Title: Taste preference, food neophobia and nutritional intake in children consuming a cows' milk exclusion diet: a prospective study

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Abstract

Background: Taste exposure in infancy is known to predict food preferences later in childhood. This is particularly relevant in children with cows' milk allergy, who consume a substitute formula and/or cows' milk exclusion (CME) diet early in life. This prospective study aimed to show whether there is a long term effect of consuming a substitute formula and CME diet on taste preferences and dietary intake.

Methodology: Children were predominantly recruited from two large birth cohort studies in the UK. 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 completed a food neophobia questionnaire and an estimated prospective food diary. Children completed a taste preference test and their growth was assessed.

Results: 101 children of mean age 11.5 years were recruited (28 CME and 73 controls). Children in the CME group had a significantly higher preference for bitter taste than those in the control group ($p < 0.05$). There were significant differences between groups for intake of some micronutrients including riboflavin, iodine, sodium and selenium. Food neophobia did not differ between groups. 28% of the CME group were overweight/obese compared to 15% of the control group, however this difference was not statistically different.

Conclusion: Consuming a substitute formula and/or CME diet in infancy has a long term effect on preference for bitter taste. Differences exist for intake of some micronutrients but not for macronutrients. There was a non-significant trend towards overweight and obesity in children in the CME group.

Introduction

Cows' milk allergy (CMA) affects nearly 3% of young children in the UK [1–3]. Its management requires a strict cows' milk exclusion (CME) diet, usually in combination with a substitute infant formula, with or without breastfeeding [4,5]. Substitute infant formula used in CMA are composed of extensively hydrolysed peptides, amino acids or occasionally soya protein and are known for their bitter taste [6–8]. Milk, whether formula or breast milk, is the first infant food and becomes the standard against which all other new flavours are evaluated [9]. This is particularly salient when the milk has an altered or unusual flavour. In the majority of children, CMA will resolve by the age of two years, when cows' milk products can successfully be tolerated [1,3]. The natural history of CMA therefore provides an opportunity to explore the effect of dietary exclusion in infancy on later dietary outcomes.

New-born infants are responsive to different taste stimuli. Generally, a sweet taste evokes a positive reaction, whereas both sour and bitter tastes provoke negative reactions[10]. Despite the fact that these preferences are inbuilt, they can be modified through exposure in utero, during early infancy, in childhood and in adolescence[11]. A systematic review assessing the effect of infant taste experiences on later acceptance concluded there is a clear programming effect for bitter but studies on sweet and salty were equivocal[12]. The altered taste of substitute formula used in CMA have been shown to affect preference for savoury, sour and bitter foods in infancy[13] and up to of 4-5 years of age[14]. It is said that the characteristic flavour of a formula is "imprinted" from an early age[15]. However, in other conditions that use substitute formula from infancy, such as phenylketonuria (PKU), there has been disagreement [15,16].

In addition to theoretical changes to taste preferences caused by substitute formula, the dietary exclusion of foods or food groups in early life, in combination with adverse symptoms can cause changes in food behaviour and preferences [17–20]. Food neophobia, meaning "a fear of new food", often presents in normally developing children as a reluctance to eat unfamiliar foods, peaking between the ages of two to six years [21]. Heightened levels of fussy eating have been demonstrated in CMA[22], with higher levels of neophobia reported in PKU [16], however it remains unclear if there is a long term effect of CMA on neophobia or whether there are nutritional implications.

Several studies have demonstrated differences in nutritional intake and growth in children consuming exclusion diets, mostly reporting lower micronutrient intake and poorer growth[23–27]. Although milk allergy is usually outgrown, it is known that a proportion of food

allergic children never fully reintroduce the culprit food into their diet once the allergy has resolved, possibly due to anxiety [28,29]. This has potential to influence dietary intake if the food/food group is ubiquitous and nutrient dense. This study will therefore aim to investigate if there is a long-term impact of consuming substitute infant formula and excluding cows' milk in early infancy on taste preferences, food neophobia, nutritional intake and growth.

