Effects of vitamin D and high dairy protein intake on bone mineralization and linear growth in 6- to 8-year-old children: the *D-pro* randomized trial

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ABSTRACT

Background: Vitamin D and dairy protein may stimulate bone mineralization and linear growth in children, but previous studies show inconsistent results and have not examined their combined effects.

Objectives: To investigate combined and separate effects of vitamin D supplementation and high-protein (HP) compared with normal-protein (NP) yogurt intake on children's bone mineralization and linear growth.

Methods: In a 2 × 2–factorial trial, 200 healthy, 6- to 8-year-old, Danish, children with light skin (55°N) were randomized to 20 μg/d vitamin D_3 or placebo and to substitute 260 g/d dairy with HP (10 g protein/100 g) or NP (3.5 g protein/100 g) yogurt for 24 weeks during an extended winter. Outcomes were total body less head (TBLH) and lumbar spine bone mineral density (BMD), bone mineral content (BMC), and bone area (BA) by dual-energy X-ray absorptiometry, height, and biomarkers of bone turnover and growth. The primary outcome was TBLH BMD.

Results: In total, 184 children (92%) completed the study. The baseline serum 25-hydroxyvitamin D was 80.8 ± 17.2 nmol/L, which increased by 7.2 ± 14.1 nmol/L and decreased by 32.3 ± 17.5 nmol/L with vitamin D and placebo, respectively. The baseline protein intake was 15.4 ± 2.4 energy percentage (E%), which increased to 18.3 ± 3.4 E% with HP. There were no vitamin D–yogurt interactions and no main effects of either intervention on TBLH BMD. However, vitamin D supplementation increased lumbar spine BMD and TBLH BMC compared to placebo, whereas HP groups showed lower increments in lumbar spine BMD, TBLH BMC and BA, and plasma osteocalcin compared to NP groups. Height, growth factors, and parathyroid hormone levels were unaffected.

Conclusions: Although there were no effects on whole-body BMD, vitamin D increased bone mass and spinal BMD, whereas high compared with normal dairy protein intake had smaller incremental effects on these outcomes. This supports a recommended vitamin D intake of around 20 μg/d during winter but not use of HP dairy products for improved bone mineralization among healthy, well-nourished children. This trial was registered at clinicaltrials.gov as NCT03956732. *Am J Clin Nutr* 2021;114:1971–1985.

Keywords: bone mineralization, DXA, height, insulin-like growth factor, cholecalciferol, vitamin D status, serum 25(OH)D, milk protein, pediatric nutrition, school-age

Introduction

Optimal bone mineralization during childhood and adolescence is an important determinant of adult bone health, as peak bone mass is inversely associated with later risks of osteoporosis and fractures (1). Vitamin D and high-quality protein from dairy products are considered important for childhood bone health (2, 3) and linear growth (4, 5). However, results from randomized trials are inconsistent (3, 6–11) and the specific effect of dairy protein, alone or in combination with vitamin D, has not been investigated in children.

A high prevalence of vitamin D deficiency (serum 25-hydroxyvitamin D (s-25(OH)D) <30 nmol/L) and insufficiency

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Supplemental Table 1 and Supplemental Figure 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: BA, bone area; BMAD, bone mineral apparent density; BMC, bone mineral content; BMD, bone mineral density; COVID-19, coronavirus disease 2019; DXA, dual-energy X-ray absorptiometry; ESPGHAN, European Society for Paediatric Gastroenterology Hepatology and Nutrition; E%, energy percentage; FFQ, food frequency questionnaire; HP, high protein; IGF-I, insulin-like growth factor I; IGFBP-3, IGF binding protein-3; IOM, Institute of Medicine; LC-MS/MS, liquid chromatography–mass spectrometry; NP, normal protein; PTH, parathyroid hormone; Q-Q, quantile-quantile; s-25(OH)D, serum 25-hydroxyvitamin D; SUN, serum urea nitrogen; TBLH, total body less head.

