

Micronutrient Status and Nutritional Intake in 0- to 2-Year-old Children Consuming a Cows' Milk Exclusion Diet

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ABSTRACT

Objectives: To study micronutrient status and nutritional intake from complementary feeding in children on a cows' milk exclusion (CME) diet.

Methods: Fifty-seven children with cows' milk allergy, younger than 2 years, were included in a cross-sectional study. Blood was analyzed for micronutrient status. Complementary feeding was defined as all solids and liquids except of breast milk, and assessed by 3-day food diary. The results were analyzed according to 3 feeding patterns: mainly breast-fed (mBF), partially breast-fed, and no breast milk group (nBM).

Results: The children had a median age of 9 months and micronutrient status was within normal range for total homocysteine (p-tHcy), s-B12, s-folate, b-Hb, s-ferritin, s-zinc, and s-25(OH)D. There were no significant differences between feedings groups, except for B12-biomarkers. The mBF had higher p-tHcy ($P < 0.000$) and lower s-B12 ($P = 0.002$) compared nBM. Vitamin B12 deficiency (p-tHcy $> 6.5 \mu\text{mol/L}$ combined with s-B12 $< 250 \text{pmol/L}$) was found in 12% of participants, most frequently among the mBF (36%) and none in nBM group ($P = 0.009$). Vitamin B12 intake from complementary feeding was negatively correlated with p-tHcy ($r = -0.479$, $P = 0.001$) and positively with s-B12 ($r = 0.410$, $P = 0.003$). Iron deficiency anemia was found in 5%. Iron intake correlated positively with b-Hb ($r = 0.324$, $P = 0.02$). Zinc deficiency was found in 7% and low 25(OH)D in 9%. Vitamin D intake was positively correlated with the use of supplements ($r = 0.456$, $P = 0.001$).

Conclusion: The risk of B12 deficiency was high in mBF infants on CME diet, and complementary feeding was associated with better B12 status. Iron, zinc, and vitamin D deficiencies were present in all feeding groups. Complementary feeding should be introduced at 4 to 6 months of age. Vitamin D supplement is recommended to ensure adequate intake.

Key Words: breast-feeding, children, complementary feeding, cow's milk protein-free diet, feeding patterns, micronutrients

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What Is Known

- Children on cows' milk exclusion diets are at risk of nutritional deficiencies.
- Data on biomarkers for micronutrient status are scarce in children on cows' milk exclusion diets.

What Is New

- The risk of B12 deficiency was high in mainly breast-fed infants on cows' milk exclusion diet, and complementary feeding was associated with better B12 status.
- Iron, zinc, and vitamin D deficiencies were present in all feeding groups.
- Appropriate complementary feeding should be introduced at 4 to 6 months of age.
- Vitamin D supplement is recommended to ensure adequate intake.

Cows' milk allergy (CMA) affects 1.9% to 4.9% of infants and toddlers in industrialized countries, and is the most prevalent food allergy in this age group in Europe (1,2). Cows' milk exclusion (CME) diet is the treatment, and if the child is breast-fed the nursing mother is often recommended to consume a CME diet (3,4). Recent Norwegian surveys found that 80% of healthy infants were breast-fed at 6 months of age (5,6). CME may negatively affect nutritional quality of children's diets, and hence their nutritional status (7–9).

Mari Borge Eskerud works for Nestle Nutrition when submitting this manuscript, but during the study period she was a master student at the University of Oslo. The remaining authors report no conflicts of interests to declare.

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There is a void in the literature on micronutrient status among children on CME diet. In healthy Danish infants, exclusive breastfeeding (eBF) for >4 months have been shown to increase risk of B12 deficiency (10). Dairy products are the most important B12 sources for toddlers (11), and there are no published studies on B12 status and intake on CME diet. Iron deficiency (ID) is the most common micronutrient deficiency in European children (12), related to high requirement because of rapid growth. ID has been associated with late introduction of iron-rich foods (meat and iron-fortified cereals) in infants (12). Dairy foods are important dietary sources of zinc. In CMA low intakes of zinc have been reported (13,14), and zinc deficiency has been detected (14). Adequate vitamin D status is crucial to secure bone health (15), but there is little knowledge of vitamin D status and CME diet. Conflicting data have been published regarding nutrient intake and nutritional status in children on elimination diets (9,13,16–20). The aim of this study was to assess micronutrient status and nutritional intake from complementary feeding (CF; all solids and liquids except of breast milk) in 0- to 2-year-old children on CME diet.