Methodology

Study design and participants

This was a cross sectional study of 7-13 year old children from the Isle of Wight and Winchester area, UK. Figure 1 summarises the study design. 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 food allergens (e.g. egg) 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)[1] and Prevalence of Infant Food Allergy (PIFA)[30] studies, born in 2001-2002 and 2006-2008 respectively. For both of these studies, detailed prospective information was obtained about feeding practices in infancy. A small number of participants (n =5) were recruited from NHS allergy clinics from the Isle of Wight to increase the sample size. Children with current food allergy or any condition requiring a special diet were excluded. The study was approved by Berkshire NHS ethics committee (reference 13/SC/0194). Written informed consent was obtained from both parent and child.

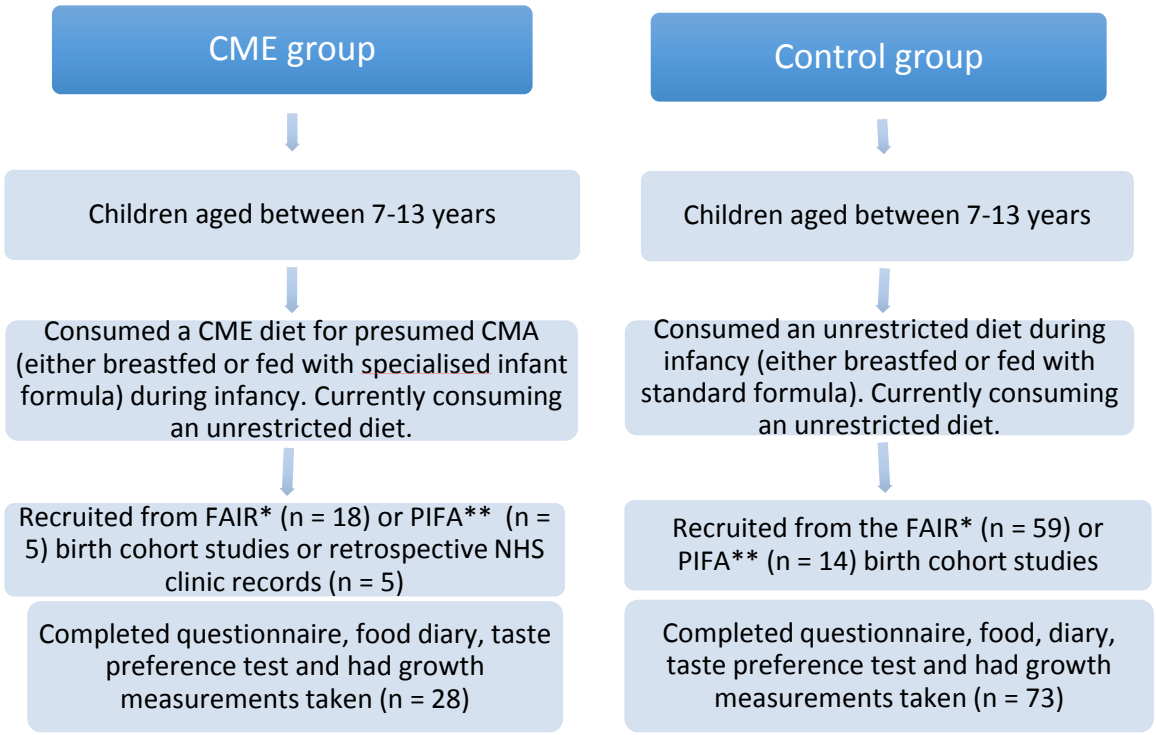

Figure 1 Summary of study design

*The FAIR study recruited infants born on the Isle of Wight[1].

**The PIFA study recruited infants born in the Winchester area[30].

Data collection

Participants eligible for inclusion in the CME group were identified by the study coordinators of the FAIR and PIFA studies. Control participants were identified as the consecutive study participants to each identified CME participant in the database. 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, information was extracted from medical notes.

Food neophobia

Food neophobia was measured using the Child Food Neophobia Scale[31] a validated parentally completed questionnaire. In the current study the Cronbach alpha correlation was 0.921, indicating good internal consistency.

Taste preference

Preference was assessed for the five main tastes: sweet, salty, bitter, savoury and sour, based on the methodology of Knof et al.[32] and Liem & Mennella[14]. Participants were asked to taste and rate five different flavoured waters using a child-orientated rating scale[33]. A sixth sample consisted of plain water. Samples were prepared in advance using bottled water and kept refridgerated until immediately before the test. The dilution of each substrate is shown in supplementary file 1. Samples were identical in appearance and presented individually in opaque cups in a counterbalanced order.