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(s-25(OH)D <50 nmol/L) is evident globally, with estimates of about 13% and 40%, respectively, in Europe (12) and 5% and 18%, respectively, in the United States (13). Vitamin D increases intestinal absorption and renal reabsorption of calcium (14), which is an important constituent of bone matrix, and vitamin D deficiency may therefore contribute to nonoptimal bone mineralization. Vitamin D supplementation has been shown to have inconsistent effects on bones in healthy children (8–11) and to increase insulin-like growth factor I (IGF-I) and linear growth in children with rickets (15). During its extended winter (October–March), Denmark is suitable for investigating effects of vitamin D supplementation due to minimal UVB-induced vitamin D synthesis, little fortification of foods, and, until December 2020, no recommendation for vitamin D supplements for children with light skin. In the Nordic countries and the United States, the RDAs in children are 10 μg/d (16) and 15 μg/d (17), respectively. Yet, a recent meta-analysis showed that about 20 μg/d is required to maintain winter s-25(OH)D levels ≥50 nmol/L (18), which is defined as sufficiency by the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) (14) and the Institute of Medicine (IOM) (19).

Intake of milk and dairy products may also increase bone mineralization (3) and linear growth (4). Specifically, consumption of dairy protein has been shown to stimulate secretion of IGF-I (20), which regulates numerous signaling pathways in bone cells (21). The effect of dairy protein per se on bones has not been investigated in children, but observational studies generally show positive associations between the two (22–24). However, high protein intake may increase urinary calcium excretion and, thereby, potentially bone demineralization (25).

Combined effects of vitamin D and milk protein have been indicated, as whey protein supplements with vitamin D, calcium, and leucine increased bone mineral density (BMD) and IGF-I in sarcopenic elderly (26). Further, milk proteins were recently shown to enhance vitamin D absorption in rats (27). Therefore, we aimed to investigate combined and separate effects of vitamin D supplementation and high-protein (HP) compared with normal-protein (NP) yogurt on bone mineralization and linear growth in 6- to 8-year-old children during extended winter. The primary outcome was BMD of the total body less head (TBLH). Secondary outcomes were BMD, BMD *z*-scores, bone mineral content (BMC), and bone area (BA) of the TBLH and lumbar spine, as well as height and biomarkers of bone turnover and growth. We hypothesized that vitamin D and high dairy protein intakes would increase these outcomes and that the effects would be greater when combined.

Methods

Study design

The *D-pro* study ("Effects of milk protein and vitamin D on children's growth and health") was a 2 × 2–factorial, randomized, 24-week trial in 200 healthy 6- to 8-year-old children living in Denmark (55°N) during the extended winter period. The children were randomly allocated to receive blinded tablets with either 20 μg/d of vitamin D_3 or placebo and to substitute 260 g/d of their usual milk and yogurt in the diet with either HP or NP yogurt. This resulted in 4 groups: placebo-HP, placebo-NP, vitamin D–HP, and vitamin D–NP, where the placebo and NP treatments corresponded to the children's habitual intakes. Baseline visits were conducted from 19 August to 25 October 2019, when vitamin D status was at its highest after summer. Endpoint visits were conducted by the end of the vitamin D winter—that is, when UVB-induced synthesis was still minimal—from 3 February to 3 April 2020.

All study activities were conducted at the Department of Nutrition, Exercise, and Sports, University of Copenhagen, Frederiksberg, Denmark. Biomedical measurements followed standard operational procedures and were conducted at baseline and endpoint by the same trained investigators or a maximum of 2 different investigators. The study was approved by the Committees on Biomedical Research Ethics for the Capital Region of Denmark (no. H-19008199) and was registered with clinicaltrials.gov (NCT03956732) on 21 May 2019.

