Clinical and Translational Allergy

Nutritional adequacy of a cows' milk exclusion diet in infancy

--Manuscript Draft--

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Full Title:	Nutritional adequacy of a cows' milk exclusion diet in infancy	
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Abstract:	Abstract Background: Infants with suspected Cows' Milk Allergy (CMA) are required to follow a strict milk exclusion diet which may lead to nutritional deficiencies, especially if not supervised by a healthcare professional. The aim of this study was to assess the nutritional adequacy of a cows' milk exclusion diet in a group UK infants over a period of six months. Methods: Participants in this study are a subgroup of the Prevalence of Infant Food Allergy (PIFA) study, a prospective food allergy birth cohort study from the South of England. Each infant consuming a milk free diet, following advice from a specialist allergy dietitian, was matched to two control infants who were consuming an unrestricted diet, forming a nested matched case-control study. Detailed food diaries completed prospectively for one week per month over a five month period, were coded and analysed according to a standard protocol. Results: The diets of 39 infants, (13 milk-free and 26 controls) were assessed. Mean age at diet commencement was 14 weeks. Two of the eleven infants started on an extensively hydrolysed formula (EHF) did not tolerate it and required an amino acid formula (AAF) for symptom resolution. All infants had mean intakes in excess of the Estimated Average Requirement (EAR) for energy and the Recommended Nutrient Intake (RNI) for protein, calcium, iron, selenium, zinc, vitamins A, C and E. Vitamin D intake was in excess of the RNI at all time-points, except at 44 weeks of age. Across the study period, selenium intake was higher for infants consuming a milk free diet whilst Vitamin C intake was higher for infants consuming an unrestricted diet. Differences were found between the two groups for protein, calcium, iron and vitamin E intakes at differing time points. Conclusion: This study demonstrated that although infants consuming a milk-free diet have a nutritional intake that is significantly different to matched controls who are eating an unrestricted diet, this difference is not constant and it is not seen for all nutrients. Further research in infants without dietetic input is needed to explore the nutritional implications of unsupervised cows' milk exclusion diets.	
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Response to Reviewers:	Dear Editor-in-chief, Thank you for reviewing this manuscript and for the input of the reviewers. We have taken account of your comments and rewritten the manuscript into a brief communication. Please find below the detailed list of reviewer comments and our responses. Reviewer #1: As I think I understand it, children were selected for inclusion into the study on the basis of completing 3 diaries over a 12 week period. The data that was analysed were the diaries recorded between 24 and 44 weeks of age (5 months, as opposed to the 6 months mentioned in the abstract - this needs correcting). This has been changed to five months (line 16). The methods section needs further clarification. It would be improved by including separate sections on; + Selection of participants - are these patients who had already completed diary cards earlier in the study?? As part of the birth cohort study protocol, all participants were asked to complete a food diary in the 4th week of every month between birth and on year of age. The data from the early food diaries has not been included in this paper as dietary intake at that age was limited to just milk (formula or breast milk). Inclusion of participants in this study was based on infants who reported an adverse reaction to cow's milk and met the inclusion criteria to have at least 3 quantitative diaries collected over 12 weeks available for analysis. It is not possible to explain this in detail due to word count, however the inclusion criteria is specified in lines 105-107, including a new subheading of "selection or participants", as recommended. + Dietetic support - I think this was only provided for the milk exclusion group, but it needs to be specified. Yes this is correct. This has been clarified by adding a sentence: "Children who did not report an adverse reaction to cows' milk did not receive any dietetic input" (see lines 101-102). A new subheading of "dietetic support" has been added as recommended. + Data collection - diary cards in both groups were completed from 24 weeks to 44 weeks of age. This is included in line 119. Were breastfeeding rates the same in both groups - could this be included in Table 1? The control infants were matched to their symptomatic infant for age, number of weeks food diaries were returned and breastfeeding status. For example, if the milk-excluding child was breastfed to 6 months then their control infants were also breastfed to 6 months. This was to ensure any nutrient differences seen between the groups were due to the exclusion diet and not to the differing composition of breast milk and infant formula. This is included in the methods section (lines 108-109). There was no significant difference between duration of breastfeeding and if an infant had ever been breastfed, which has been added to Table 1. Only two of the 13 milk-free infants included in this study were being breastfed at commencement of the milk free diet. Their control infant would also have been breastfed at this age. Was formula intake the same in both groups? Unfortunately we are not able to access the data on exact volume of formula consumed. We are aware that some of the differences in micronutrient intakes between groups may be due to the volume of formula consumed, however due to word count limits, it is not possible to discuss this in detail. We have discussed the nutritional composition of follow on formula and how the differences in formula usage between groups could contribute to micronutrient differences between groups throughout the