METHODS

Design

An observational cross-sectional study on biomarkers of micronutrients and nutritional intake in CME diet in children 0 to 2 years of age was performed. Inclusion, by informed consent from both parents, was done from January 2014 to May 2015. Children treated with CME diet for more than 3 weeks at the Department of Paediatric medicine, Oslo University Hospital (OUH), were invited to participate. The diagnosis of CMA was done by doctors on basis of clinical history and symptom relief upon CME diet. Provocation was done if uncertainty about the diagnosis. Exclusion criteria were premature birth, thyroid disease, dependence of enteral- or parenteral nutrition, and other illnesses or procedures affecting nutrient metabolism or iodine status. Iodine status was also examined in this study and results are previously published (7). The Regional Committee for Medical and Health Research Ethics in Norway (REC nr. 2013/1579), the Head of the Department and the Research committee, OUH, approved the study.

Blood Samples

Venous blood samples were performed according to routine procedures at inclusion. Biomarkers analyzed were total homocysteine (p-tHcy), s-B12, s-folate, b-Hb, s-iron, mean corpuscular volume (b-MCV), mean corpuscular haemoglobin (b-MCH), s-ferritin, s-transferrin saturation, s-transferrin receptor, C-reactive protein (s-CRP), s-zinc, s-albumin, and s-25(OH)D. The analyses were done at the Department of Medical Biochemistry, OUH, except for s-25(OH)D that was analyzed at the Hormone Laboratory, OUH.

Diagnosis of B12 deficiency was defined by an algorithm including p-tHcy >6.5 $\mu\text{mol/L}$ and s-B12 <250 pmol/L, in patients with normal s-folate. In infants' tHcy has been shown to be a sensitive marker for B12 deficiency (21). The metabolic conversion of tHcy to methionine is inhibited if the coenzyme methionine synthase is not saturated with B12, and this result in increased tHcy in plasma. Specificity of increased tHcy is low, as folate deficiency also will lead to increased values (22,23). The cut-offs for p-tHcy and B12 were chosen based on suggestions in recent literature (23–25). Total-Hcy >6.5 $\mu\text{mol/L}$ represents the 97.5 percentile in 4-month-old infants given a single intramuscular dose of 400 μg hydroxycobalamin at 6 weeks, rendering them to be cobalamin optimized (24). S-B12 has low sensitivity and specificity as a biomarker of vitamin B12 deficiency, and deficiency cannot be excluded by a normal s-B12 alone (21). S-B12 is included in the

algorithm as this is the only marker used in screening of vitamin B12 status. The cut-off for s-B12 <250 pmol/L was chosen as suggested by the Norwegian Paediatric Association (25).

ID was defined as s-ferritin <12 $\mu\text{g/L}$ if age >6 months, <20 $\mu\text{g/L}$ if 4 to 6 months and <40 $\mu\text{g/L}$ if 2 to 4 months. Anemia was defined as Hb <10.5 g/dL. Iron deficiency anemia (IDA) was diagnosed if both ID and anemia were present. These criteria are recommended by ESPGHAN (12). Zinc deficiency was defined as s-zinc <10 $\mu\text{mol/L}$ according to cut-offs at OUH laboratory and literature (26). Sufficient s-25(OH)D was defined as values >50 nmol/L, deficiency if 25 to 50 nmol/L and severe deficiency if values <25 nmol/L based on suggestions by ESPGHAN (15). High values were defined >250 nmol/L.