Subjects

Boys and girls living in the Capital Region of Denmark were identified through the Danish Civil Registration System and recruited via invitation letter to the parents. The children had to be 6–8 years old at baseline, of European origin, and have light skin (be considered White), since a vitamin D supplement was recommended for children with darker skin in Denmark. In addition, the children had to have a habitual intake of at least 0.25 liters per day of milk/yogurt products and the child and at least 1 parent had to be able to speak and understand Danish. Only 1 child per household could participate, and the child could not be included if the family was planning a winter vacation south of a latitude of 50°N. Exclusion criteria were an allergy or intolerance to milk or milk components, chronic disease or the use of medication that might affect study outcomes, habitual use of vitamin D–containing supplements >3 days/week for the prior 2 months and at all in the month immediately preceding the start of the intervention, and concomitant participation in other studies involving dietary interventions or blood sampling. Parents and children received written and oral information about the study, and written consent was obtained from all custody holders of each child.

Randomization and allocation

Randomization was performed in blocks of 12 children to ensure equal distribution into the 4 study groups throughout the fall. A staff member not involved in the study created the randomization list using R (version 3.6.2), and 236 consecutive numbers were each linked to 1 of the 4 intervention groups. Another impartial staff member created 230 sealed envelopes containing the dairy product allocation and marked them and the tablet bottles with the randomization numbers. The order in which the children completed their baseline visit determined their randomization number, and thus their allocation.

Intervention, compliance, and blinding

Chewable tablets with either 20 μg of vitamin D_3 (Minisun) or placebo (both from Oy Verman Ab) of identical appearance and taste were provided in identical, white tablet bottles containing 200 tablets. Tablets were analyzed for content of vitamin D by

liquid chromatography–mass spectrometry (LC-MS/MS) at the National Food Institute, Søborg, Denmark, by triplicate analysis of 5 tablets from each dose. These analyses showed a content of 25 ± 1.6 μg/tablet in the vitamin D tablets, whereas vitamin D was undetectable in the placebo tablets. Parents were instructed to give the child 1 tablet per day in connection with a meal, to record the child's intake of tablets in precoded recording sheets, and to store the tablets out of the reach of children. The tablet intervention arm was double blinded, which was verified by asking investigators and parents to guess the intervention group at the endpoint. Tablet compliance was assessed from the vitamin D biomarker s-25(OH)D, as well as from the recording sheets and number of tablets left in the bottles, which the parents returned at the endpoint.

The HP groups received drained, low-fat yogurt ("skyr") with a high protein content of 9–11 g protein/100 g, and the NP groups received regular yogurt with a normal protein content of 3.0–3.9 g protein/100 g from Arla Foods amba (**Supplemental Table 1**). All yogurts were fermented with the same bacterial strains: *Streptococcus thermophilus* and *Lactobacillus bulgarius*. Parents picked up the yogurts at the department free of charge every second or third week and could choose from unflavored and fruit-flavored variants in 1-L containers, as well as 150-mL cups with granola on top, all in the original packaging. Parents were instructed that the children should consume 300 g/d for 6 days per week, corresponding to approximately 260 g/d, and substitute this for the corresponding amount of milk or yogurt in the habitual diet. Otherwise, the children were to maintain their habitual dietary habits. Parents recorded the child's daily intake of the specific yogurts during the intervention in the recording sheets. These data were used to calculate the child's mean daily yogurt intake as a measure of compliance and its contributions of protein, calcium, sugar, fat, and SFAs. In addition, serum urea nitrogen (SUN) was used as a short-term indicator of protein intake. Blinding of the yogurts was not possible due to differences in taste and texture, but the investigators were blinded prior to the data analysis.

Household characteristics and pubertal stage

At baseline, the parents answered an electronic questionnaire about their education level and income. Pubertal stage was evaluated by the parents using a self-administered questionnaire with illustrations for the Tanner stages of pubic hair for boys and breast development for girls. Menarche status (yes/no) was also obtained for girls. Puberty was defined as being in Tanner stage 2–5 or having reached menarche.

