Original Article

The Effect of Unadjusted Mineral Supplementation on Bone Health of Preterm Infants Fed Fortified Human Milk: An Exploratory Analysis

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Abstract

Background: In standard fortification of human milk (HM), the HM macronutrient content is assumed, and a fixed amount of a multinutrient fortifier is added to achieve recommended nutrient intakes. In target fortification, the HM macronutrient content is regularly measured, guiding the addition of modular macronutrient supplements to the fortified HM, to achieve the nutritional targets more precisely. **Objective:** The study aimed to investigate whether this addition of modular supplements, unaccompanied by mineral supplementation, predispose to metabolic bone disease (MBD). **Methods:** This is a secondary analysis of a larger study of infants born with <33 weeks gestational age. Fortifications based on the assumed (Group 1) or measured (Group 2) of the HM macronutrient content were compared, using low serum phosphate levels as an indicator of MBD, and length growth as a surrogate of bone growth. **Results:** Eighty-four infants were included, 35 in Group 1 and 49 in Group 2. During the exposure period, infants of Group 2 received higher mean fat (6.1 vs. 5.3 g/kg/day, $P < 0.001$) and carbohydrate (13.0 vs. 11.7 g/kg/day, $P < 0.001$) intakes; in addition, they exhibited lower mean serum phosphate (5.5 vs. 6.0 mg/dL, $P = 0.022$) and faster mean length velocity (1.06 vs. 0.89 cm/week, $P = 0.003$). **Conclusions:** These findings suggest that feeding fortified HM with extra fat and carbohydrate content, unaccompanied by mineral supplementation, promotes increased bone growth, as indicated by accelerated length growth, but with insufficiently mineralized osteoid, indicated by low serum phosphate levels. Intervention studies using direct biomarkers of bone mass content and mineral density are necessary to corroborate our findings.

Keywords: *Bone mineralization, human milk fortification, length growth, metabolic bone disease, preterm infants, target fortification*

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Introduction

Osteomalacia is a form of metabolic bone disease (MBD) of prematurity, characterized by deficient mineralization of bone matrix (osteoid).[1-3] Risk factors for MBD include the absence of mechanical stimulation and the use of systemic.[1,4-7] Bronchopulmonary dysplasia, severe forms of necrotizing enterocolitis, and cholestasis are preterm comorbidities that have been associated with MBD.[5,7,8]

Preterm infants are susceptible to MBD, and they require a balanced supply of energy and macronutrients for osteoid synthesis, as well as minerals for osteoid mineralization.[9,10] Indeed, the nutrients involved in growth and bone mineralization have a complex interrelationship, as their effects on these processes are interconnected and interdependent.[11] The basic approach to prevent MDP of prematurity is the provision of high amounts of minerals and an adequate amount of Vitamin D.[12]

Human milk (HM) alone is insufficient to meet the high nutritional requirements of growing preterm infants. Therefore, the supplementation of HM with multinutrient fortifiers is necessary to provide adequate nutrition to these infants.[13] For this purpose, fortifiers are formulated to provide additional nutrients to enhance growth, including minerals essential for bone health.[14,15] The method of HM fortification can impact the growth of preterm infants.[15] In "standard fortification," the macronutrient content of HM is assumed, and a fixed amount of HM multinutrient fortifier is added to achieve recommended nutrient targets.[15] On the other hand, "target fortification" involves regularly measuring the macronutrient content of HM, which then guides the addition of modular macronutrient supplements to fortified HM. This approach allows for a more precise and accurate achievement of the desired nutrient targets.[13,15] In this regard, studies have reported that target fortification provides higher energy intake and supports better overall growth, particularly length growth, compared to fortification based on assumed HM macronutrient content.[16-18]

Length growth is considered a surrogate marker of skeletal growth and reflects bone nutrition in infants.[19] Therefore, monitoring linear growth can provide valuable insights into the overall bone health and nutritional status of preterm infants.

Low phosphate and elevated alkaline phosphatase serum levels are commonly used biochemical markers for screening of MBD of prematurity,[20] although resorting to reference imaging methods is needed for diagnostic accuracy.[21]

Based on previous reports, we hypothesize that the provision of additional energy and macronutrients through modular macronutrient supplementation of fortified HM, without individualized mineral supplementation,[12,22] predispose to the accumulation of compromised mineralized osteoid.[1,2,23]

This study aimed to investigate the variations in bone mineralization and bone growth among preterm infants who are fed fortified HM. It compared two approaches to HM fortification: one based on assumed HM macronutrient content and the other based on measured HM macronutrient content. The two approaches were anticipated to result in different levels of extra energy and macronutrient intake. By examining these differences, the study sought to understand the impact of fortification methods on bone health and growth outcomes in preterm infants.