Nutritional intake

Parents and children were asked to jointly complete an estimated food diary, adapted from the National Diet and Nutrition Survey (NDNS), UK [34] for four consecutive days, including one weekend day. Clear instructions of how to complete the diary were given orally and in writing, including estimating portion sizes, detailing cooking method, wastage, snacks and condiments consumed both at home and outside the home. Parents were provided with a stamped envelope to return the diary. If the diary was completed in insufficient detail, contact was made to clarify details.

Food diary coding and analysis

All diaries were coded by the researcher (KM) using a predetermined protocol. Portion sizes were estimated using published age-appropriate portion sizes[35,36]. Information about supermarket foods was obtained from manufacturers' websites. Composite items were analysed by dividing the item into separate components. Food diaries were analysed using nutritional analysis software Dietplan 6 (Forestfield Software Limited, Horsham, UK). Details of dietary supplements and foods not in the database were obtained from the manufacturers' websites. Intake was compared to Estimated Average Requirements (EAR) and Recommended Nutrients Intakes (RNI) for macro and micronutrients [37].

Food groups

Frequency of intake of dairy products, dairy substitutes (i.e. soya milk), fruit, vegetables, chocolate and non-chocolate confectionary were calculated from the diaries, using published age appropriate portion sizes [36].

Growth

Weight was measured using an electronic scale in kg to one decimal place. Height was measured using a stadiometer in cm to one decimal place. Weight for age percentile was calculated using a UK growth chart [38]. Body Mass Index percentile (BMI%) was calculated and plotted on a standard UK chart. Overweight and obesity were defined as BMI% > 91st and > 98th respectively[39]. Waist circumference was measured in cm to one decimal place and plotted on a UK centile chart. It was measured as the "narrowest waist", which is the most frequently recommended site[40]. All measurements were conducted by the same researcher.

Statistical analyses

Data was analysed using SPSS software (IBM, version 20). Descriptive statistics were calculated for all variables. Differences between the CME and control groups were compared using an independent t-test, Mann Whitney or X^2 test. A two way Analysis of Variance (ANOVA) test was undertaken to compare intake of micronutrient between groups whilst controlling for gender. The significance level was set at 0.05 for all analyses.

Sample size was calculated on the basis of a detecting a 20% difference in food neophobia scores with a ratio of 1:2 CME group: control group. Using a two tailed outcome, at 80% power and significance level of 0.05 indicated that 37 CME and 74 control children were required.

This study and the preparation of the manuscript complies with STROBE guidelines for transparent and accurate reporting of observational studies.

Results

101 participants were recruited, 28 in the CME and 73 in the control group. Participant demographic characteristics are detailed in table 1. No significant difference was found between the CME and control groups for age, gender, ethnicity, number of siblings, parental education or paternal food allergy history. Significant differences were found for maternal and sibling food allergy history ($p < 0.05$), with those in the CME group having higher rates of both.

Table 1 Demographic characteristics of participants

	All (*N* =101)	CME group (*n* =28)	Control group (*n* = 73)
Median age in years (minimum-maximum)	11.5 (7.04 – 13.83)	11.33 (7.25 – 13.83)	11.58 (7.04 – 12.44)
Male (%)	53 (52.5)	12 (42.9)	41 (56.2)
Median number of siblings (minimum-maximum)	1 (0-5)	1 (0-4)	1 (0.5)
Ethnicity			
White British (%)	98 (97)	28 (100)	70 (95.9)
Median maternal age in years (minimum-maximum)	42.5 (29-53)	43 (32-51)	42 (29-53)
Maternal education			
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)
Family history of food allergy			
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)*

*$p < 0.05$

Infant feeding and dietary exclusion

Detailed infant feeding data has previously been published [17]. In brief, substitute formula was initiated at a median age of 11.5 weeks (range 2-40) in the CME group, with a median duration of usage of 67.5 weeks (range 16-205). The majority of the CME group were fed soya formula (50%), followed by extensively hydrolysed casein formula (21.4%), extensively hydrolysed whey formula (17.8%) and amino acid formula (10.7%). Within the CME group, 50% excluded only cows' milk during infancy, 39.3% excluded two foods during infancy and 10.7% excluded three foods during infancy. All participants were consuming unrestricted diets at the time of the study.

