

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

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6 **Key words:** taste preference, cows' milk allergy, dietary intake, food neophobia

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38 **Author contributions:** KM designed the study, collected and analysed the data and drafted  
39 the manuscript. KG was study co-ordinator of the PIFA study, Co-PI of the PIFA birth study  
40 and iFAAM follow-up study and assisted with recruitment and design of the follow up study.  
41 EO was the study coordinator for the follow up study. GR was the PI for the PIFA study and  
42 lead PI of the follow up study. TD was the PI for the FAIR birth cohort study and contributed  
43 to study design of the follow up study. SHA was involved in the design of the FAIR birth  
44 cohort study and supervised the design of the follow up study. JG and GG were involved in  
45 recruitment of participants and organisation of data collection for the FAIR birth cohort and  
46 follow up study. CV co designed this study, supervised the operation of the study and  
47 contributed to manuscript writing. All authors critically reviewed and approved the final paper.

48

#### 49 **Acknowledgements**

50 The authors would like to thank the families involved in the FAIR and PIFA studies.

#### 51 **Funding**

52 Supported by the UK Food Standards Agency (Project TO7046) as part of the EU  
53 EuroPrevall Project (contract no. FOOD-CT-2005-514000).

54 The authors declare there are no conflicts of interest.

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56

57

58 **Abstract**

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

64 **Methodology:** Children were predominantly recruited from two large birth cohort studies in  
65 the UK. Two groups were recruited: an experimental group of children who had consumed a  
66 CME diet during infancy and a control group, who had consumed an unrestricted diet during  
67 infancy. Parents completed a food neophobia questionnaire and an estimated prospective food  
68 diary. Children completed a taste preference test and their growth was assessed.

69  
70 **Results:** 101 children of mean age 11.5 years were recruited (28 CME and 73 controls).  
71 Children in the CME group had a significantly higher preference for bitter taste than those in  
72 the control group ( $p < 0.05$ ). There were significant differences between groups for intake of  
73 some micronutrients including riboflavin, iodine, sodium and selenium. Food neophobia did  
74 not differ between groups. 28% of the CME group were overweight/obese compared to 15%  
75 of the control group, however this difference was not statistically different.

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

80

## 81 **Introduction**

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

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

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

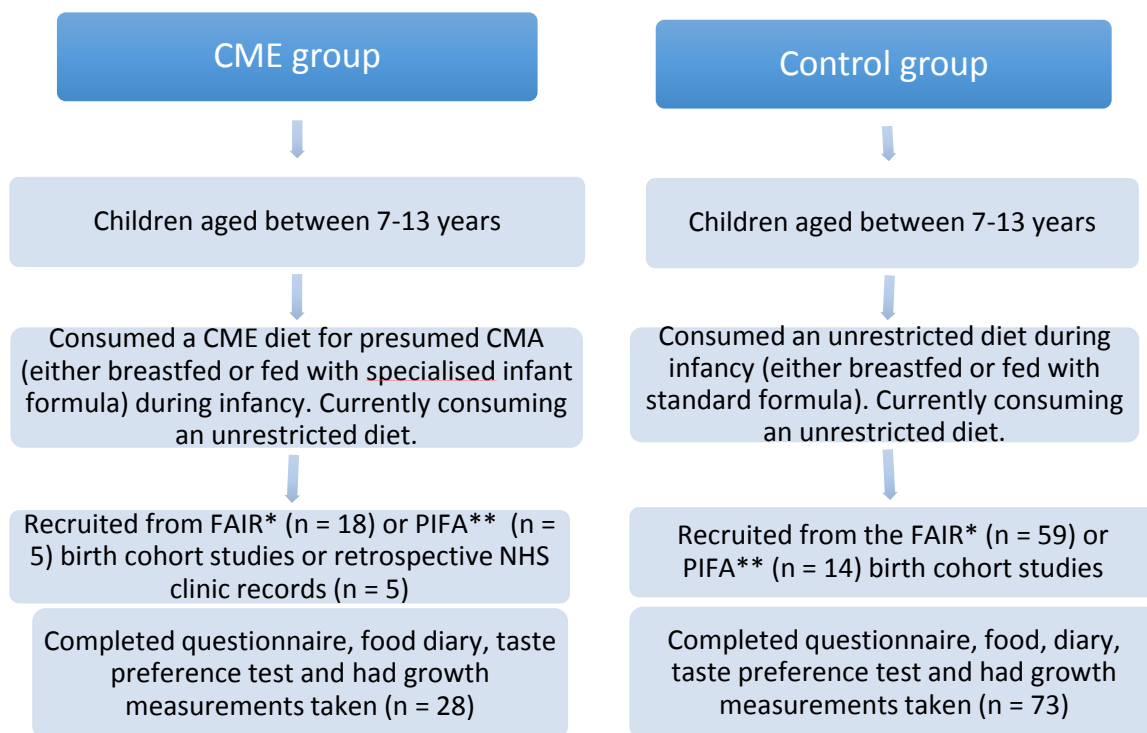
110 Several studies have demonstrated differences in nutritional intake and growth in  
111 children consuming exclusion diets, mostly reporting lower micronutrient intake and poorer  
112 growth<sup>(23-27)</sup>. Although milk allergy is usually outgrown, it is known that a proportion of food

