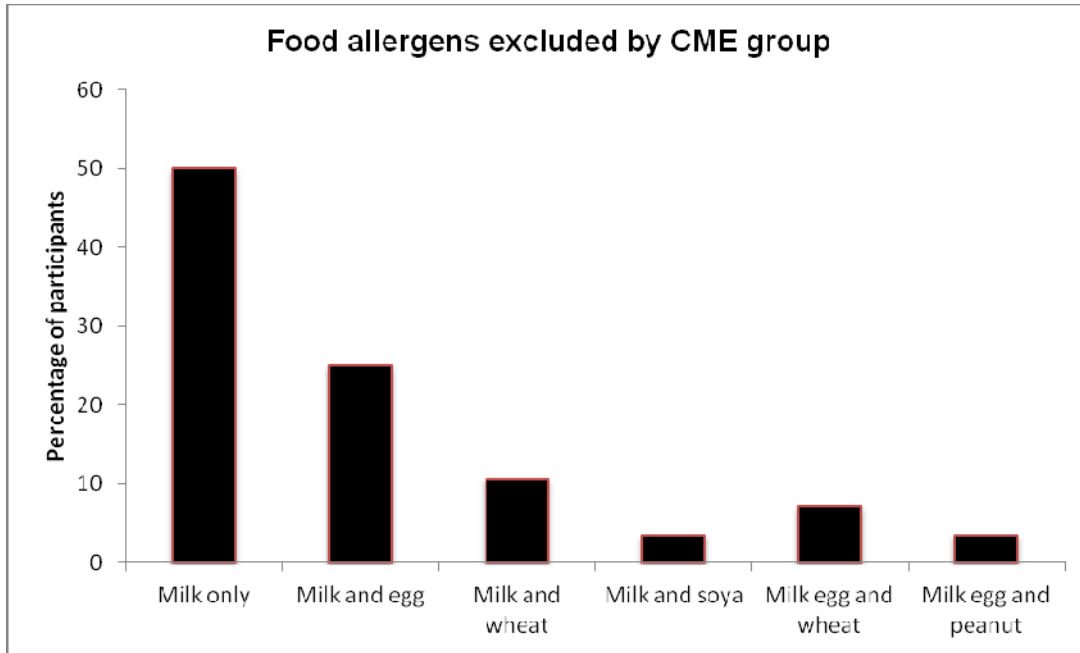


Figure 2. Type of food allergens excluded by CME group

Fussy Eating

There were no significant differences found for individual subscales of the CEBQ by gender or family history of food allergy and no association between questionnaire scores and participant age, parental education or occupation status or any infant feeding factors. Differences between the CME and control group for the fussy eating questionnaire are shown in Figure 3. The CME group had significantly higher scores for the “slowness in eating” subscale ($p < 0.001$). No significant difference was found for the other three subscales individually (food responsiveness, fussiness and enjoyment of food), however when the three negative subscales were combined to give a measure of “avoidant food behaviour”, there were significantly higher scores observed in the CME group ($p < 0.001$). There was no significant difference in CEBQ scores between participants in the CME group according to type of formula consumed.