Taste preference

Results of the taste preference test are shown in figure 2. The most preferred taste overall was plain water, followed by sweet. Boys rated sweet, umami and salty tastes significantly worse than girls ($p < 0.05$). The CME group rated bitter taste significantly better than the control group ($p < 0.05$), but there was no difference between groups for other tastes. Within the CME group, bitter taste preference was not significantly correlated with age of introduction of substitute formula, duration of substitute formula usage, age of introduction of solids, duration of breastfeeding or number of foods excluded. Bitter taste preference did not differ per type of substitute formula used. There was no association found between taste preference and any growth measurement.

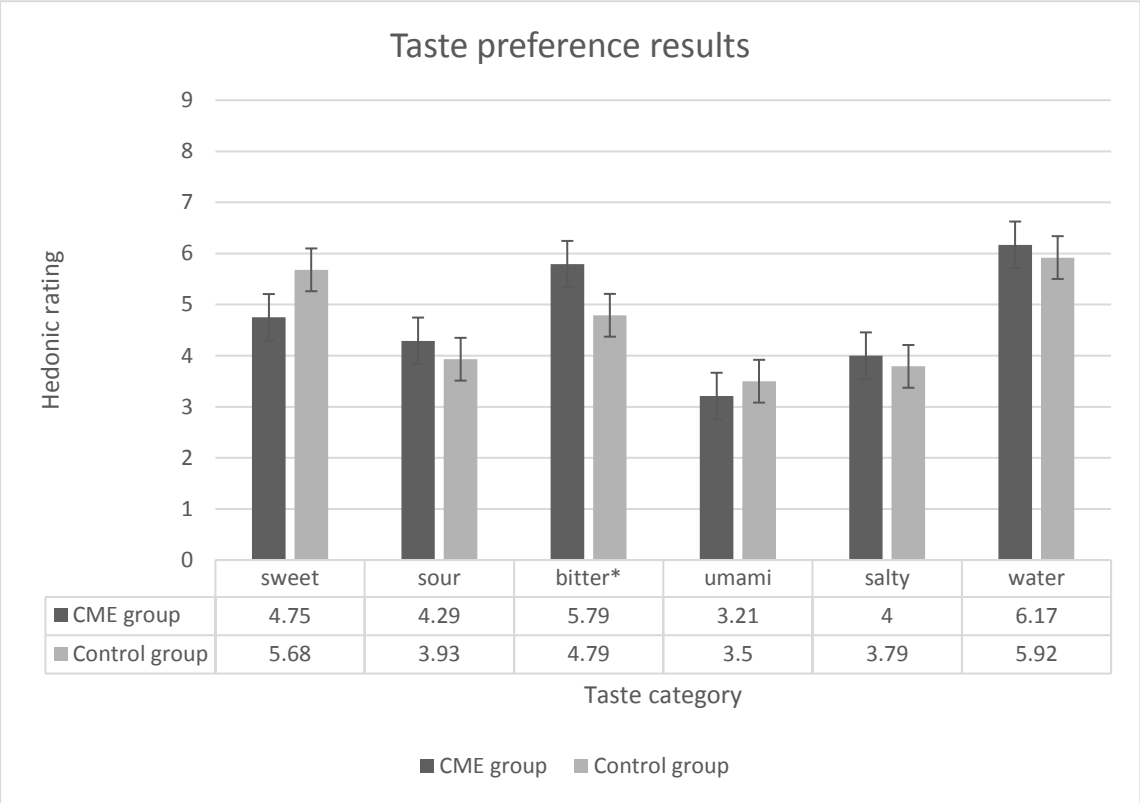

Figure 2. Taste preference results. *significant difference between groups < 0.05. Higher scores indicate a better perceived taste and vice versa.