Dietary intake

Energy and macronutrient intake.

Energy and macronutrient intake was measured by a 4-day dietary record covering 3 consecutive weekdays and 1 weekend day prior to the baseline and endpoint visits. At these 2 time points, 100% and 96% of participants, respectively, completed the dietary recording with a minimum of 3 recording days. Parents were instructed to weigh and record everything the child ate and drank (except water) in the web-based software Madlog (17). Household measures were used when weighing was not possible. The dietary records were checked for completeness and corrected after dialogue with the parents at the visit. Parents were instructed to choose the generic variants of the foods consumed to ensure as much nutritional information from the software as possible. If a variant lacked information on sugar or saturated fat content, a trained study investigator changed it to a variant with a similar energy and macronutrient content, but which comprised this information.

Vitamin D and calcium intake.

At the baseline and endpoint visits, parents completed an electronic food frequency questionnaire (FFQ) to assess the child's intake of vitamin D and calcium the preceding month. The FFQ was based on a semi-quantitative FFQ previously validated for vitamin D intake (28, 29), which was digitalized and updated for this study with new fortified products on the Danish market, such as plant-based alternatives to dairy products. The FFQ consisted of 16 questions covering milk and dairy products, cheese, eggs, bread, cereals, meat, fish, fortified fats, and dietary supplements. Most food categories were subcategorized; for example, yogurts were divided into "skyr," other yogurt products, and plant-based alternatives to yogurt. The FFQ measured 9 intake frequencies ranging from "rarely/never" to "4 or more times per day." Milk was reported in deciliters, and bread and bread toppings were reported in slices. Portion sizes for yogurt, cheese, breakfast cereals/porridge, meat, and fish were estimated from photos from the Danish National Survey of Diet and Physical Activity (30). Within each food category, vitamin D and calcium contents were obtained from the Danish food composition database, Frida (31), supplemented with information from product labels for fortified fats and plant-based chocolate drinks and yogurts. At endpoint, 7 participants had not included the intervention yogurts in the FFQ, so these were corrected based on the recording sheets and dietary record.

Anthropometry and bone mineralization

Standing height was measured 3 times to the nearest millimeter with the head in the Frankfurt plane using a Seca 216 stadiometer. The children were weighed once in the fasted state in underwear on a Tanita BWB-800 digital scale. From mean height and weight, the BMI was calculated and categorized as "underweight," "normal weight," or "overweight or obese" using the International Obesity Task Force criteria described by Cole et al. (32, 33). In addition, age- and sex-adjusted *z*-scores for height and BMI were calculated using the WHO Anthro Software (version 1.0.4).

To obtain measures of bone mass, bone size, and bone density, we measured the BMC, BA, and BMD, respectively, of the whole body and lumbar spine (vertebra L1–L4) by dual-energy X-ray absorptiometry (DXA) using a GE Lunar Prodigy (GE Healthcare) scanner with GE Healthcare software version 17, SP1. The software computed age- and sex-specific *z*-scores for BMD, and we used the TBLH of the whole-body scans. Scanning took place before noon, after a standardized breakfast meal, and after the children had emptied the bladder. The children wore light clothes without metal. For the lumbar spine scan, a firm pillow

was placed under the children's knees. Daily and weekly quality assurance were conducted with a quality assurance–phantom and a Spine Phantom no. 17466, respectively, which had CVs during the study period of 0.4% and 0.3% for BMC and BMD, respectively.

Blood sampling and analysis

Venous blood samples of 30 mL were taken in the morning after an overnight fast. Parents were instructed to apply local anesthetic patches (emla, AstraZeneca) to the children's arms before sampling to minimize discomfort. Lithium-heparinized plasma tubes for analyses of osteocalcin, IGF-I, and IGF binding protein-3 (IGFBP-3) and EDTA plasma tubes for analysis of parathyroid hormone (PTH) were kept on ice. Serum tube samples for analyses of calcium, albumin, 25(OH)D, and SUN were left to coagulate at room temperature for 30 minutes. All samples were centrifuged for 10 minutes at 2300 × *g* at 4°C and stored at –80°C until analysis.