	discussion section (lines 235-267). Table 5 refers to 'symptomatic' and 'control' infants - This is confusing as you are comparing infants who are asymptomatic on a milk free diet, to the controls. Could 'symptomatic' be changed to 'milk-free' in Table 5 and elsewhere in the paper. This has been done. All infants had mean intake in excess of requirements for energy. Do you have any growth data? As the emphasis of this study was on nutritional intake rather than growth, we have not included growth data in this manuscript, however there was no difference between groups. We have cited papers that focus on growth in the introduction section (lines 67-68) (Vieria et al., 2010 and Agostini et al., 2007). Due to word count limitations and the request by the editor to reformat the manuscript into a brief communication, it is not possible to include data and discussion on growth. I enjoyed reading the discussion Thank you for your comments Reviewer #2: The paper is well written statistical good and highlights the potential pitfalls of a cmpa free diet or not as this paper suggests. The prospective and in depth analysis adds. The paper needs only minor revision but the following need clarifying 1. What is the allergic status of the patients were they confirmed Non-IgE by challenge The cumulative incidence of CMA in the whole birth cohort in the first two years of life is 2.4%, of which 1.7% is non IgE CMA. This has recently been published in this journal (reference: Grimshaw et al. Incidence and risk factors for food hypersensitivity in UK infants: results from a birth cohort study. Clin Transl Allergy. 2016 Jan 26;6:1). Due to reformatting this manuscript into a brief communication it is not possible to go into any detail about diagnostic food challenges, but we have cited the Grimshaw CTA (2016) paper in the first paragraph of the introduction for readers' attention (line 48). 2. Was the same weaning schedule used and is yes what other foods were identified for example soya was this restricted for the 3 months that these children were followed up for after weaning (if mean was 14 weeks then assume most were followed to 9 months of age ?) Yes all infants in the milk-free groups received the same weaning advice by the same specialist allergy dietitian. Soya was not restricted. This has been clarified by adding a sentence to the methods: "These infants were not excluding any other food from their diet (e.g. soya)." (line 99). Was there any feeding symptoms that were being treated as this symptom could impact on intake and is a feature of allergy? None of children presented with feeding problems, this may be because they received individualised dietetic input at an early age which may have prevented problems occurring. We have added a sentence to the discussion to suggest this: "including timely advice to encourage a varied diet, which may have helped prevent fussy eating and feeding problems" (see lines 204-206). 4. What was the prevalence of CMPA in this cohort as the number seems very small here 13/1140 seems very low was this a recording of identification concern? In total 74/1140 infants were required to follow a milk free diet as part of the birth cohort study. Of the 74 infants, 13 infants met the inclusion criteria to have at least 3 quantitative diaries collected over 12 weeks available for analysis. This has been added to the results section (lines 136-139): "In total 74 infants were required to follow a milk free diet as part of the birth cohort study. Of the 74 infants, 13 infants met the inclusion criteria to have at least 3 quantitative diaries collected over 12 weeks available for analysis". The cumulative incidence of CMA in the whole birth cohort in the first two years of life is 2.4%, of which 1.7% is non IgE CMA. This has recently been published in this journal and is cited in the first paragraph of the introduction (reference: Grimshaw et al. Incidence and risk factors for food hypersensitivity in UK infants: results from a birth cohort study. Clin Transl Allergy. 2016 Jan 26;6:1). Reviewer #3: In this paper the authors tried to assess the nutritional intake in infant following a cow's milk exclusion because of suspected having an adverse reaction to cow's milk. The study cohort was not proven to be allergic to cow's milk.