Methods

Study design, setting, and recruitment

This entails a *post hoc* secondary analysis within a mixed-cohort study conducted at the maternity ward of a tertiary university hospital. The STROBE reported guidelines were followed.

A comparison of the effects on growth between two methods of HM fortification in infants born appropriate-for-gestational age at <33 weeks of gestation was conducted. In Group 1, HM was fortified based on assumed HM macronutrient content, whereas in Group 2, HM was fortified based on measured HM macronutrient content.[18]

For this secondary analysis, infants who had serum and/or urine measurements of calcium, phosphate, and alkaline phosphatase, as biochemical markers of MBD, were initially eligible. However, on conducting an exploratory search, it was found that serum phosphate was the only marker that had been measured consistently. Therefore, only infants who had their serum phosphate measured during fortified HM feeding, defined as the exposure period, were included in this analysis.

Ethics statement

This *post hoc* secondary analysis, nested within a mixed-cohort study, was approved by the hospital ethics committee (Nr 558/2018). The recruitment of participants in this study required the written informed consent from the parents or legal representatives of the infants.

Nutritional support

The same institutional protocol, following international and national guidelines for parenteral and enteral nutrition, was used in both groups as thoroughly

explained elsewhere.[18,24] Briefly, both fortification methods started with "standard fortification," by adding a predetermined dose of multinutrient fortifier to HM (4.4 g/100 mL). The same multinutrient HM fortifier (Aptamil FMS; Danone GmbH, Friedrichsdorf, Germany) was utilized, containing the following nutrient composition per 1 g of powder: 3.47 kcal of total energy, 0.25 g of protein, 0.62 g of carbohydrates, 19.41 mg of calcium, 8.72 mg of phosphorus, and 1.15 mg of magnesium. The consistent use of this fortifier ensured that the additional nutrients provided to infants through fortified HM were standardized across both groups. In addition, further adjustments to desired nutrient intakes were left to the clinical discretion of physicians. In Group 1, the addition of modular macronutrient supplements to fortified HM was based on assumed HM macronutrient content as reported in the literature.[25] In Group 2, the addition of modular macronutrient supplements to fortified HM was guided by the measured macronutrient content of the administered HM. To guide this procedure, HM samples that included both mothers' own milk (MOM) and donor HM (DHM) were subjected to macronutrient content analysis using a real-time HM analyzer (Miris AB, Uppsala, Sweden).[18] Therefore, whenever necessary the targets recommended by the ESPGHAN 2010 guidelines[26] were reached by adding modular protein (Aptamil Protein Supplement; Danone GmbH, Friedrichsdorf, Germany) containing 3.38 kcal of total energy and 0.821 g of protein per 1 g of powder and/or modular fat, through medium chain fatty acids (MCT Oil; Danone, GmbH, Friedrichsdorf, Germany) containing energy of 8.6 kcal and 0.95 g of fat per 1 mL, were added to fortified HM as previously described.[18]

Data on energy, protein, protein-to-energy ratio (PER), fat, and carbohydrate intakes were retrieved from the principal cohort study.[18]

Estimates for calcium, phosphorus, and magnesium intakes were retrospectively calculated from the recorded volumes of administered MOM and DHM as well as the amounts of added HM fortifier. The average mineral content previously reported in preterm HM (for MOM) and term HM (for DHM)[27] was utilized. In addition, the mineral content of HM fortifier provided by the manufacturer was incorporated for the purpose of calculations in the study. These values were essential for accurately determining the mineral composition of fortified HM.

According to the institutional nutrition protocol, infants in this study received a daily dose of 160 IU/kg of Vitamin D_2 during the period of parenteral nutrition. Once the infants transitioned to full enteral feeding, they received a daily enteral supplementation of 670 IU of vitamin D_3.

Serum phosphate levels

During the exposure period, serum phosphate levels were regularly monitored and measured. Two different cutoff points were used to define hypophosphatemia: ≤5.6 mg/dL and ≤3.72 mg/dL, as previously documented.[21] These cutoff values were utilized to identify and classify individuals with lower-than-normal serum phosphate levels, indicating potential hypophosphatemia.