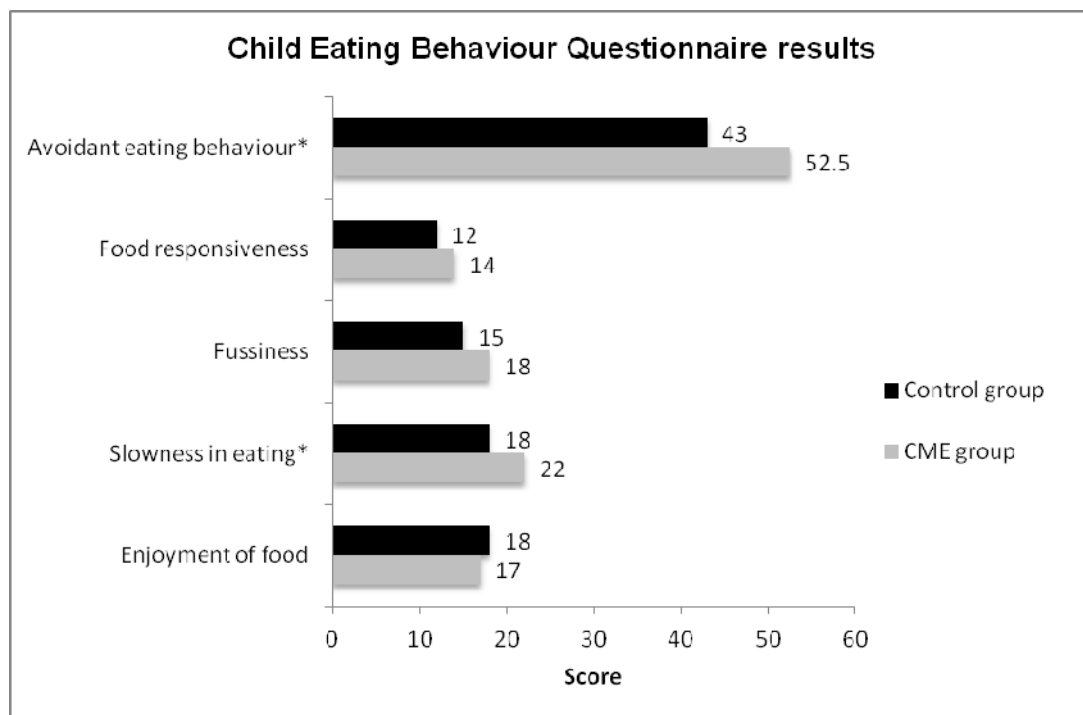


Figure 3. Results of the Child Eating Behaviour Questionnaire. *significant difference between CME and control group $p < 0.05$

The number of reported symptoms was moderately correlated with higher levels of avoidant eating behaviour, across all participants ($\rho = 0.272$, $p = 0.006$). The number of food allergens excluded was moderately positively correlated with slowness in eating ($\rho = 0.283$, $p = 0.04$) and avoidant eating behaviour ($\rho = 0.345$, $p = 0.000$).

As breastfeeding duration and number of symptoms were found to be significantly different between the CME and control groups, two separate one-way ANCOVA calculations were conducted to compare the avoidant eating behaviour scores between dietary exclusion groups, whilst controlling for these two factors. After adjusting for breastfeeding duration, a significant difference between the CME and control groups persisted ($p = 0.001$), with dietary exclusion status explaining 11.2% of the variance in avoidant eating behaviour ($p = 0.263$, partial eta squared = 0.013). Likewise, after adjusting for the number of symptoms, a significant difference between the CME and control groups persisted ($p = 0.005$), with dietary exclusion status explaining 7.8% of the variance in avoidant eating behaviour ($p = 0.230$, partial eta squared = 0.015).

Food preference

Total liking for all foods was not found to be associated with age, number of symptoms or any infant feeding variables. Liking for all foods was inversely related to food fussiness ($\rho = -0.473$, $p = 0.0001$) and slowness in eating ($\rho = -0.340$, $p = 0.001$), but positively correlated to enjoyment of food ($\rho = 0.314$, $p = 0.002$). The most preferred category by all participants was the sweet and fatty foods category and the least preferred category was vegetarian substitutes, followed by vegetables.

No difference was found between the CME and the control group for any category of food. However, looking at individual milk containing foods, significant differences were found between the CME and control groups for a number of foods, with the control group rating them more positively. This is shown in table 3. As was the case with fussy eating, there was no significant difference in food preference scores between participants in the CME group according to type of formula consumed.

Table 3. Difference between CME and control group preference scores for dairy foods.

*Rated more positively by control group, significance of $p < 0.05$

Food	p value
Full fat milk*	0.004*
Semi skimmed milk	0.164
Yoghurt	0.168
Butter*	0.043*
Margarine	0.243
Cream*	0.016*
Cheese	0.853
Ice cream*	0.030*
Chocolate*	0.024*

Discussion

This study set out to compare the eating habits of two groups of children: one group who had consumed a CME diet for CMA as infants and one group who had consumed an unrestricted diet as infants, but who are all now consuming unrestricted diets. As the majority of participants (96/101) were recruited from two large birth cohort studies it was possible to measure the effect of infant feeding variables on current eating behaviours using prospectively collected data from infancy. In terms of fussy eating, the CME group scored

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significantly higher on “slowness of eating” and on the combined “avoidant eating behaviour” construct. There was no difference according to gender or infant feeding, but a higher number of excluded food allergens and symptoms were associated with more negative eating behaviour. The CME group rated liking of several dairy foods (butter, cream, chocolate, full fat milk and ice cream) significantly lower than the control group, although there were no significant differences seen for the overall dairy category or for any other category of food.