Nutritional Intake

Food diaries were returned for 64 participants (63.3%); 17 from the CME group (60.7%) and 47 (74.6%). from the control group. There was no difference between those who did and did not return the diary for age, gender, parental education, maternal age, food exclusion history, family history of food allergy, growth or food neophobia. A summary of nutritional intake is

shown in table 2. Using the 7-10 year old age bracket as a guide, overall participants met the Estimated Average Requirement (EAR) for all nutrients. Looking at energy intake, there was no significant difference in % EAR consumed between groups. However, when examining proportions of participants meeting the DRV for energy, 41% of participants in the CME group (n =7) consumed >100% of the EAR, compared to 14.9% of participants in the control group (n =7) ($p = 0.032$). Intakes of some minerals appeared suboptimal (iron 72% of RNI, zinc and magnesium both 74% of RNI), however they were above the EAR. Boys had significantly higher intakes than girls for protein, sodium, iron, zinc, magnesium, iodine and phosphate ($p < 0.05$ for all).

Looking at dietary exclusion groups separately, the CME group's intake of zinc and iodine was below the EAR, but above the Lower Reference Nutrient Intakes (LRNI). The control group met the EAR for all nutrients. Both groups had remarkably similar intakes of energy, protein, fat, saturated fat and vitamin D. The control group had significantly higher intakes of iodine ($p < 0.01$) and riboflavin ($p < 0.05$). The CME group had significantly higher intakes of sodium ($p < 0.05$) and selenium ($p < 0.05$).

As the intake of some nutrients was found to be significantly different between boys and girls, a two way between groups ANOVA was conducted to compare sodium and iodine intakes between groups, controlling for gender. After adjusting for the gender, a significant difference between groups persisted for iodine intake ($p < 0.01$). Gender was not found to be significantly related to iodine intake whilst controlling for dietary exclusion group ($p = 0.068$, partial eta squared = 0.057). In terms of sodium intake, the same trend emerged. After adjusting for the gender, a significant difference between the CME and control groups persisted ($p < 0.01$).

Table 2. Median intakes of selected nutrients from food diary analysis

	All (N = 64)	CME group (n = 17)	Control group (n = 47)
Energy (kcal)	1687 (82%)	1668 (85%)	1688 (82%)
Protein (g)	62.1 (156%)	62.4 (152%)	62.05 (156%)
Fat (g)	63.8 (84%)	63.9 (83.0%)	63.8 (87.0%)
Saturated fat (g)	24.85 (107%)	24.9 (107%)	24.8 (104.5%)
Fibre (g)	14.3 (N/A)	15.4 (N/A)	13.9 (N/A)
Sodium (mg)*	2252 (155%)	2819 (176%)*	2166 (144.0%)*
Calcium (mg)	704.5 (84%)	587 (74%)	717 (88.5%)
Iron (mg)	9.1 (72%)	8.2 (61%)	9.31 (75.5%)
Zinc (mg)	6.39 (74%)	5.3 (66%)	6.5 (75%)
Selenium (mcg)*	34.85 (80%)	42.4 (98%)*	34.2 (78%)*
Magnesium(mg)	194 (74%)	188.0 (74%)	194.0 (75%)
Iodine (mcg)*	108 (86.5%)	67.1 (55.0%)*	118.4 (93%)*
Phosphorous (mg)	1077 (164%)	986.5 (158.5%)	1082 (165%)
Vitamin A (mcg)	517 (103%)	538 (107%)	479 (95.8%)
Thiamin (mg)	1.37 (175%)	1.29 (175%)	1.40 (175%)
Riboflavin (mg)*	1.28 (116%)	1.09 (93%)*	1.42 (124%)*
Niacin(mg)	15.2 (114%)	15.9 (136%)	15.19 (107.5%)
Vitamin B6 (mg)	1.54 (248%)	1.58 (248%)	1.52 (252%)
Vitamin B12 (mcg)	3.0 (273%)	2.1 (187%)	3.04 (291.5%)
Folate (mcg)	192 (104%)	185 (101%)	195 (104%)
Vitamin C (mg)	84.0 (244%)	114 (325%)	78.0 (236%)
Vitamin D (mcg)	1.83 (NO DRV)	1.92 (NO DRV)	1.83 (NO DRV)
Vitamin E (mg)	6.32 (NO DRV)	7.97 (NO DRV)	6.31 (NO DRV)

%Reference nutrient intake is shown in brackets. *significant difference between groups using a Mann Whitney test $p < 0.05$. Analysis includes nutritional supplements.