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

## 118 **Methodology**

### 119 Study design and participants

120 This was a cross sectional study of 7-13 year old children from the Isle of Wight and  
121 Winchester area, UK. Figure 1 summarises the study design. Children were eligible for  
122 inclusion in the CME group if they had consumed a substitute formula and/or a CME diet in  
123 the first year of life for  $\geq 3$  months. Children excluding other food allergens (e.g. egg) in  
124 addition to cows' milk were also eligible for inclusion. Participants were primarily recruited  
125 from two birth cohort studies; the Food Allergy and Intolerance Research (FAIR)<sup>(1)</sup> and  
126 Prevalence of Infant Food Allergy (PIFA)<sup>(30)</sup> studies, born in 2001-2002 and 2006-2008  
127 respectively. For both of these studies, detailed prospective information was obtained about  
128 feeding practices in infancy. A small number of participants (n =5) were recruited from NHS  
129 allergy clinics from the Isle of Wight to increase the sample size. Children with current food  
130 allergy or any condition requiring a special diet were excluded. The study was approved by  
131 Berkshire NHS ethics committee (reference 13/SC/0194). Written informed consent was  
132 obtained from both parent and child.



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136

Figure 1 Summary of study design

137 \*The FAIR study recruited infants born on the Isle of Wight<sup>(1)</sup>.

138 \*\*The PIFA study recruited infants born in the Winchester area<sup>(30)</sup>.

139 Data collection

140

141 Participants eligible for inclusion in the CME group were identified by the study coordinators  
142 of the FAIR and PIFA studies. Control participants were identified as the consecutive study  
143 participants to each identified CME participant in the database. Extensive information about  
144 social demographics, infant feeding, family and allergy history was available from the original  
145 birth cohort dataset. For participants recruited from NHS allergy clinics, information was  
146 extracted from medical notes.

147

148 Food neophobia

149 Food neophobia was measured using the Child Food Neophobia Scale<sup>(31)</sup> a validated parentally  
150 completed questionnaire. In the current study the Cronbach alpha correlation was 0.921,  
151 indicating good internal consistency.

152 Taste preference

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

160 Nutritional intake

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

168 Food diary coding and analysis

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

177 Food groups

178 Frequency of intake of dairy products, dairy substitutes (i.e. soya milk), fruit, vegetables,  
179 chocolate and non-chocolate confectionary were calculated from the diaries, using published  
180 age appropriate portion sizes <sup>(36)</sup>.

181 Growth

182 Weight was measured using an electronic scale in kg to one decimal place. Height was  
183 measured using a stadiometer in cm to one decimal place. Weight for age percentile was  
184 calculated using a UK growth chart<sup>(38)</sup>. Body Mass Index percentile (BMI%) was calculated  
185 and plotted on a standard UK chart. Overweight and obesity were defined as BMI% > 91<sup>st</sup> and  
186 > 98<sup>th</sup> respectively<sup>(39)</sup>. Waist circumference was measured in cm to one decimal place and  
187 plotted on a UK centile chart. It was measured as the “narrowest waist”, which is the most  
188 frequently recommended site<sup>(40)</sup>. All measurements were conducted by the same researcher.

189 Statistical analyses

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

195

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

199

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

## 202 **Results**

203 101 participants were recruited, 28 in the CME and 73 in the control group. Participant  
204 demographic characteristics are detailed in table 1. No significant difference was found  
205 between the CME and control groups for age, gender, ethnicity, number of siblings, parental  
206 education or paternal food allergy history. Significant differences were found for maternal and  
207 sibling food allergy history ( $p < 0.05$ ), with those in the CME group having higher rates of  
208 both.