Food Diary

Dietary intake of all solids and liquids except of breast milk (CF) was recorded by household measures (eg, mL formula, teaspoon) in a diary for 3 consecutive days, as this is a feasible method in infants and children (27). All participants got advice on CME diet and instructions on dietary record by a dietitian (J.A.K., R.A.T., M.B.E.). Information on homemade foods, including cooking methods, was noted in the diary. Formula type and the recipe were recorded. The dietitians clarified questions regarding records. Two dietitians (R.A.T., M.B.E.) coded diaries. Information from the manufacturer was obtained if necessary. Composite items were analyzed by dividing into separate components. Grams of formula per day was calculated and used in the analysis. The data on CF and dietary supplements were entered into an online Norwegian nutrition analysis tool (Kostholdsplanleggeren) (28), based on the Norwegian Food Composition Table (29). Results were compared to recommended daily intake (RDI). In the present study RDI refers to Nordic Nutrition Recommendations 2012 (30). Breast milk was not included in the calculations due to methodological and financial limitations.

The study population was divided into three subgroups according to feeding pattern, reflecting the different stages of weaning during the first year of life. The feeding group not receiving breast milk was termed no breast milk (nBM). The breast-fed children were divided into mainly breast-fed (mBF), receiving <50% of their estimated energy requirement from CF, or partially breast-fed (pBF), more reliant on CF for energy and nutrients. World Health Organization/The Food and Agriculture Organization of the United Nations energy recommendations for breast-fed children under the age of 12 months, 80 kcal/kg/day, was used as reference value (31).

Statistics

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 22 (Armonk, NY). Nonparametric methods were used for all data. Medians with interquartile range (25th–75th percentile) were presented. Groups were compared using the Kruskal-Wallis test when more than 2 groups. If significant difference ($P < 0.05$), analysis between pairs of groups were performed by Mann-Whitney *U* test and Bonferroni adjustment ($P < 0.17$). Categorical data were presented as frequencies, and tested with chi-square or Fisher exact test. To explore differences between 2 related groups Wilcoxon signed-rank test were applied. Spearman rho correlation coefficient was used to assess the relationship between variables. Missing variables were excluded pairwise. The level of significance for all analysis was 2 sided and set to 0.05. Sample size was calculated for the endpoint urine iodine concentration. With 80% power and significance lever of 5% a sample size of 46 children was necessary to detect a difference of 20 $\mu\text{g/L}$ from

the cut-off level of 100 µg/L. Results have been previously published (7).

RESULTS

Subjects

Eighty-two children were invited and 57 (70%) participated in the study. When dividing in 3 feeding patterns 24% was mBF, 33% was pBF, and 43% was nBM. Only 1 child was eBF. Median age was 9 months (range 2–23 months) and 54% were boys. The mBF was significantly younger (7 months) than the pBF (10 months) and nBM (11 months). Median weight was 8650 g (7465–9788) and length 72.0 cm (69.0–75.7). Median weight standard deviations (SDS) was -0.41 (-1.23 , 0.22), length SDS was -0.39 (-0.93 , 0.46) and BMI SDS was -0.44 (-1.41 , 0.26). The majority were of Scandinavian ethnicity (84%), and 89% of mothers had higher education. Median age of the mothers was 33 years, and all were nonsmokers. Almost all infants (98%) had gastrointestinal symptoms (colic, reflux, loose stools, obstipation, or bloody stools). Frequent symptoms reported were feeding difficulties (70%), sleeping disturbances (47%), eczema/skin symptoms (46%), and failure-to-thrive (38%). Baseline

characteristics of the study population are described in an earlier publication (7).

Micronutrient Status

Biomarkers of micronutrient status for the participants are presented in Table 1.

Diet

More than half of the children (57%) were breast-fed. All mothers were on CME diet. Assessment of maternal diet was not done, except for supplements which was used by 88%, thereof 34% containing B12. Median duration of CME diet at inclusion were 17 (range 4–84 weeks). CF was introduced at median age 4 months. Among participants 28% avoided only cows' milk (23% of mBF, 22% of pBF, and 39% of nBM), whereas 16% reported other food allergies besides milk. Median number of food groups avoided were 3, most commonly nuts (65%), eggs (39%), soy (37%), and fish (22%). Nutrient intakes from CF are presented in Table 2. CF did not meet RDI for vitamin B12, iron, and zinc in mBF infants. In pBF, CF did not meet RDI for iron and zinc. Vitamin D intake was