	My comments: 1. The children were suspected of having an adverse reaction to cow's milk (line 153) therefore cow's milk was excluded (line 153-156). What about goat, sheep's milk etc and relevant products? This has been clarified by addition of the sentence: “Advice was provided to avoid other mammalian milk and milk products (e.g. sheep, goat) as there is known cross reactivity.” (lines 96-99). 2. The scope of this study was built on facts based on milk-allergic individuals. This individual may have different gastrointestinal characteristics that may affect bioavailability of the different nutrients consumed than other individual with other disorders. Therefore the study's inferences are not based on a cohort of allergic individuals and therefore it is not know if they can be extrapolated to allergic children. As part of the protocol for the birth cohort study, all children with a clinical history of food hypersensitivity were challenged after a period of dietary exclusion. However there was a mean time lag of 4 months between dietary exclusion and the food challenge taking place. You are therefore correct that it is not possible to say whether these children were milk allergic or not at the time of dietary exclusion, as food challenge had not all yet taken place when this data was collected. This has been discussed in a previous publication (Schoemaker et al. 2015). Because of the diagnostic uncertainty, we have deliberately not referred to the milk-free group as milk allergic throughout the document and have sought only to compare their nutritional intake, and not their allergic status or symptoms per se. Schoemaker et al. (2015) Incidence and natural history of challenge-proven cow's milk allergy in European children--EuroPrevall birth cohort. Allergy. 2015 Aug;70 (8):963-72. doi: 10.1111/all.12630. Epub 2015 May 18. 3. It is not clear to me if only 13 infants (197) out of the 1140 recruited in total (line 146) met the inclusion criteria. If this is the case, can only 13 infants provide enough statistical power to discriminate non-significant nutritional differences from randomly occurring significant ones (and the opposite)? If yes, then at least provide a post hoc power analysis. We have added the following sentence to the results section to give more detail about the response rate and inclusion criteria (lines 136-139): “In total 74 infants were required to follow a milk free diet as part of the birth cohort study. Of the 74 infants, 13 infants met the inclusion criteria to have at least 3 quantitative diaries collected over 12 weeks available for analysis”. It known that food diary completion places considerable burden on the respondent, which may lead to a response bias. This has been acknowledged as a limitation in the discussion section and the small sample size has also been mentioned. See lines 285-289. “Additionally, since the data set is relatively small, there is potential for sampling error and response bias, but as the data is prospective and longitudinal, the patients well defined and the analysis completed across all the time points, this potential is reduced. Overall, the sample size of 39 is comparable to other published studies of dietary intake in CMA (21,22,28).” 4. How many of the recruited children were breastfeeding at the time of recruitment in addition to formula milk? Do they differ between the two examined groups? The control infants were matched to their symptomatic infant for age, number of weeks food diaries were returned and breastfeeding status. For example, if the milk-excluding child was breastfed to 6 months then their control infants were also breastfed to 6 months. This was to ensure any nutrient differences seen between the groups were due to the exclusion diet and not to the differing composition of breast milk and infant formula. This is included in the methods section (lines 108-109). There was no significant difference between duration of breastfeeding and if an infant had ever been breastfed, which has now been added to Table 1. Only two of the 13 milk-free infants included in this study were being breastfed at commencement of the milk free diet. Their control infant would also have been breastfed at this age. 5. The statement in lines 268-270 should be mentioned in the abstract in addition to the fact that all recruited children were given dietary advice from a specialist allergy dietitian (lines 272-273, 283-285). This phrase “following advice from a specialist

	allergy dietitian" has been added (lines 12-13), however due to word limit constraints, it is not possible to include all the information suggested in the abstract. We hope you are satisfied with these changes and the reformatting of the documents. Yours sincerely, Kate Maslin, PhD, RD Kate Grimshaw PhD, RD

Nutritional adequacy of a cows' milk exclusion diet in infancy

Abstract

Background: Infants with suspected Cows' Milk Allergy (CMA) are required to follow a strict milk exclusion diet which may lead to nutritional deficiencies, especially if not supervised by a healthcare professional. The aim of this study was to assess the nutritional adequacy of a cows' milk exclusion diet in a group UK infants over a period of six months.

Methods: Participants in this study are a subgroup of the Prevalence of Infant Food Allergy (PIFA) study, a prospective food allergy birth cohort study from the South of England. Each infant consuming a milk free diet, **following advice from a specialist allergy dietitian**, was matched to two control infants who were consuming an unrestricted diet, forming a nested matched case-control study. Detailed food diaries completed prospectively for one week per month over a **five** month period, were coded and analysed according to a standard protocol.

Results: The diets of 39 infants, (13 milk-free and 26 controls) were assessed. Mean age at diet commencement was 14 weeks. Two of the eleven infants started on an extensively hydrolysed formula (EHF) did not tolerate it and required an amino acid formula (AAF) for symptom resolution. All infants had mean intakes in excess of the Estimated Average Requirement (EAR) for energy and the Recommended Nutrient Intake (RNI) for protein, calcium, iron, selenium, zinc, vitamins A, C and E. Vitamin D intake was in excess of the RNI at all time-points, except at 44 weeks of age. Across the study period, selenium intake was higher for infants consuming a milk free diet whilst Vitamin C intake was higher for infants consuming an unrestricted diet. Differences were found between the two groups for protein, calcium, iron and vitamin E intakes at differing time points.

Conclusion: This study demonstrated that although infants consuming a milk-free diet have a nutritional intake that is significantly different to matched controls who are eating an unrestricted diet, this difference is not constant and it is not seen for all nutrients. Further research in infants without dietetic input is needed to explore the nutritional implications of unsupervised cows' milk exclusion diets.

Key words: cows' milk allergy, dietary exclusion, nutritional intake, infant

Background

Cows' milk allergy (CMA) is the most common infant food allergy with an estimated prevalence of 1.26-2.9% in the United Kingdom (1,2), the majority of which is non-IgE mediated (3). Parents of reactive children are advised that their child should follow a special weaning diet avoiding all forms of cows' milk until the allergy is outgrown. This avoidance should ideally be supported by input from an allergy dietitian to monitor and optimise the nutritional content of the diet and to maintain potential growth (4,5).