209

210

211



212 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)
<i>Ethnicity</i>			
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)
<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>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)*

213 \*p < 0.05

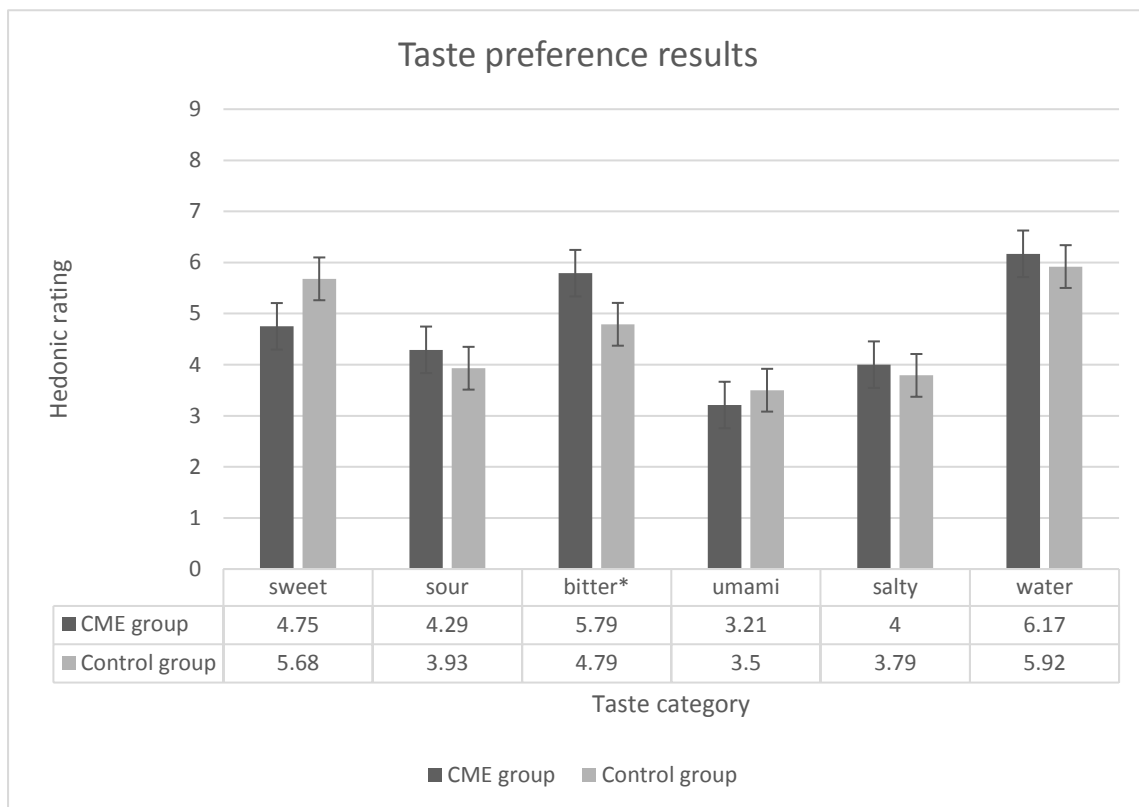
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215 Infant feeding and dietary exclusion

216 Detailed infant feeding data has previously been published <sup>(17)</sup>. In brief, substitute formula was  
 217 initiated at a median age of 11.5 weeks (range 2-40) in the CME group, with a median duration  
 218 of usage of 67.5 weeks (range 16-205). The majority of the CME group were fed soya formula  
 219 (50%), followed by extensively hydrolysed casein formula (21.4%), extensively hydrolysed  
 220 whey formula (17.8%) and amino acid formula (10.7%). Within the CME group, 50% excluded  
 221 only cows' milk during infancy, 39.3% excluded two foods during infancy and 10.7% excluded  
 222 three foods during infancy. All participants were consuming unrestricted diets at the time of  
 223 the study.

224 Taste preference

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



234  
 235 Figure 2. Taste preference results. \*significant difference between groups  $< 0.05$ . Higher scores  
 236 indicate a better perceived taste and vice versa.

237 Nutritional Intake

238 Food diaries were returned for 64 participants (63.3%); 17 from the CME group (60.7%) and  
 239 47 (74.6%). from the control group. There was no difference between those who did and did  
 240 not return the diary for age, gender, parental education, maternal age, food exclusion history,  
 241 family history of food allergy, growth or food neophobia. A summary of nutritional intake is

242 shown in table 2. Using the 7-10 year old age bracket as a guide, overall participants met the  
243 Estimated Average Requirement (EAR) for all nutrients. Looking at energy intake, there was  
244 no significant difference in % EAR consumed between groups. However, when examining  
245 proportions of participants meeting the DRV for energy, 41% of participants in the CME group  
246 (n =7) consumed >100% of the EAR, compared to 14.9% of participants in the control group  
247 (n =7) (p = 0.032). Intakes of some minerals appeared suboptimal (iron 72% of RNI, zinc and  
248 magnesium both 74% of RNI), however they were above the EAR. Boys had significantly  
249 higher intakes than girls for protein, sodium, iron, zinc, magnesium, iodine and phosphate (p <  
250 0.05 for all).