TABLE 1. Micronutrient status (median, 25–75 percentiles) in 0- to 2-year-old children consuming a cows' milk exclusion diet

	n	All	Mainly breastfed n = 13	Partially breastfed n = 18	No breast milk n = 23	P
b-Hb, g/dL	56	11.8 11.1–12.4	11.2 11.1–11.5	11.9 11.1–12.5	12 11.7–12.4	*ns
b-MCV, fL	52	77 75–80	77 74–78	77 75–79	78 75–79	*ns
b-MCH, pg	52	26.4 24.8–27.0	25.9 24.6–26.4	25.9 25–26.9	26.6 24.7–27.0	*ns
s-Ferritin, µg/L	55	30 22–43	28 15–47	26 18–38	33 28–43	*ns
s-Soluble transferrin receptor, mg/L	46	4.8 4.0–5.5	5.2 4.4–5.5	4.8 4.4–5.5	4.6 3.8–5	*ns
s-Transferrin saturation	37	0.13 0.10–0.18	0.15 0.13–0.16	0.12 0.08–0.13	0.14 0.12–0.19	*ns
s-Folate, nmol/L	54	40 34–45	38 31–45	37 31–45	41 38–45	*ns
s-B12, pmol/L	55	447 350–565	392 228–423	444 278–565	526 447–619	*0.009 †0.082 ‡0.002 §0.119
p-tHcy, µmol/L	50	5.9 4.9–7.8	8.3 5.9–9.7	6.6 4.7–8.0	5.1 4.6–6.4	*0.001 †0.029 ‡<0.000 §0.093
s-Zinc, µmol/L	45	11 11–12	12 11–13	11 10–11	11 11–13	*ns
s-Albumin, g/L	52	46 44–48	46 45–47	46 45–48	45 44–48	*ns
s-CRP, mg/L	55	0.6 0.6–0.6	0.6 0.6–0.6	0.6 0.6–0.6	0.6 0.6–0.6	*ns
s-25(OH)D, nmol/L	54	79 61–88	86 68–103	72 61–88	78 62–87	*ns

*Kruskal-Wallis test.

†Mann-Whitney test mainly breast-fed versus partially breast-fed.

‡Mann-Whitney test mainly breast-fed versus no breast milk.

§Mann-Whitney test partially breast-fed versus no breast milk. Bonferroni adjustment ($P < 0.17$).

TABLE 2. Daily nutritional intake from complementary feeding (all solids and liquids except of breast milk) (median, 25–75 percentiles) in 0- to 2-year-old children consuming a cows' milk exclusion diet

	All* n = 54	Mainly breast-fed n = 13	Partially breast-fed n = 18	No breast milk n = 23	NNR 2012 ^{†‡}
Energy, KJ	2601	1129	2525	3298	342–330 KJ/kg
	1578–3360	862–1308	2174–3208	2688–4031	
Energy, kcal	620	269	602	781	
	376–803	205–313	519–765	632–963	
kcal/kg	77	32	67	92	82–79 kcal/kg
	39–94	26–36	52–90	80–107	
Protein, g	19.3	8.4	19.4	24.1	
	13.3–27.5	4.3–10	14.8–25.5	18.7–31.5	
g/kg	2.1	1.0	2.1	2.9	1.1 [†] –1.0 [‡] g/kg
	1.5–3.0	0.6–1.2	1.8–2.8	2.1–3.3	
Vitamin B-12, µg	1.2	0.4	1.1	1.7	0.5 [†] –0.6 [‡] µg
	0.6–1.9	0.2–1	0.7–1.5	1–3.2	
Folate, µg	94	52	114	103	50 [†] –60 [‡] µg
	68–134	28–78	75–134	87–156	
Iron, mg	8.1	3.3	7.6	9.9	8 mg
	4.4–10.1	1.6–4	5.2–9.7	8.9–11.7	
Zinc, mg	5.0	2.2	4.7	6.5	5 mg
	3.1–6.5	1.1–2.8	3.2–5.7	5–7.7	
Vitamin D, µg	13.2	11	12	14.8	10 µg
	8.1–16.2	6.2–14.6	9.4–16.1	8.0–17.4	
- without supplements	5.3	1.8	4.1	7.5	
	2.9–7.7	0.5–3.1	3.0–6.7	5.5–9.0	
Calcium, mg	338	134	320	630	540 [†] –600 [‡] mg
	203–633	60–226	214–415	372–715	
Formula, g [§]	23.0	0.0	20.2	63.0	
	0.0–61.7	0.0–5.5	0.0–36.0	32.8–88.0	

*Missing for 3 participants.