Length growth

Length growth data were retrieved for the contemporary cohort, from a previous mixed-cohort study, in which accurate measurements were assured.[18,24] Specifically, measurements were conducted by the same observer (MC) following the recommended technique.[28-30] Specifically, the crown-heel length was measured weekly to the nearest millimeter, using a rigid length board, with the infant in the supine position. Two observers participated in measurements: one holding the infant's head in the Frankfurt plane against the fixed headboard and aligned with the trunk, whereas the other observer (MC) gently pressed the infant's knees down, fully extending the lower limbs. The feet were held vertically at a right angle to the length board, and the footboard was moved up against the heels. Consistency was ensured by performing three consecutive measurements, and the mean value of these measurements was utilized for the analysis. This approach helped minimize measurement variability and enhance the accuracy of the length data obtained in this study. The mean coefficient of variation, calculated as the standard deviation (SD) divided by the mean multiplied by 100, was 0.19.

Length growth was assessed using both differential (Δ) length z-scores and length gain velocity.[18]

Statistical analysis

The demographics and clinical characteristics of infants are presented using frequencies (percentages) for categorical variables, and mean values with SD or median and interquartile range (25^{th} percentile–75^{th} percentile) for continuous variables, as appropriate. Differences between groups were assessed using tests, such as the independent sample *t*-test, Mann–Whitney *U*-test, Chi-squared test, or Fisher's exact test, as determined suitable for the specific comparisons. A significance level (α) of 0.05 was used to establish statistical significance. Data analysis was performed using SPSS version 29 software (SPSS Inc., Chicago, IL, USA).

Results

Demographic data

Out of the 115 participants from the principal cohort study,[18] a total of 84 individuals who had serum phosphate measured during the exposure period were included in this study, 35 individuals in Group 1 and 49 individuals in Group 2.

The demographic data of included participants are shown in Table 1. Infants of Group 2 were significantly shorter and exhibited smaller head circumference at birth, compared to Group 1. There were no significant differences between groups in the prevalence of bronchopulmonary dysplasia or the use of postnatal steroids. There were no cases of necrotizing enterocolitis or cholestasis in either group.

Exposure period

In Group 1, the median postnatal age (P_{25}; P_{75}) at the beginning of the exposure period was 11.0 (9.0; 13.0) days, which corresponds to a median of 1.6 (1.3; 1.9) weeks. The median postnatal age at the end of the exposure period was 37.0 (23.0; 50.0) days, which corresponds to a median of 5.0 (4.95; 5.24) weeks.

In Group 2, the median postnatal age (P_{25}; P_{75}) at the beginning of the exposure period was 12.0 (9.0; 14.0) days, which corresponds to a median of 1.7 (1.29; 2.0) weeks. The median postnatal age at the end of the exposure period was 35.0 (29.0; 49.0) days, which corresponds to a median of 5.1 (4.86; 5.2) weeks.

Nutrient intakes during the exposure period

The mean total energy, protein, PER, fat, and carbohydrate intakes, and the median calcium, phosphorus, and magnesium intakes fell within the ranges recommended in the updated ESPGHAN 2022 guidelines.[13]

To account for missing nutrient intake values, the average of the weekly mean values recorded was calculated and compared between groups. No significant differences were observed in nutrient intakes, except for significantly higher fat and carbohydrate intakes in Group 2, as indicated in Table 2. Data on the energy and macronutrients content in HM (MOM and DHM) during the exposure period, as well as the daily protein and fat intake provided by modular protein and fat supplements during the exposure period, are provided in Tables 1 and 2, respectively. In Group 2, HM was significantly denser in carbohydrates and fat [Table 1], and modular fat supplementation had provided significantly higher daily fat intake, compared with Group 1 [Table 2].

Table 1: The demographic and clinical characteristics of infants included on the *post hoc* analysis

	Group 1 (*n*=35)	Group 2 (*n*=49)	*P*
Gestational age (weeks), mean (SD)	29.5 (1.94)	29.5 (2.20)	0.845
Females, *n* (%)	15 (42.9)	22 (44.9)	0.853
Twins, *n* (%)	11 (31.4)	21 (42.9)	0.288
Birth weight *Z*-score, mean (SD)	−0.06 (0.78)	−0.21 (0.63)	0.344
Birth length *Z*-score, mean (SD)	−0.21 (0.77)	−1.08 (0.70)	<0.001
Birth HC *Z*-score, mean (SD)	−0.52 (1.05)	−1.58 (0.77)	<0.001
Prenatal steroids, *n* (%)	34 (97.1)	45 (91.8)	0.396
SNAPPE II severity index	10 (0–22)	10 (1–27)	0.538
Bronchopulmonary dysplasia, *n* (%)	3 (8.6)	6 (12.2)	0.729
Postnatal steroids, *n* (%)	0	4 (8.2)	0.137

Student's *t*-test, Chi-square test, Fisher's exact test, median test, or Mann–Whitney test as appropriate. HC - Head circumference; SNAPPE - Score for neonatal acute physiology perinatal extension; SD - Standard deviation

Serum phosphate levels

To cover the entire exposure period in both groups, all serum phosphate measurements between the 2nd and 5th postnatal weeks were considered, provided that the infants were still fed fortified HM. The mean serum phosphate value for each week was calculated per participant.