Dietary supplements

In total 21 (20.7%) participants took dietary supplements, 7 (25%) from the CME group and 14 (19.2%) from the control group. Two of the CME group took calcium/vitamin D supplements, with the remainder taking multivitamin/mineral combinations. All 14 of the control group took multivitamin/mineral supplements.

Food group intake

Intakes of selected food groups are shown in table 3. Two participants in the CME group consumed dairy substitutes (soya milk and yoghurt), in addition to dairy products. The CME group consumed significantly less dairy products and chocolate than the control group ($p < 0.01$), but significantly more dairy substitute products ($p < 0.05$). There was no difference in consumption of fruit, vegetables or non-chocolate confectionary between groups. Consumption of food groups was not associated with neophobia, infant feeding variables or any growth measure. There was an inverse correlation between bitter taste preference and dairy intake (rho = -0.382, $p < 0.01$) and also between chocolate intake and sour taste preference (rho = -0.331, $p < 0.05$).

Table 3 Consumption of selected food categories over a 4 day period.

	All food diaries (*n* = 63)	CME group (*n* = 16)	Control group (*n* = 47)	p value
Dairy products	6 (0-15)	3 (0-11)	7 (0-15)	0.000*
Dairy substitute products	0 (0-8)	0 (0-8)	0 (0-0)	0.015*
Fruit	5 (0-17)	6 (0-11)	5 (0-17)	0.697
Vegetables	6 (0-15)	6 (0-15)	6 (0-10)	0.956
Chocolate	2 (0-7)	0.5 (0-6)	3 (0-7)	0.008*
Non-chocolate confectionary	3 (0-6)	4 (0-6)	3 (0-6)	0.425

Median number of portions consumed. Minimum-maximum values in brackets.*Mann Whitney test p value significant < 0.05.

Growth

Anthropometric measurements are shown in table 4. There was no difference between dietary exclusion groups for any of the measurements. Overall participants had very high waist circumference centiles (median of 98.8%). Twenty participants were classified as overweight

or obese, with no difference observed for age, gender, number of siblings or parental education. There was no difference between healthy weight and overweight/obese children for food neophobia, nutritional intake or taste preference. Comparing dietary exclusion groups, 28.6% (n = 8) of the CME group compared to 15% (n = 11) of the control group were classified as overweight/obese, however this difference was not statistically significant.

Table 4 Anthropometric measurements of participants

	All (*N* = 101)	CME group (*n* = 28)	Control group (*n* = 73)
Weight (kg)	38.8 (20.1 – 74.5)	38.9 (22.2 – 74.5)	38.7 (20.1 – 69.9)
Height (cm)	147.7 (118.8 – 165.5)	143.3 (120.6 – 163.1)	148.0 (118.8 – 165.5)
Weight for age percentile	106.7 (72.5 – 201.3)	103.8 (77.8 – 201.3)	107.4 (72.5 – 174.75)
BMI percentile	58.15 (2.0 -99.9)	56.1 (15.9 – 99.8)	59.8 (2.0 – 99.9)
Waist (cm)	58.95 (46.2 – 90.3)	58.95 (48.3 – 79.0)	58.95 (46.2 – 90.3)
Waist percentile	98.8 (84.2 – 145.0)	97.85 (87.2 – 135.0)	99.1 (84.2 – 145.0)
% Normal weight participants	80.2	67.9	84.9
% Overweight participants	8.9	14.3	6.8
% Obese participants	10.9	17.9	8.2

Minimum – maximum values shown in brackets.

Food neophobia

The median food neophobia score was 34 (ranging from 10-70). The minimum and maximum possible scores on this questionnaire are 10 and 70 respectively. There was no difference for food neophobia score by gender or family history of food allergy and no association between food neophobia score and participant age, parental education/occupation status, maternal age or any infant feeding factors. There was no difference between CME and control groups, with the CME group scoring a median of 36 (12-60) and the control group scoring a median of 34 (10-70). There was no association found for number of foods excluded. Food neophobia was not correlated with any macro or micronutrient intake or growth measurement.