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

257         As the intake of some nutrients was found to be significantly different between boys  
258 and girls, a two way between groups ANOVA was conducted to compare sodium and iodine  
259 intakes between groups, controlling for gender. After adjusting for the gender, a significant  
260 difference between groups persisted for iodine intake (p < 0.01). Gender was not found to be  
261 significantly related to iodine intake whilst controlling for dietary exclusion group (p = 0.068,  
262 partial eta squared = 0.057). In terms of sodium intake, the same trend emerged. After adjusting  
263 for the gender, a significant difference between the CME and control groups persisted (p <  
264 0.01).

265

266 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)

267 %Reference nutrient intake is shown in brackets. \*significant difference between groups using  
 268 a Mann Whitney test  $p < 0.05$ . Analysis includes nutritional supplements.

269

270

271

272 Dietary supplements

273 In total 21 (20.7%) participants took dietary supplements, 7 (25%) from the CME group and  
 274 14 (19.2%) from the control group. Two of the CME group took calcium/vitamin D  
 275 supplements, with the remainder taking multivitamin/mineral combinations. All 14 of the  
 276 control group took multivitamin/mineral supplements.

277 Food group intake

278 Intakes of selected food groups are shown in table 3. Two participants in the CME group  
 279 consumed dairy substitutes (soya milk and yoghurt), in addition to dairy products. The CME  
 280 group consumed significantly less dairy products and chocolate than the control group ( $p <$   
 281  $0.01$ ), but significantly more dairy substitute products ( $p < 0.05$ ). There was no difference in  
 282 consumption of fruit, vegetables or non-chocolate confectionary between groups. Consumption  
 283 of food groups was not associated with neophobia, infant feeding variables or any growth  
 284 measure. There was an inverse correlation between bitter taste preference and dairy intake ( $\rho$   
 285  $= -0.382$ ,  $p < 0.01$ ) and also between chocolate intake and sour taste preference ( $\rho = -0.331$ ,  
 286  $p < 0.05$ ).

287 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

288 Median number of portions consumed. Minimum-maximum values in brackets.\*Mann

289 Whitney test p value significant  $< 0.05$ .

290

291 Growth

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

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

300 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

301 Minimum – maximum values shown in brackets.

302

303 Food neophobia

304 The median food neophobia score was 34 (ranging from 10-70). The minimum and maximum  
 305 possible scores on this questionnaire are 10 and 70 respectively. There was no difference for  
 306 food neophobia score by gender or family history of food allergy and no association between  
 307 food neophobia score and participant age, parental education/occupation status, maternal age  
 308 or any infant feeding factors. There was no difference between CME and control groups, with  
 309 the CME group scoring a median of 36 (12-60) and the control group scoring a median of 34  
 310 (10-70). There was no association found for number of foods excluded. Food neophobia was  
 311 not correlated with any macro or micronutrient intake or growth measurement.

312

313 **Discussion**

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

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

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

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

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

361 The food diary response rate in this study was good, being similar to other food allergy  
362 studies<sup>(23,24)</sup> and superior to the NDNS response rate of 56%<sup>(34)</sup>. Because UK nutritional  
363 requirements are grouped into two age brackets that did not precisely match this study, the 7-  
364 10 year age bracket was used<sup>(37)</sup>. Overall, participants met the EAR for all nutrients. Intakes of  
365 some minerals appeared suboptimal, however all exceeded the LRNI. This is very similar the  
366 most recent NDNS which reported that in children under 11 years old intakes of all minerals  
367 were at or above the RNI<sup>(34)</sup>. Median vitamin D intakes were low in all participants (1.83  
368 mcg/day). Likewise the NDNS reported mean daily intake for children and adolescents of 2.7  
369 mcg and 2.4 mcg respectively, with 20% of children having low serum vitamin D<sup>(34)</sup>. Although  
370 there is no DRV in the UK for vitamin D for children over five years old, using the arbitrary  
371 value of 10 mcg/day<sup>(50)</sup>; it can be concluded that intake in all participants is insufficient.

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



377 possible that the CME group take dietary supplements to compensate for the possible deficit  
378 of calcium incurred, however this is only speculation. Dairy products are an important dietary  
379 source of calcium, phosphorus, magnesium, zinc, iodine, potassium, vitamin A, vitamin D,  
380 vitamin B12, and riboflavin. In this study, the significantly lower intakes for iodine and  
381 riboflavin in the CME group could be attributed to a lower intake of dairy products. In the  
382 NDNS, the major contributor to riboflavin intake was ‘milk and milk products’, accounting for  
383 41% of daily intake in children aged 4-10 years. Similarly ‘milk and milk products’ was the  
384 largest contributor to iodine, providing 51% of intake<sup>(34)</sup>.

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

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

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

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

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

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

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