Among the 84 participants included in the study, 142 serum phosphate measurements were obtained during the exposure period. Out of these measurements, 52 were from Group 1 and 90 were from in Group 2.

The mean (SD) serum phosphate levels were found to be significantly lower in Group 2 (n = 49) than in Group 1 (n = 35): 6.0 (0.9) mg/dL vs. 5.5 (0.9) mg/dL, P = 0.022.

Table 3 presents the comparison between groups of the prevalence of hypophosphatemia during the exposure period, using the cutoff points ≤5.6 mg/dL or ≤3.72 mg/dL. It is important to note that during the total exposure period, some participants may have experienced hypophosphatemia on multiple occasions. Therefore, the prevalence stated for the total period may be less than the sum of cases reported in all weeks [Table 3].

Although no significant differences were found in the prevalence of hypophosphatemia between the two groups, there was weak evidence (P = 0.051) suggesting a higher prevalence in Group 2, during week 3, using the cutoff ≤5.6 mg/dL, as shown in Table 3.

Length growth

The analysis of length growth included the period from birth to discharge, including the periods before and after

Table 2: Daily energy, protein, protein-to-energy ratio, fat, carbohydrate, calcium, phosphorus, and magnesium intake during the exposure period

Daily nutrient intakes	Group 1 (*n*=35)	Group 2 (*n*=49)	*P*
Total energy (kcal/kg), mean (SD)	117.1 (15.2)	121.6 (10.1)	0.130
Protein (g/kg), mean (SD)	3.9 (0.5)	4.0 (0.4)	0.297
PER (g/100 kcal), mean (SD)	3.4 (0.6)	3.3 (0.3)	0.357
Fat (g/kg), mean (SD)	5.3 (0.9)	6.1 (1.0)	<0.001
Carbohydrate (g/kg), mean (SD)	11.7 (1.6)	13.0 (1.2)	<0.001
Calcium (mg/kg), median (P_{25}–P_{75})	124.6 (91.7–133.0)	120.7 (92.1–128.9)	0.565
Phosphorus (mg/kg), median (P_{25}–P_{75})	72.7 (66.9–79.2)	70.1 (55.7–75.9)	0.060
Magnesium (mg/kg), median (P_{25}–P_{75})	11.9 (11.2–12.9)	11.6 (9.5–12.4)	0.072

Student's *t*-test or Mann–Whitney test as appropriate. PER - Protein-to-energy ratio; SD - Standard deviation

Table 3: Prevalence of hypophosphatemia in both groups, during the exposure period, using serum phosphate levels ≤5.6 mg/dL or ≤3.72 mg/dL as cut-off points

	Week 2	Week 3	Week 4	Week 5	Total period
≤5.6 mg/dL					
Group 1, *n* (%)	7 (53.8)	4 (25.0)	4 (30.8)	3 (30.0)	14 (40.0)
Group 2, *n* (%)	5 (23.8)	16 (55.2)	14 (58.3)	7 (43.8)	27 (55.1)
P	0.139	0.051	0.109	0.683	0.172
≤3.72 mg/dL					
Group 1, *n* (%)	0	0	0	0	0
Group 2, *n* (%)	0	2 (6.9)	1 (4.0)	2 (12.5)	4 (8.2)
P	-	0.531	1.000	0.508	0.137

Chi-square test or Fisher's exact test, as appropriate

the exposure period. The weeks were defined as follows: the 1st week comprised 1–10 postnatal days, the 2nd week comprised 11–17 postnatal days; the 3rd week comprised 18–24 postnatal days; the 4th week comprised 25–31 postnatal days; the 5th week comprised 32–38 postnatal days; the 6th week comprised 39–45 postnatal days; the 7th week comprised 46–52 postnatal days; the 8th week comprised 53–59 postnatal days; the 9th week comprised 60–66 postnatal days; and the 10th week comprised 67–73 postnatal days.