Discussion

This study is the first to investigate the long term effect of consuming a substitute infant formula and CME diet in infancy on taste preference, food neophobia, nutritional intake and growth. We have demonstrated significant differences in bitter taste preference between groups, in addition to differences in intakes of some micronutrients (iodine, riboflavin, selenium and sodium) and some foods/food groups (dairy products, dairy substitute products and chocolate). This demonstrates that consuming a substitute formula and exclusion diet for CMA in infancy has a persistent effect, even once cows' milk has been reintroduced into the diet several years previously. There is also a trend that a higher proportion of children in the CME group are now overweight or obese compared to the control group, which although not statistically significant, is both novel and concerning.

The significant difference in bitter taste preference between groups is an important finding. It is supported by previous studies in young children[14,41]. It concurs with the hypothesis that feeding infants altered tasting hydrolysed or soya formulae during a period of developmental plasticity in the first few months of life can manipulate preferences to like innately disliked sour and bitter tastes associated with fruit and vegetables[14,42]. Although a genetic tendency to reject bitter tastes and possibly prefer sweet taste exists, it is thought to only have limited influence on weight status and food preferences in daily life[43,44]. Therefore the early origins of chronic diseases such as obesity may derive from taste and food preferences that are "imprinted" from infancy [9,10,45]. This is relevant from a public health perspective as excess intake of salty and sweet foods is related to many long-term conditions. The lack of correlation between any taste preference and any growth measurement, infant feeding variable or number of foods excluded is not surprising given the sample size.

Only one study was identified in the literature that assessed taste preference in children older than seven years previously fed substitute formula[8]. This study (n = 833) found a positive association between feeding hydrolysed formula in infancy and the acceptance of extensively hydrolysed casein formula at age ten; although the data distribution was extremely skewed as all children rated the taste of the formula very negatively[8]. Due to the timing of the FAIR and PIFA studies, the majority of children in the CME group were fed soya formula, which is not currently indicated as first line treatment of CMA in infant under six months old [4,5]. However as we did not detect any difference between formula groups, it is not possible to say whether being fed an extensively hydrolysed, amino acid or soya formula has any greater

effect on bitter taste preference. Additionally amongst the CME group, because bitter taste preference was not found to be significantly correlated with age of introduction/duration of substitute formula, age of introduction of solids, duration of breastfeeding or number of foods excluded, it is difficult to draw any firm conclusions.

The results of the food neophobia questionnaire demonstrated no difference between dietary exclusion groups. This could be due to the age of the participants, as neophobia is thought to peak between 2-6 years old[21] or the sample size. Existing research on food neophobia and previous dietary exclusion is sparse, with only one study identified. Rigal et al.[46] compared food neophobia in children of mean age 7-9 years who had outgrown their food allergy to a sibling, concluding that previously food allergic children are more reluctant to try new foods than their non-allergic sibling. It is not possible to directly compare our questionnaire scores to that study as different questionnaires were used. We did not find any association between neophobia and nutritional or food group intake, which is in contrast to other literature [47,48]. This could be because all participants in the CME group received nutritional advice and dietetic input is known to improve nutritional outcomes in food allergy or because the study was underpowered[24,49].

The food diary response rate in this study was good, being similar to other food allergy studies[23,24] and superior to the NDNS response rate of 56%[34]. Because UK nutritional requirements are grouped into two age brackets that did not precisely match this study, the 7-10 year age bracket was used[37]. Overall, participants met the EAR for all nutrients. Intakes of some minerals appeared suboptimal, however all exceeded the LRNI. This is very similar the most recent NDNS which reported that in children under 11 years old intakes of all minerals were at or above the RNI[34]. Median vitamin D intakes were low in all participants (1.83 mcg/day). Likewise the NDNS reported mean daily intake for children and adolescents of 2.7 mcg and 2.4 mcg respectively, with 20% of children having low serum vitamin D[34]. Although there is no DRV in the UK for vitamin D for children over five years old, using the arbitrary value of 10 mcg/day[50]; it can be concluded that intake in all participants is insufficient.

Calcium has been identified as the key at-risk nutrient in children consuming exclusion diets[26], although more recent research highlights that other micronutrients are at risk of deficiency and excess, with under and over supplementation a concern[50,51]. The results of food category analysis show that the CME group consumed significantly less dairy products over a four day period. As there was no difference in calcium intake between groups, it is

possible that the CME group take dietary supplements to compensate for the possible deficit of calcium incurred, however this is only speculation. Dairy products are an important dietary source of calcium, phosphorus, magnesium, zinc, iodine, potassium, vitamin A, vitamin D, vitamin B12, and riboflavin. In this study, the significantly lower intakes for iodine and riboflavin in the CME group could be attributed to a lower intake of dairy products. In the NDNS, the major contributor to riboflavin intake was 'milk and milk products', accounting for 41% of daily intake in children aged 4-10 years. Similarly 'milk and milk products' was the largest contributor to iodine, providing 51% of intake[34].