It is thought that perceived food allergy could be ten times higher than that confirmed by appropriate tests (6). This is particularly the case in paediatric food allergy, where parents may incorrectly perceive their child to have experienced an adverse reaction to a food (7) . With allergy services considered inadequate to meet demand in many countries (8), unwarranted exclusion diets are often initiated by parents (9–12). This heightens the likelihood of unsupervised exclusion diets at a time in life that is critical for growth, development and establishment of eating habits.

Adequate nutritional intake in infancy is essential to ensure appropriate physiological and mental development (13). Exclusion of any food group can result in a nutritionally deficient diet, but the elimination of dairy in infancy is particularly likely to cause nutritional deficiencies (14). This is highly significant as both reduced dietary variety (15,16) and deficiencies of specific micronutrients (17) are postulated to be implicated in food allergy development. Exclusion diets, in particular cows' milk exclusion diets, have been associated with poor growth in childhood (18,19).

Studies from various countries have investigated the nutritional intake of children consuming an exclusion diet secondary to cows' milk and other food allergies, demonstrating differences in both macro and micro nutrient intakes (20–30). However, most of the previous literature in this area is cross sectional. Since the assessment of dietary intake during infancy is complicated by changing development and food refusal (31), a snapshot of dietary intake is unable to accurately represent the changing infant diet. This study will compare the dietary intake of infants consuming a cows' milk exclusion diet for CMA to those consuming an unrestricted diet, with the aim of assessing adequacy of micro and macronutrient intake over a period of twenty weeks.

Methodology

Overview of birth cohort study: The data reported in this paper consists of a sub group of infants who were recruited as part of a prospective birth cohort study. The PIFA study, the UK arm of the EuroPrevall project (32), recruited 1140 infants between 2006 and 2008 in the Southampton/Winchester area in the South of England. Infants were followed up to 2 years of age in order to assess the prevalence and natural history of food allergies.

Data collection: As part of the study, parents kept prospective food diary data. Food diaries were completed until the age of one and returned every 4 weeks (33,34). Every fourth week the diaries were more detailed which allowed the infants macro and micronutrient intake to be calculated.

Dietetic support: Infants suspected of having an adverse reaction to cows' milk were given advice to follow a cows' milk exclusion diet to determine if their symptoms resolved. The advice, given by a specialist allergy dietitian, detailed strict and complete cows' milk avoidance, with accompanying written information and details of milk-free products and recipes provided. **Advice was provided to avoid other mammalian milk and milk products (e.g. sheep, goat) as there is known cross reactivity with cows' milk** (35)**. These infants were not excluding any other foods from their diet (e.g. soya)**. If symptoms improved on the exclusion diet, the infant continued with the diet and were termed "**milk-free**". **Children who did not report an adverse reaction to cows' milk did not receive any dietetic input.**

Selection of participants:

Each infant following a milk exclusion diet who had returned at least 3 weeks of quantitative diet data covering a period of twelve weeks had their dietary intake data analysed. Each reactive infant was matched to two control infants (who were consuming an unrestricted diet for their age), **according to age, number of food diaries available and breastfeeding status**, thus forming a nested matched case-control study.

Dietary analysis: Dietary analysis was performed with the dietary analysis package 'CompEatPro' (Nutrition Systems, 2008). Breast milk intake was estimated by age using average values obtained from previous published literature (36,37). Portion sizes were recorded in household measures and converted into weights using published data or by weighing the stated portion-size for that food. Food diaries were coded according to a standard protocol by two nutritionists and a dietitian. To ensure the most data was available for the RM-ANOVA, **diaries 6-11 (24 weeks to 44 weeks of age)** were analysed.

Statistical Analysis: Mean daily values for nutrient intake were calculated by the dietary analysis package, imported into Statistical Package for the Social Sciences version 18 (SPSS Inc) and compared to UK Recommended Nutrient Intakes (RNI)(38). A General Linear Model Repeated Measures analysis of variance with between subject factors (RM-ANOVA) was carried out to determine whether there was a difference in dietary intake between the groups for macronutrients and selected micronutrients. Specific time point analyses were carried out *post hoc*.

Ethical, consent and permissions: The North and Mid Hampshire Local Research Ethics Committee approved the study protocol (reference O5/Q1703/34). Written consent was provided for each participant by their parent/guardian.

Results

Participant characteristics: **In total 74 infants were required to follow a milk free diet as part of the birth cohort study. Of the 74 infants**, 13 infants met the inclusion criteria to have at least 3 quantitative diaries collected over 12 weeks available for analysis.

Mean age of infants at diet commencement was 14 weeks (range 5-36 weeks). Each **milk-free** infant was matched to 2 control infants, resulting in dietary analysis of 13 milk-free and 26 control infants. Baseline characteristics are detailed in table 1.