Accurate length measurements were available for 17 out of 35 participants in Group 1 and for all 49 participants in Group 2.

Δ length *z*-scores

Differential (Δ) length *z*-scores did not differ significantly between groups during the exposure period [Table 4]. However, infants of Group 2, despite being born significantly shorter [Table 1], had a significantly smaller *z*-score decline from birth to discharge, reflecting a better linear growth [Table 4].

Length gain velocity

To account for missing length gain velocity measurements, the average of mean length velocities recorded in each week, during the exposure period, was considered for each participant.

During the exposure period, the mean (SD) length gain velocity was significantly higher in Group 2 (n = 49) than in Group 1 (n = 17): 0.89 (0.23) cm/week versus 1.06 (0.19) cm/week, P = 0.003.

Due to excessive missing length measurements before and after the exposure period, it was not possible to accurately calculate length gain velocity from birth to discharge.

Discussion

In this study, infants of Group 2 receiving fortified HM with added modular supplements, guided by measured HM macronutrient content, received significantly higher fat and carbohydrate intakes, compared with infants of Group 1 who underwent fortification based on the assumed HM macronutrient content. Furthermore, infants of Group 2 exhibited significantly better length growth, which is indicative of bone growth according to research.[31] However, they also had significantly lower levels of serum phosphate, suggesting inadequate bone mineralization.[21] This finding raises the hypothesis that the fortification method used in Group 2 may promote bone growth without appropriate mineralization of osteoid, a condition known as osteomalacia.

The higher fat intake observed in Group 2 was attributed to the intake of HM denser in fat and to the higher fat intake provided by the modular MCT oil supplementation. Both fortification methods used the same multinutrient fortifier without any modular carbohydrate supplement inclusion. The higher carbohydrate intake in Group 2 can be attributed to the higher carbohydrate content in HM within this group.

Bone nutrition

Adequate bone nutrition involves providing sufficient energy and protein to support bone matrix formation as well as adequate mineral supply to facilitate

Table 4: Differential (Δ) length *Z*-score during the exposure period and from birth to discharge, in both groups

Δ Length *Z*-score, median (P_{25}–P_{75})	Group 1 (*n*=17)	Group 2 (*n*=49)	*P*
From birth to discharge	−0.71 (−1.74–−0.40)	−0.34 (−0.76–−0.15)	0.007
During exposure period	−0.34 (−0.69–−0.14)	−0.25 (−0.40–−0.05)	0.182

Student's *t*-test or Mann–Whitney test, as appropriate

mineralization.[9,10] It is recognized that extremely preterm infants with rapid growth may require higher calcium and phosphorus intakes compared to infants with slower growth rates.[32]

In preterm infants fed fortified HM, bone mineralization benefits from currently available HM multinutrient fortifiers, which contain energy, macronutrients, and minerals.[14,33] However, concerns arise when modular macronutrient supplements are added to fortified HM without additional mineral supplementation.

There are some mechanisms that may explain the association between higher fat and carbohydrate intakes and better length growth (which reflects bone growth) in our infants who were fed fortified HM with added modular supplements. In animal models, it was found that osteocytes (not osteoblasts) directly build mineralized bone structures.[34] Osteoblasts serve as a precursor of osteocytes[34] and fatty acids and glucose are important energy sources for their function and differentiation.[35] In a longitudinal study of breastfed term infants, it was found that higher total carbohydrate concentration in HM was significantly associated with greater infant length in the first 12 months of life.[36] This is in line with the results of our study, in which infants of Group 2, who received higher carbohydrate intake through HM denser in carbohydrates, had better length growth.

In a cohort study of very preterm infants, it was reported that fat intake provided during the first four postnatal weeks was positively associated with bone mineral content (BMC) at term equivalent age.[10] Noteworthy, the effect of dietary fat on bone health is dependent on the quality of fat consumed. Excessive intake of saturated fatty acids and *n*-6 polyunsaturated fatty acids may promote bone loss and osteoporosis. Conversely, monounsaturated fatty acids, particularly linolenic acid and *n*-3 polyunsaturated fatty acids, have been found to have beneficial effects on bone health.[37,38] In the context of this study, we speculate that the addition of modular MCT oil to fortified HM may have provided additional energy resources while sparing *n*-3 polyunsaturated fatty acids from HM for bone metabolism. This hypothesis suggests that the inclusion of modular MCT oil in the diet of preterm infants could have contributed to the observed positive effects on bone growth.