[†]Nordic Nutrition Recommendations 2012 children 6 to 11 months.

[‡]Nordic Nutrition Recommendations 2012 children 1 to 2 year.

[§]13.5 g powder = 100 mL liquid formula.

secured by supplements (Table 2). Calcium intake from CF was lower than RDI for mBF and pBF, but adequate for nBM group (Table 2). Most children (69%) used formula in their diet, 62% an extensively hydrolysed formula, and 38% an amino acid formula, respectively. Median intake of formula was 23 g/day (0–61.7 g) (Table 2).

B12 Status and Dietary Intake

Median p-tHcy was 5.9 µmol/L in all participants. P-tHcy was significantly higher in mBF (8.3 µmol/L) compared to pBF (6.6 µmol/L) ($P=0.03$) and nBM (5.1 µmol/L) ($P<0.000$) (Table 1). Median s-B12 was (447 pmol/L), significantly lower in mBF (392 pmol/L) compared to nBM (526 pmol/L) ($P=0.002$) (Table 1). Vitamin B12 deficiency (p-tHcy >6.5 µmol/L and s-B12 <250 pmol/L) was found in 12% of participants (Fig. 1). A significantly higher prevalence was found in mBF (36%) compared to pBF (12%) and nBM (0%) ($P=0.009$) (Fig. 2). Vitamin B12 intake from CF met RDI for pBF and nBM, but not for mBF (Table 2). Vitamin B12 intake from CF was negatively correlated with p-tHcy ($r=-0.479$, $P=0.001$) and positively correlated with s-B12 ($r=0.410$, $P=0.003$). Formula (g/day) was negatively correlated to p-tHcy ($r=-0.365$, $P=0.01$) and positively correlated to B12 intake ($r=0.423$, $P=0.002$). No significant correlations between intake and B12 deficiency were found. S-folate was within normal reference values for all participants (Table 1), and folate intake was above RDI (Table 2).

Iron Status and Dietary Intake

Median b-Hb (11.8 g/dL) and s-ferritin (30 µg/L) were normal, and no significant differences between feeding groups were detected (Table 1). ID was found in 13% and IDA in 5% of participants (Fig. 1). Anemia was present in 9%. Iron intake positively correlated to b-Hb ($r=0.324$, $P=0.02$). Consumption of formula (g/day) was correlated to iron intake ($r=0.501$, $P=0.000$) and s-ferritin ($r=0.378$, $P=0.006$). Iron intake from CF was lower than RDI for mBF and pBF, but not for nBM (Table 2).

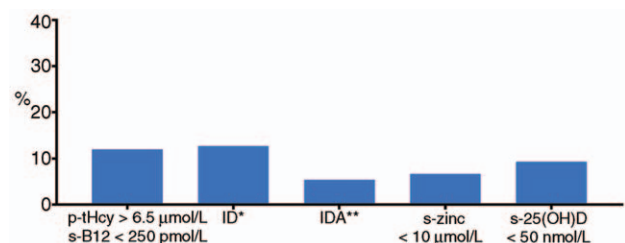


FIGURE 1. Micronutrient deficiencies (%) in 0- to 2-year-old children consuming a cows' milk exclusion diet. ID = iron deficiency; IDA = iron deficiency anemia.

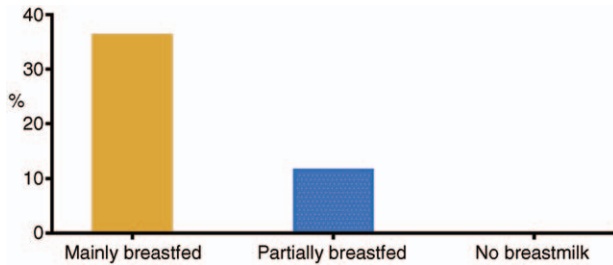


FIGURE 2. Vitamin B12 deficiency (p-tHcy $>6.5 \mu\text{mol/L}$ and s-B12 $<250 \text{pmol/L}$) according to feeding pattern in 0- to 2-year-old children consuming a cows' milk exclusion diet.