The significant difference observed between dietary exclusion groups for avoidant eating behaviour is a novel finding. It demonstrates a long-term effect of modifying the infant diet, persisting approximately 7-10 years, even whilst controlling for breastfeeding duration and number of allergic symptoms. The moderate correlations observed between both number of symptoms and number of avoided food and worse eating behaviour are also novel findings. The finding that those in the CME group are slower eaters is of note. Mealtime duration is associated with problem eating behaviour, with prolonged mealtimes known to worry parents (16). Although this study measured behaviour based on parent report rather than child report or direct observation of eating, the questionnaire used has been validated against behavioural measures of food intake and demonstrated to have good internal consistency, reliability and construct validity (17). In a recent study of eating behaviour, Adamson *et al.* found parents to be accurate reporters of mealtime duration and reported that children who were “problem eaters” spent more time engaged in aversive behaviour and less time eating.

Food preference is due to a combination of several properties (e.g. taste, olfaction, texture and temperature). The categorisation of foods into nine broad categories is arbitrary and is related to food type (e.g. meat, vegetables) not necessarily to taste or sensory properties. Because of the limitations with the grouping of individual foods into categories, a sub analysis was carried out on individual milk containing foods. Although there was found to be no difference in preference ratings between the groups for some milk containing foods (e.g. semi skimmed milk, yoghurt) significant differences were found for five milk containing foods (full fat milk, butter, cream, ice cream and chocolate). This is particularly surprising, considering that in Cooke *et al.*'s study of children consuming unrestricted diets (15) chocolate, pizza and ice cream were the three most favoured foods. Indeed, recent national UK dietary data reports that amongst young people aged 4-18 years, mean weekly intakes of chocolate and ice cream are 63-84g (~two standard bars) and 56-91g (~1.5 scoops) respectively (18). A high percentage of children's food preferences are formed by the age of 2-3 years (19) therefore if exposure and liking of these dairy products was not established in infancy due to CMA, it is understandable that they are now disliked. We hypothesise that

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cheese, yoghurt and semi skimmed milk were introduced to the diet of children with resolving CMA as these were deemed to be nutritious foods, yet butter, cream, ice cream, full fat milk and chocolate were introduced at a later stage as they were perceived to be less healthy or less important.

Milk aversion is well documented in adults with suspected lactose intolerance and it is likely a learned trait caused by experiencing unpleasant side effects (20). The literature regarding children outgrowing food allergy has not specifically studied the role of food dislike or aversion. Kim *et al.*'s (12) study of children who had undergone baked milk challenges, observed that 12% of those who had tolerated baked milk products chose to avoid the food when reviewed five years later. Mostly this was attributed to a fear of recurrence of symptoms and all declined to have a further supervised challenge in hospital. A study of 210 children with CMA in Finland found that although 120 participants had introduced milk products by the age of three years, with 87% drinking milk, 67% consuming cheese and 45% consuming yoghurt; the total amounts of milk products consumed daily were small and less than the national average (21).

Limitations

There are some limitations to this study. Firstly the CME group was a heterogenous group as the two birth cohorts took place five years apart, involving two separate research protocols. However both cohorts took place in the same region and by incorporating both studies, allowed a broader age range and larger sample to be recruited. The most commonly used formula (soya), is no longer recommended as first line treatment of CMA (22). The lack of ethnic diversity and high maternal educational level means the study may not be generalisable to other populations, however previous international studies have shown that food preferences and fussy eating in children are consistent across cultures. Despite the power calculation indicating 129 participants were required, it was only possible to recruit 101. Although we did detect some differences between groups, the lack of power could explain why others were not observed. For example we did not observe any differences between formula types (soya compared to extensively hydrolysed) within the CME group. Maternal food preferences and food availability were not measured. Although we have reported differences in eating behaviour, we have not reported nutritional intake.

Conclusion

Overall this study demonstrated that consuming a CME diet during infancy has an effect on some, but not all, eating habits that were under investigation. However, given that the avoidance of cows' milk in the short term is currently the only management option for CMA,

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this will not fundamentally change its management in routine clinical practice, although it does underline the importance of a robust diagnosis. The implications of these findings are that problem eating habits in children consuming CME diets need to be addressed early and appropriate dietetic intervention occurs during both the exclusion and reintroduction period.

Acknowledgements/funding source: We would like to thank the participants of the study. The PIFA cohort was supported by the UK Food Standards Agency (project FS305019) and European Union's FP7 iFAAM Project (312147)

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