Table 1 Baseline characteristics of participants

	Milk free group (n = 13)	Control group (n = 26)	p
Caucasian ethnicity	12 (92.3)	26 (100)	0.333†
Female sex	4 (30.7)	11 (42.3)	0.728†
Mothers' mean age, years	32.0	32.4	0.872^
Fathers' mean age, years	34.2	34.9	0.988^
Highest education of parents			0.598§
Low (up to 12y)	3 (23)	8 (30.7)	
Intermediate (>12y, e.g. college)	5 (38.5)	6 (23)	
High (e.g. university)	5 (38.5)	12 (46.1)	
Allergies in family			
Maternal atopy (A, AR or E)*	11 (84.6)	16 (61.5)	0.269^
Paternal atopy (A, AR or E)*	7 (53.8)	16 (61.5)	0.736^
Maternal food hypersensitivity	2 (15.4)	3 (11.5)	1.000^
Paternal food hypersensitivity	2 (15.4)	7 (26.9)	0.689^
Urban living environment	2 (15.4)	2 (7.7)	0.589†
Mean number of siblings	0.6	0.3	1.000^
Mean birth weight (g)	3538	3476	0.738^
Mean duration breastfeeding (months)	**1.75**	**2.68**	**0.189^**
Ever breastfed	**7 (53.8)**	**22 (57.8)**	**0.742†**

Data are expressed as number (percentage) unless indicated
† Chi-square test of homogeneity unless indicated
Mann Whitney U test^
§ ANOVA F test

Eleven infants were initially put onto the same Extensively Hydrolysed Formula (EHF, Nutramigen, Mead Johnson), two of these then progressed onto an Amino Acid Formula (AAF, Neocate, Nutricia) as their symptoms did not improve on the EHF. Two infants had already been commenced onto a soya infant formula (Wysoy, Nutricia) by their General Practitioner. From 26 weeks, all infants consuming EHF were changed to an extensively hydrolysed follow-on formula. In the control group, 16 infants consumed a follow on formula from 26 weeks onwards, whilst 10 remained on their standard formula.

All infants had mean intakes in excess of the requirements for energy and the recommended intakes for protein, calcium, iron, selenium, zinc, vitamins A, C, D and E. RM-ANOVA 'between subject' analysis indicated that the mean daily intake differed significantly between the groups across the whole time period for selenium (p=0.003) and vitamin C (p=0.01) (shown in Figures 1 & 2). At all time-points, selenium intake was higher for infants following a milk free diet than for infants following an unrestricted diet (p=0.003).

Observed vitamin C intake decreased for both groups from the start of the 20 week period (24 weeks of age) compared to the end (44 weeks of age) and was higher for infants following an unrestricted diet than for infants following a milk free diet at all-time points (p = 0.001).

Figure 1 Estimated means for daily selenium intake (*µg)*

Figure 2 Estimated means for daily Vitamin C intake (mg)

Differences were also found between the two study groups at differing time periods for protein, calcium, iron and vitamin E. A summary of significant differences between groups is shown in Table 2.

Table 2. Time points between which there was a significant difference in nutrient intake between food allergic milk-free and matched control infants and nature of the difference observed.

Nutrient	Age between specific time points (weeks)	p value	Nature of difference in intake
Protein	28-32	p=0.039	Intake higher in **milk-free** infants compared to control infants between these weeks
Fat	32-36	p=0.023	Intake increases in **milk-free** infants at a greater rate than intake in control infants between these weeks
Calcium	36-40	p=0.025	Intake decreases in **milk-free** infants but increases in control infants between these weeks
Iron	24-28	p=0.028	Intake increases slightly in **milk-free** infants but increases sharply in control infants between these weeks
Selenium	24-28	p=0.049	Intake increases dramatically in **milk-free** infants but only slightly in control infants between these weeks
Vitamin E	32-36	P=0.044	Intake increases dramatically in **milk-free** infants but decreases slightly in control infants between these weeks

Discussion

This study aimed to compare the nutritional intake of a group of infants consuming a cows' milk free diet to a matched control group of infants consuming an unrestricted diet over a period of five months. All participants had mean dietary intakes in excess of the recommended levels (with the exception of vitamin D at age 44 weeks) and this is in agreement with data from the UK Diet and Nutrition Survey of Infants and Young Children (DNSIYC) (39). Whilst it is reassuring that both groups of infants met their requirements for most nutrients at all time points, it must be highlighted that the majority of infants in this study were born to well-educated mothers, who may be more likely to follow recommended feeding advice than less well-educated mothers (40).

It is well known that some parents may implement restricted diets without medical supervision (10) and previous research suggests that that infants consuming exclusion diets who had not received nutritional advice were likely to have diets deficient in vitamin D and calcium compared to those who had received nutritional advice (24). A recent study from Italy (30) confirmed that dietetic input has a positive significant effect on anthropometric and laboratory biomarkers of nutritional status in young children with CMA. In this study cows' milk avoidance advice was provided by a specialist allergy dietitian, **including timely advice to encourage a varied diet, which may have helped prevent fussy eating and feeding problems.** Therefore our findings cannot be extrapolated to infants not receiving individualised dietetic advice.