Zinc Status and Dietary Intake

Median s-zinc was normal ($11 \mu\text{mol/L}$), and no differences were found between feeding groups. Three subjects (7%) had zinc deficiency (Fig. 1). The mBF and pBF had a zinc intake below RDI (Table 2). There was no significant correlation between zinc intake and s-zinc. A positive correlation between formula consumption and zinc intake was found ($r=0.542$, $P<0.000$).

Vitamin D Status and Dietary Intake

Median 25(OH)D was 79nmol/L , and no significant differences were found between the feeding groups (Table 1). Three (9%) participants had deficiency between 25 and 50nmol/L (Fig. 1), none had severe deficiency $<25 \text{nmol/L}$ or high values $>250 \text{nmol/L}$. Dietary supplements, used by 86%, significantly contributed to vitamin D intake (13.2 vs $5.5 \mu\text{g/day}$, $P<0.001$). Vitamin D intake was positively correlated to use of supplements ($r=0.456$, $P=0.001$). Vitamin D intake or dietary supplements did not correlate to 25(OH)D. Supplements used were D-drops 44%, cod-liver-oil 33%, omega-3 11%, and multivitamin-mixture 11%.

DISCUSSION

This cross-sectional study presents micronutrient status and nutritional intake from CF in a group of 0- to 2-year-old children consuming CME diet. Dietary pattern changes during the 2 first years of life, and feeding groups mBF, pBF, and nBM were therefore investigated. The study is the first to report a high risk of vitamin B12 deficiency in mBF infants consuming a CME diet, and CF was associated with better B12 status. Iron, zinc, and vitamin D deficiencies were present in all feeding groups. Median s-values for all biomarkers were normal. No significant differences were found between feeding groups, except for p-tHcy and s-B12.

Results indicate that B12 supply from CF was important for securing B12 status. This is in line with studies of healthy infants (10,24,32,33). However, Hay et al (33) suggested low s-B12 in breast-fed infants to be a normal finding related to physiologic mechanisms. Vitamin B12 in breast milk has been found to correlate to maternal serum concentrations (34,35); Hence, breast-fed children's intake depends on this. Greibe et al (10) found a significant decrease both in breast milk B12 and infant serum levels at 4 months, compared to values at birth and at 9 months in healthy mother-child pairs. They concluded that introducing weaning foods from 4 months of age was important to secure status. In the present study median age of mBF children was 7 months, an age at which breast milk must be complemented sufficiently by solids to secure nutrient supply (36). The breast-feeding frequency in the study group was high (57%), and similar to healthy Norwegian infants at the same age (37). Formula was used

by 69%, but only in small amounts by the mBF (Table 2). However, based on the present study, we cannot conclude whether risk of B12 deficiency was related to CME diet or low amounts of CF rich in B12.

B12 deficiency may also be a result of malabsorption, which might be relevant in CMA. Acquired defects or inborn errors of B12 metabolism are rare (23), and not suspected in this study group. Low birth stores can also contribute to low B12 status in infancy (38,39).

Vitamin B12 is an essential nutrient, and deficiency negatively affects neurological development due to reduced myelination, a process most active in the first months of life (32,40,41). The early functional symptoms of B12 depletion are unspecific, and in infancy frequent symptoms are feeding problems, failure-to-thrive, vomiting, diarrhea, developmental delay, hypotonia, fatigue, and seizures. B12 deficiency may lead to permanent neurological sequelae (22,42,43). Results from a recent randomized study found that an injection with hydroxycobalamin improved motor development and reduced regurgitation in vitamin B12 deficient healthy infants (40). Further studies are needed to evaluate if B12 deficiency may be a differential diagnosis to CMA as similar symptoms are frequently reported.

The lack of consensus for diagnostic criteria for B12 deficiency is a challenge (23). S-B12 most often is the screening biomarker used in clinical settings, and most hospital laboratories have reference values that are even lower than chosen in the present study. This may result in B12 deficiency in infants not being detected. More research is needed on diagnostic criteria for B12 deficiency, and on which biomarkers to use (23).