Plasma osteocalcin, IGF-I, IGFBP-3, and PTH were analyzed on an Immulite 2000 Xpi Systems Analyzer (Siemens Healthcare GmbH). The intraassay CV was 3%–4% and the interassay CV was 2%–3%. To estimate free IGF-I, we calculated the IGF-I:IGFBP-3 molar ratio (IGF-I, 1 μg/L = 0.133 nM; IGFBP-3, 1 mg/L = 33 nM). Plasma osteocalcin was used as a biomarker of bone formation, and plasma IGF-I and IGFBP-3 were used as biomarkers of growth. SUN, calcium, and albumin were analyzed on a Pentra 400 (HORIBA ABX) analyzer with intra- and interassay CVs of <3% and <2%, respectively. Albumin-corrected serum calcium was calculated as total serum calcium + 0.020 × (41.3 − serum albumin), and hypercalcemia was defined as a total serum calcium level >2.6 mmol/L, as recommended by Lietman et al. (34). S-25(OH)D_2 and 25(OH)D_3 were analyzed by LC-MS/MS at University College Cork, Ireland, as described elsewhere (35). The total s-25(OH)D concentration was calculated as the sum of 25(OH)D_2 and 25(OH)D_3 concentrations. The intra- and interassay CVs were <6% and <5%, respectively, for both s-25(OH)D metabolites.

Protocol changes during the national lockdown

Due to the coronavirus disease 2019 (COVID-19) pandemic, University of Copenhagen entered partial lockdown from 13 March 2020 until the study completion, which affected the endpoint visits of 66 children. Of these, 35 completed fasting morning visits, including blood sampling; 25 completed non-fasting visits without blood sampling; and 6 were not able to attend, in accordance with the regulations, and were therefore lost to follow-up. During the lockdown, the children were weighed in their clothes, which were then subtracted from the measured weight by using standardized weights of clothes, and the fasting children were DXA scanned in the fasted state, as we were not allowed to serve them breakfast.

Adverse events

Parents were asked to report any health-related events during the intervention to the study staff. At the endpoint, they were further asked whether there had been any adverse events since baseline. All adverse events and use of medication during the intervention were recorded and evaluated for severity and possible relation to the intervention by the responsible clinician.

Sample size calculation

The sample size calculation was based on detecting a relevant BMD accretion difference of 0.0085 g/cm^2 between any 2 groups, which corresponds to 0.8 SD of the previously observed BMD accretion of 0.0197 ± 0.0105 g/cm^2 during 6 months among 368 Danish 8- and 9-year-old children (36). With a 5% significance level and 80% power, we would need 49 children in each of the 4 intervention groups ($n = 196$ in total). Testing the main effects of vitamin D and HP dairy intakes with 2 groups ($n = 98$ children per group) enabled us to detect a BMD accretion difference of 0.006 g/cm^2, corresponding to 0.6 SD.

Statistical analysis

Data were analyzed using Stata/IC 16.1, and statistical significance was established at a P value < 0.05. Normal distribution of variables was assessed with histograms and Q-Q (quantile-quantile) plots. Values are presented as means ± SDs, medians (IQRs), *n*, or percentages as appropriate. ANCOVA models were validated with residual histograms and Q-Q plots, and all met the model assumptions. As we wished to test the biological effects of the intervention, data were analyzed as completer's cases. Differences in baseline characteristics between dropouts and completers were tested with Pearson's chi-squared, Wilcoxon rank-sum, or 2-sample *t*-test, as appropriate. Within-group changes from baseline to endpoint were tested with paired *t*-tests.