Conversely, the significantly higher intakes in the CME group for sodium and selenium could be explained by proportionately higher intakes of non-dairy foods, specifically soya products are a good source of selenium. NDNS data indicates that approximately one third of both sodium and selenium intakes in 4-10 year olds is derived from cereal products, followed by meat/meat products[34]. We showed that the CME group consume slightly more fruit than the control group over a 4 day period, however this difference was not significant. The trend of higher intakes of fibre, vitamin A and vitamin C in the CME group, would concur with this hypothesis as these are nutrients that are typically found in fruit. Indeed it has previously been suggested that children with a food allergy history have a tendency to establish "healthier" eating habits[52]. Overall it is unlikely that the differences between groups would have a meaningful health significance as both groups met the EAR for all nutrients. However, the suboptimal vitamin D content across all participants is of concern.

Growth of children with CMA and other food allergens has been thoroughly investigated across many countries[23,53–57]. The only study comparing long term growth of children fed substitute formula for CMA did not show any difference in growth at age 10 years [58]. A Japanese study of 7-15 year olds (n = 14669)[52] reported that those with a history of consuming an exclusion diet had lower weight z scores, with an overall lower incidence of overweight and obesity; however the data on food avoidance was collected retrospectively. The lack of significant difference detected between dietary exclusion groups in the present study could be expected given the sample size, the multitude of factors that influence growth and because most macro and micro nutrient intakes did not differ significantly between groups**.** The finding that a higher percentage of participants in the CME group consumed >100% of the EAR for energy, is a novel finding and is worth further exploration.

The high median waist circumference centile observed is possibly a reflection of the rising rate of central obesity and that waist circumference charts rely on data collected in 1990 [59]. The overall percentage of children classified as overweight or obese (19%) is lower than national statistics, with the most recent data indicating 19.1% of children aged 10-11 are obese and a further 14.4% are overweight[60]. However it is particularly interesting that proportionately nearly double the amount of children in the CME group were overweight/obese compared to the control group, although this difference was not statistically significant. Meyer et al.[55] has previously identified that obesity is an increasing concern in children with food allergy and that the emphasis should not always be on under nutrition. As we did not measure body composition or account for physical activity, it is not possible to determine the reason for the larger proportion of overweight and obese children in the CME category. However, it is clearly an area that requires further examination.

There are both limitations and strengths to this study. The taste preference methodology used, although basic and simple in approach and exploratory in nature, used validated scales and dilution of taste substrates that have previously been identified as appropriate in this age group[32,61]. Perhaps using food rather than flavoured water would have provided more meaningful implications, however sensory research in children is complex and labour intensive[33]. We did not measure genetic perception of bitter taste. As with any dietary assessment method, food diary recording and analysis are subject to error and bias and there are difficulties using proxy respondents for children[62–64]. Use of electronic tools may yield improved accuracy and response rates. However, all analyses and measurements were conducted by the same researcher to minimise error. Unfortunately the study was less well powered than planned, particularly the CME group, which was composed of participants with a history of consuming both single and multiple exclusion diets. Due to the small sample size of this group (n = 28), there may be limitations with the analyses when looking at the CME group alone or in comparison to the control group, particularly when comparing different substitute formulas consumed. Although the study took place in the South of England, infant feeding and dietary intake data were extremely similar to national data, suggesting the participants habits are representative of the rest of the country. The unique strengths of the study are the availability of prospectively collected infant feeding data, long term follow up and a well matched control group.

In conclusion, this study provides preliminary evidence that use of a substitute formula and exclusion diet for CMA has a long term effect on bitter taste preference and dairy product

intake persisting into early adolescence, with potential to track into adulthood. Nutritional intake may be affected, particularly the intake of some less obvious micronutrients, but not calcium as may be expected. There may also be a long term effect on the risk of overweight and obesity, although this topic requires more in depth research with a larger sample size.

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