Individualized mineral supplementation, utilizing organic calcium and phosphate formulations, has been proposed as a preventive measure against inadequate bone mineralization in extremely preterm infants[3,32] and as a treatment for preterm infants with low serum phosphate levels.[14] However, the extent to which individualized mineral supplementation, in addition to extra energy and macronutrients during the fortification of HM, can effectively prevent the rapid growth of insufficiently mineralized osteoid remains unknown.[23]

Serum phosphate as a marker of bone mineralization

Preterm infants are susceptible to MBD, which can manifest as osteopenia due to a decrease in the organic bone matrix (osteoid), or osteomalacia resulting from deficient mineralization and subsequent accumulation of nonmineralized osteoid.[1-3] Following preterm birth, there is evidence of decreased BMC and BMD at term equivalent age.[1] This reduction may be attributed to various factors, such as low bone mineral reserves at birth, limited nutrient intake, and immature endogenous endocrine system affecting proper utilization of minerals to match intrauterine bone accretion at equivalent gestational age.[1] MBD remains silent until significant demineralization occurs.[1,39] Low serum phosphate levels and elevated serum alkaline phosphatase levels have been identified as early markers of disrupted mineral metabolism in preterm infants.[14,20,21] These biochemical markers are recommended to be monitored on a 1–2 week basis starting from 2 to 3 postnatal weeks, with the frequency depending on the presence of risk factors for MBD.[12,22,40]

In a prospective study of extremely low birth weight infants, it was found that serum phosphate was associated with decreased BMD assessed by dual-energy X-ray absorptiometry (DXA).[41] The establishment of a specific threshold for hypophosphatemia as a marker of MBD is still pending. In a systematic review,[21] it was found that a serum phosphate level equal to or <1.8 mmol/L (≤5.6 mg/dL) exhibited a good correlation with DXA measurements, resulting in a specificity of 96% and a sensitivity of 50%.[42] The accuracy improves when the serum phosphate threshold was further lowered to equal to or <1.2 mmol/L (≤3.7 mg/dL), with a specificity of 100%, sensitivity of 33%, positive predictive value

of 100%, and negative predictive value of 57% when compared to quantitative ultrasound measurements.[43]

Length growth as a surrogate of bone growth

Based on published data, we made the assumption that body length growth could serve as a surrogate marker of long bone growth in preterm infants.

During infancy, bone growth involves the elongation of bones, which occurs concurrently with the process of skeletal maturation.[19,44]

In a longitudinal study of preterm infants, a panel of biochemical markers of collagen and bone turnover was assessed during the first 10 postnatal weeks.[45] A positive correlation between linear growth and N-terminal propeptide of type III procollagen (P3NP), a marker of soft-tissue collagen synthesis, was found.[45] These results suggest that linear growth may be a marker of osteoid growth. Another prospective study demonstrated that the growth velocity of femur length in fetuses between 19 and 34-week gestation was predictive of skeletal size during childhood at 4 years of age.[46] Nonetheless, predictive values of bone growth based on body length or segmental body lengths depend on various factors, including the individual's age, the specific body segment being assessed, the type of anthropometric and imaging measurement techniques used, and the presence of soft tissue surrounding the bone in an anthropometric measurement compared to a direct bone measurement.[31,47,48]

Study limitations

Several limitations should be acknowledged in this study. As a *post hoc* secondary analysis, missing data may have introduced bias. In addition, while excluding infants without serum phosphate measurements, the original sample of the mixed-cohort study was reduced, and probably underpowered to detect significant associations of HM fortification methods with either energy and protein intakes and the prevalence of hypophosphatemia. Nevertheless, the studied sample size was powered enough to find significant associations of HM fortification with extra energy and macronutrients with better length growth and lower serum phosphate levels. As the original sample[18] did not specifically aim for representativeness, the same intention does not apply to this reduced convenience sample. This *post hoc* secondary analysis used surrogate measurements as markers of bone nutrition, mineralization, and growth, rather than direct measurements.

Conclusions

The findings from this exploratory analysis suggest a hypothesis that the addition of fat and carbohydrate intakes through fortifying HM, without accompanying mineral supplementation, may contribute to the accumulation of insufficiently mineralized osteoid, as indicated by low serum phosphate levels, despite the presence of bone growth reflected by length growth.

To further validate this hypothesis and gain a more comprehensive understanding of the underlying mechanisms involved, future intervention studies incorporating direct biomarkers of bone mass content and mineral density are warranted.

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Conflicts of interest

There are no conflicts of interest.

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