Since this study collected nutritional intake data from diet diaries completed prospectively, the diaries were re-examined *post hoc* to collect information on the actual foods eaten to further explain the observed results. The higher selenium and vitamin C intake for infants consuming a milk exclusion diet can be explained by the use of soya products as a dairy alternative. Compared to dairy based fruit yogurts, which contributed over 50% of the daily vitamin C intake in the control group, the soya desserts eaten by the milk free group did not generally contain fruit and therefore little if any, vitamin C. The intake of fruit as a finger food increased in the milk free group from 36 weeks of age and this explains the increase in vitamin C in the diets of these children from this time-point (figure 2). This increase was not seen in the control group, as their finger foods mainly consisted of milk containing foods (e.g. biscuits). The inclusion of biscuits as a regular weaning food may have implications for future preferences for sugary snack foods. It has been shown that those who consumed milk exclusion diets in infancy have lower preference for dairy foods such as chocolate and ice cream in later childhood (41).

Infants in the control group had a higher fat intake than the milk-free group at all time points, although this difference did not reach statistical significance. This is likely to be due to the inclusion of full fat dairy products in the diets of the control group. However, mean daily vitamin E intake (a fat-soluble vitamin) was broadly similar between the two groups until week 32. After this time-point, intake increased noticeably in the **milk-free** group, possibly due to the relatively rich vitamin E content of soya products. Of note, two previous studies (20,24) have also reported that children with food allergies consume more vitamin E than controls. This may be due to a recommendation to include vegetable oil as a non-dairy source of fat and calories in children with multiple food allergies (42).

Statistical analysis showed mean daily iron intake to be significantly higher in the milk-free group compared to the control group between weeks 24 and 28 (p=0.028), which can be attributed to the higher iron content of specialised formula used for CMA, compared to standard infant formula. Infants in the **milk-free** group transitioned to the “follow on” version of the specialised formula at age 26 weeks, under the guidance of the dietitian. The transition to follow on formula in the control group tended to occur at > 26 weeks, as they were not prompted to change by a dietitian. Similar to our results, Meyer et al. reported that intake of hypoallergenic formula was correlated to micronutrient intake in a group of children with food protein induced gastrointestinal allergy (29).

The significant difference in mean daily calcium intake between the two groups between 36 and 40 weeks can be attributed to a decline in formula intake. A decline in formula intake was seen in both groups, but infants aged between 4-11 months in the UK on an unrestricted diet consume between 53g to 147g per day of milk or milk products (39), which will compensate for the reduction in calcium intake from formula. In contrast, even though infants consuming a milk free diet may be consuming some calcium containing replacement foods, these may not be eaten in large enough quantities to compensate for the decrease in formula intake. However, it must be emphasised that all infants in the milk-free group met the RNI for calcium, with none requiring a calcium supplement. Meyer et al. (2015) noted that both deficiency and over supplementation of calcium is present in children consuming exclusion diets, implying that individualised dietetic advice rather than blanket recommendation of supplementation is warranted.

Higher protein intakes were found in the **milk-free** group, which is likely to be due to the higher protein content of specialised infant formula used in CMA. Although the difference is not large per 100mls (0.5g), in younger infants when total intake can be approximately 1000mls/day, this difference could equate to as much as 5g protein per day.

Although there was no significant difference in vitamin D intake between the two groups at any time point, intake did fall marginally below the RNI for both groups at the age of 44 weeks. This could be explained by a decline in the volume of infant formula consumed by both groups. Only one breastfeeding mother took a vitamin D supplement and no infant took a vitamin D supplement, despite Department of Health recommendations. Interestingly, the recent Diet and Nutrition Survey of infants and Young Children (DNSIYC) (2011) (39) reported that although only 7% of those aged 7 to 9 months and 8% of those aged 10 to 11 months took a multivitamin supplement, 94% of those aged 5 to 11 months had 25-hydroxyvitamin D (25-OHD) above the lower threshold for vitamin D adequacy.

A major and unique strength of the study is that the dietary information was collected prospectively, which eliminates any recall bias, an inherent error in other dietary assessment methods. A further strength of the study is that food diaries were collected for each infant on a monthly basis. The main limitation of the study is whether the finding that a milk free diet can meet nutritional requirements can be applied to infants who have not seen a dietitian for exclusion advice. Additionally, since the data set is relatively small, there is potential for sampling error and **response bias**, but as the data is prospective and longitudinal, this potential is reduced. Overall, the sample size of 39 is comparable to other published studies of dietary intake in CMA (21,22,28). However, it was not a randomised study and so results cannot be considered causal, but matching of the milk-free infants with controls, means the observed differences between the groups is likely to be due to the different diets rather than confounding variables.