Differences in intervention durations between the 4 study groups were analyzed in a 1-way ANCOVA with study group (4 groups) as a fixed factor. Changes in dietary intakes and bone and growth outcomes were analyzed using a 2-way ANCOVA with the vitamin D (vitamin D and placebo) and yogurt (HP and NP) groups as fixed factors and baseline variable as a covariate. Primary analyses of bone and growth measures included the treatment interaction term: if this was nonsignificant, we collapsed the groups and tested separate effects of vitamin D and yogurts as main effects in the same additive model. For significant interactions, we performed pairwise Bonferroni-corrected comparisons. As dietary intake was not considered an outcome per se, we only investigated differences between the HP and NP groups and between the vitamin D and placebo groups.

In secondary analyses, models were adjusted for age, sex, puberty, and changes in BMI *z*-scores and calcium intakes. As recommended by Prentice et al. (37), BA (BMC only), height, weight, age, sex, and puberty were included as covariates in size-adjusted models for BMC and BA. For volume correction of spinal BMD, we used the equation by Ward et al. (38) to calculate bone mineral apparent density (BMAD) as:

$$\mathrm{BMAD} = (\mathrm{BMC}_1 + \mathrm{BMC}_2 + \mathrm{BMC}_3 + \mathrm{BMC}_4) / (\mathrm{V}_1 + \mathrm{V}_2 + \mathrm{V}_3 + \mathrm{V}_4) \quad (1)$$

Here, V_n is the volume of the *n*th vertebra = $\mathrm{area}_n^{1.5}$. To check for consistency, per-protocol analyses were conducted, excluding those who breached the protocol during the intervention: that is, those who reported southern winter vacations ($n = 4$) or