CMA children may be at increased risk if iron deficiency because of intestinal losses (colitis and inflammation) (44). In the present study, the frequency of IDA and ID was at the same level as healthy European children (12). Iron intake of the nBM children met the RDI, but the breast-fed children did not (Table 2). The use of formula was positively correlated to s-ferritin and iron intake. It is well known that breast milk must be complemented by iron sources from 6 months of age in healthy children (36). Maslin et al (18) and Meyer et al (13) also found associations between the use of formula and micronutrient intake, including iron.

Dairy products are important sources of zinc. Breast-fed infants had lower zinc intake from CF than RDI, but in the nBM intake was adequate. The study revealed low s-zinc in a few participants. Berni-Canani et al (14) reported low zinc values in 23% of Italian CMA infants and children, significantly more than in healthy controls. In addition, zinc intake was lower in CMA than healthy children. Meyer et al (13) found zinc intake linked to use of formula, and this was confirmed by the present study. However, s-zinc is not a sensitive biomarker and therefore debatable (26). Symptoms of zinc deficiency and low dietary intake may be more important when assessing zinc status.

Vitamin D and calcium are important to prevent rickets and osteomalacia (45). Vitamin D supplements were used by nearly all participants (86%), and ensured RDI to be fulfilled. Vitamin D intake was comparable to intake in healthy Norwegian 12-month-old infants (37) and allergic children younger than 3.5 years in Helsinki (19). Maslin et al (18) found vitamin D intake marginally below RDI for CMA infants in UK. Meyer et al (13) also points to the risk of vitamin D deficiency in CMA. A supplement of $10 \mu\text{g/day}$ of vitamin D is recommended for infants and for children at risk by ESPGHAN (15). Findings from the present study support $10 \mu\text{g}$ vitamin D as a supplement for children aged 0 to 2 years on CME diet.

Few studies have investigated biomarkers of micronutrient status and dietary intake from CF in CME diet in 0- to 2-year-old children in which a high proportion are breast-fed. Looking at

biomarkers and feeding patterns together increases the validity of findings. For the nBM participants energy intake was comparable to RDI (30), supporting the assumption of valid results. Total nutrient intake was not known for the breast-fed children. Collection and analyses of breast milk is demanding and expensive, and the results are affected by variations in content (46). Although it is frequently reported that maternal diet influences the nutritional composition of breast milk, the data on this are scarce (47). More studies of mother's diet and breast milk composition are needed. This study cannot conclude whether breast milk content was different on CME diet, as this was not investigated. Neither are results not necessarily representative for populations of formula-fed infants.

Children with food allergy have increased permeability of the small intestine following ingestion of the offending food and during elimination diets (48). Increased needs for nutrients may be relevant, related to decreased absorption, inflammation, or losses in the gut. Biomarkers are not affected by these mechanisms, and comparing biomarkers and dietary intake can increase knowledge regarding this.

A strength of the study was the homogeneous age group. High education level and participation rate reflect a motivated group of parents, which implicated carefully registrations of dietary intake and compliance to diet. A 3-day food diary has been found to result in reliable data on dietary intake (27,49). However, the study group was a small and selected population and not representative for all infants and toddlers on CME diet. Participants were recruited at a specialized gastroenterological ward, and they may be more troubled than those treated in primary care. This may also be the reason for frequent use of amino acid formulas. However, it could also be related to not systematically implement appropriate guidelines (50). Families got dietary advice from a dietitian, which has been shown to improve nutrient intake (14), and we cannot assume similar results in populations not followed by specialists. The finding of multiple exclusions was suspected to be related to the young age group, and results equal to findings in a national Norwegian study of 1-year-old children (37). Another weakness of the study was the lack of a healthy reference group and not investigating the mothers' micronutrient status, dietary intake and breast milk content. Further research and larger studies on micronutrients and CME diet, especially in breast-fed infants, are necessary to increase knowledge on nutritional status in infants and children with CMPA.

CONCLUSION

The risk of B12 deficiency was high in mBF infants on CME diet, and CF was associated with better B12 status. Iron, zinc, and vitamin D deficiencies were present in all feeding groups. Appropriate CF should be introduced at 4 to 6 months of age. Vitamin D supplement is recommended to ensure adequate intake.

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