Conclusion

This study demonstrates that infants consuming a milk-free diet have a nutritional intake that is significantly different to matched controls consuming an unrestricted diet, but the difference is not constant and it is not seen for all nutrients. Most of the differences are a consequence of the dairy alternatives included in the milk free diet at the recommendation of the specialist allergy dietitian. However, since the main carers of all the infants following a milk-free diet received advice from a specialist allergy dietitian, these observations cannot necessarily be applied to the general population since this level of support is not always widely available. Further research is needed to explore the nutritional implications of unsupervised cows' milk exclusion diets. However in the interim, it is important to continue to emphasise to parents and carers of infants not to restrict a child's diet without adequate medical or dietetic intervention.

Abbreviations

CMA: Cows' milk allergy

DNSIYC: Diet and Nutrition Survey of Infants and Young Children

EAR: Estimated Average Requirement

EHF: Extensively Hydrolysed Formula

PIFA study: Prevalence of Infant Food Allergy study

RNI: Reference Nutrient Intake

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Conflict of interest and funding disclosure

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Contributer's Statement

Kate Maslin was involved in statistical analysis, drafted the initial manuscript and approved the final manuscript as submitted. Erin Oliver was involved in data collection, diagnosis of milk allergy, nutritional analysis, revised the manuscript for important intellectual content and approved the final manuscript as submitted. Karen Scally was involved in data collection, nutritional analysis, revised the manuscript for important intellectual content and approved the final manuscript as submitted. Josh Atkinson was involved in statistical analysis and data management, revised the manuscript for important intellectual content and approved the final manuscript as submitted. Keith Foote was involved in the initiation and set up of the PIFA study, revised the manuscript for important intellectual content and approved the final manuscript as submitted. Carina Venter revised the manuscript for important intellectual content and approved the final manuscript as submitted. Graham Roberts was involved in the initiation and set up of the PIFA study, diagnosis of food allergy, statistical analysis and interpretation of the data, revised the manuscript for important intellectual content and approved the final manuscript as submitted. Kate Grimshaw was involved in the initiation and set up of the PIFA study, writing the original study protocol for this study, data collection, diagnosis of food allergy, statistical analysis and data management, drafted the initial manuscript and approved the final manuscript as submitted.

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503

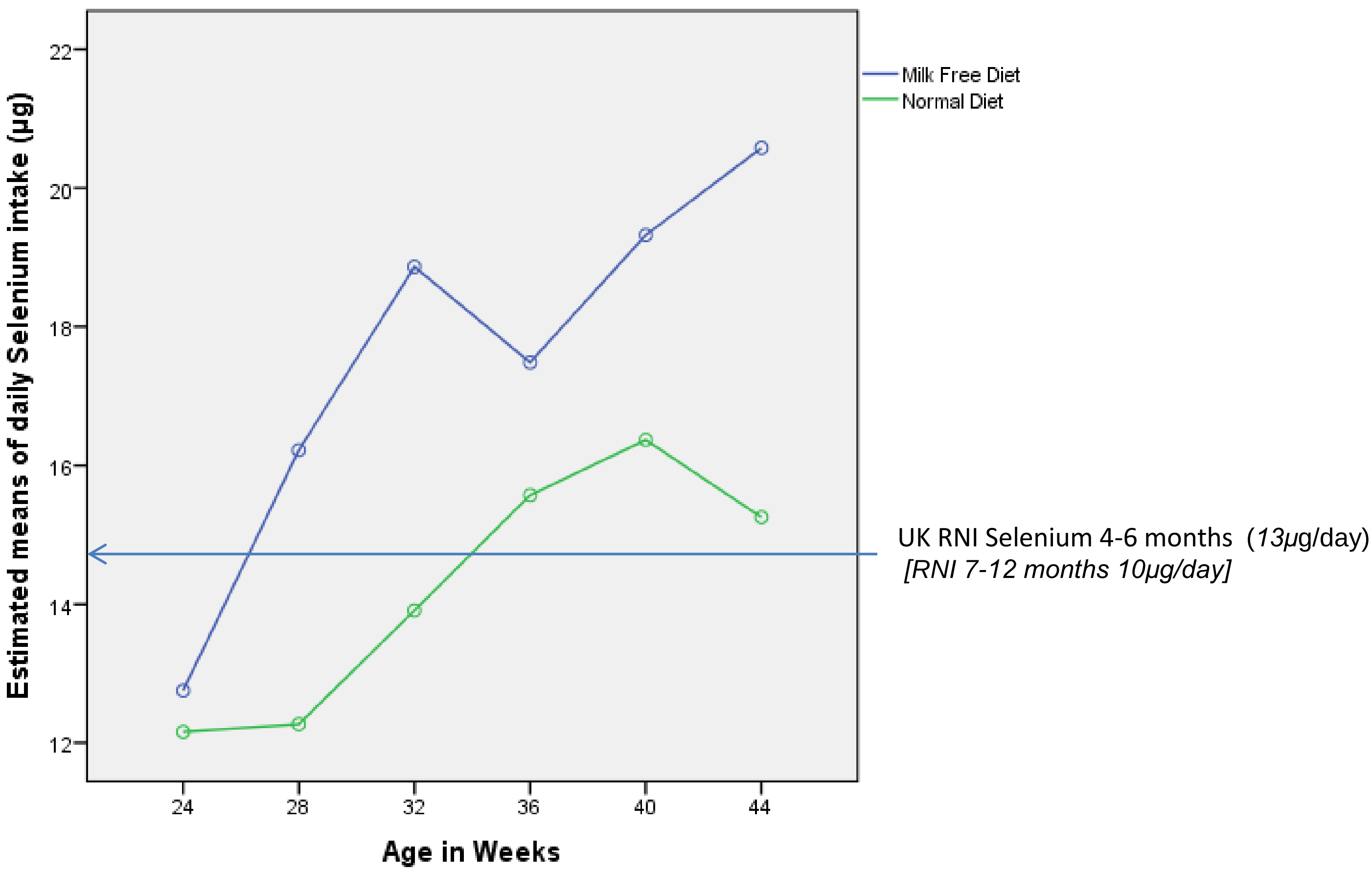
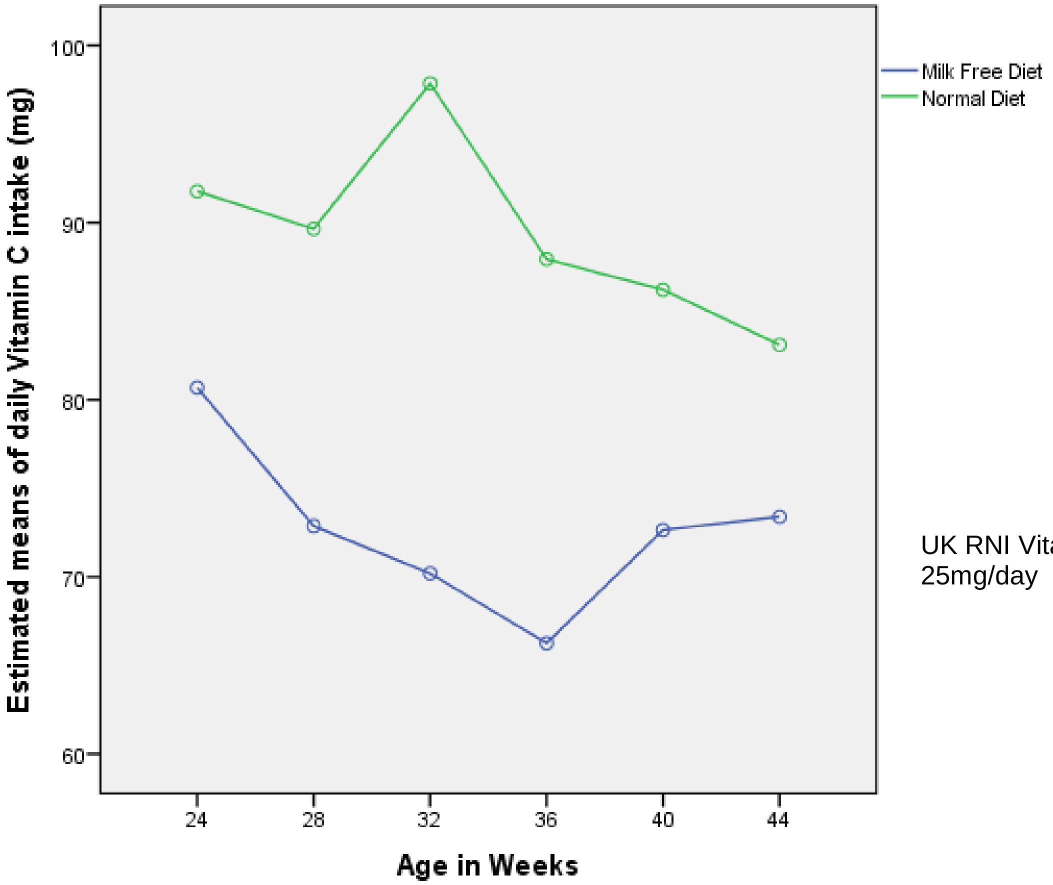

Milk Free Diet
Normal Diet
Estimated means of daily Selenium intake (µg)
22
20
18
16
14
12
24
28
32
36
40
44
Age in Weeks
UK RNI Selenium 4-6 months (13µg/day)
[RNI 7-12 months 10µg/day]

Figure 2



UK RNI Vitamin C 0-12months
25mg/day