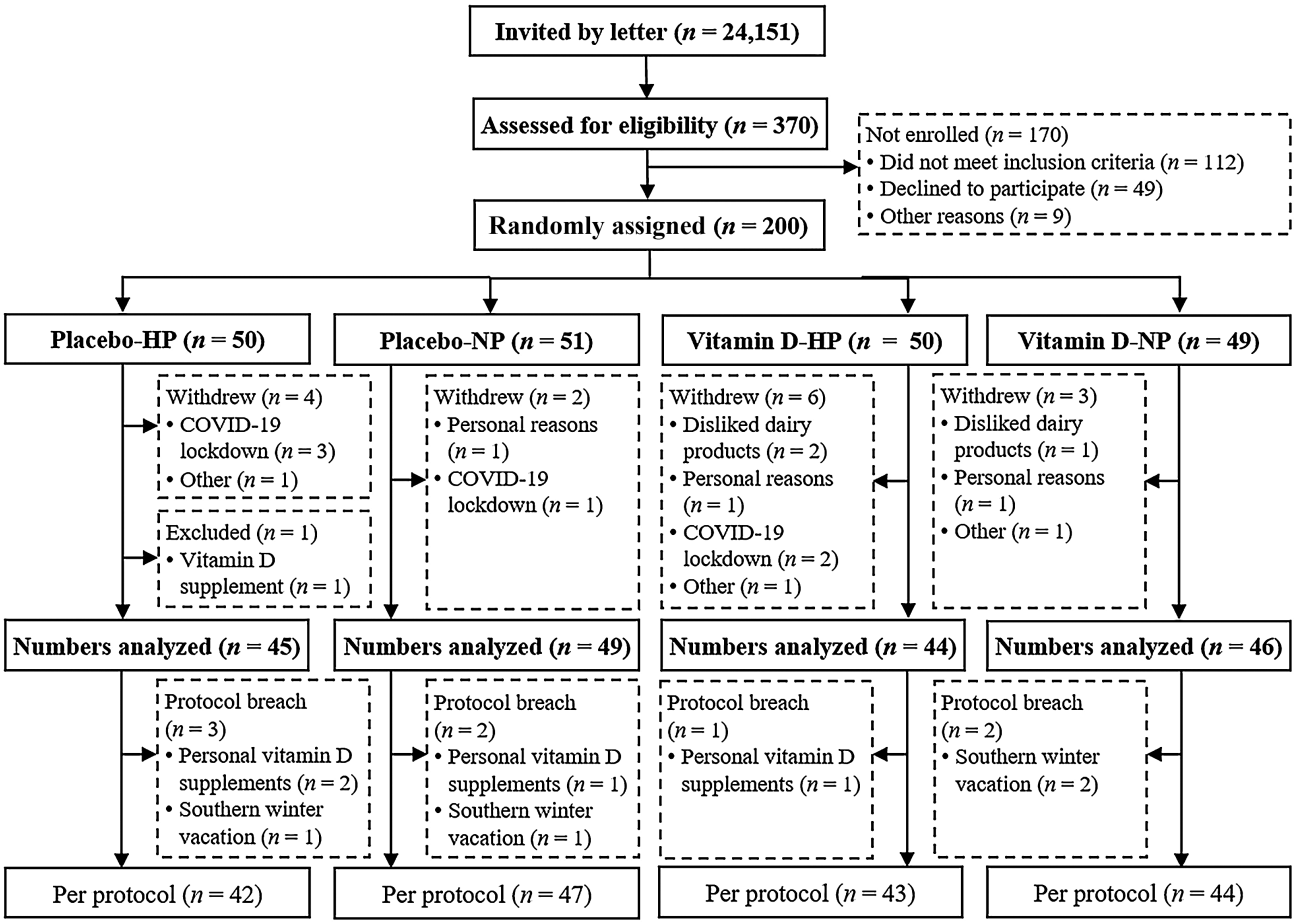

FIGURE 1 Flowchart from recruitment to analyses. Abbreviations: COVID-19, coronavirus disease 2019; HP, high-protein yogurt; NP, normal-protein yogurt.

use of non–study allocated vitamin D–containing supplements ($n = 4$). Finally, we investigated whether sex modified the effects by including the interaction terms vitamin D × sex and yogurt × sex in the baseline-adjusted, additive ANCOVA, and conducted stratified analyses when these were significant. The study was not powered to test sex × vitamin D × yogurt triple interactions.

Results

Baseline characteristics

During 8 weeks at the late summer peak in vitamin D status, we were able to recruit 200 children, 184 (92%) of whom completed the trial (**Figure 1**). The 16 noncompleters were mainly boys ($n = 14$) but did not differ from the completing children with respect to anthropometry, age, measures of bone mineralization, or parental education ($P > 0.2$). At baseline, most children had a normal weight (79%), had not entered puberty (96%), and had at least 1 parent with a bachelor's degree or higher (85%; **Table 1**). The randomization was successful, although the BMI *z*-score appeared slightly higher in the vitamin D–NP group compared to the other groups. The mean intervention duration was 24 weeks (range, 21–26 weeks), with no difference between groups ($P = 0.16$). Tablet blinding was highly successful, as 52% of participants correctly guessed their allocation, while investigators correctly guessed 55% of participants' allocations.

Compliance and dietary intake

The median tablet compliance was 95% (IQR, 90%–98%), with no difference between vitamin D and placebo groups ($P = 0.37$). The HP and NP groups consumed 241 g/d (IQR, 200–263 g/d) and 257 g/d (IQR, 234–268 g/d) of yogurt ($P = 0.005$), and the intake of dairy protein from the yogurts was more than twice as high in the HP (22.7 g/d; IQR, 18.9–24.7 g/d) compared to NP (9.2 g/d; IQR, 8.5–10.0 g/d) groups ($P < 0.001$). Tablet compliance was similar between the sexes ($P = 0.35$), but the boys consumed 21 g/d (95% CI: 3.7, 38 g/d) more yogurt than the girls ($P = 0.02$), as expected, due to a higher energy intake ($P < 0.001$).

At baseline, 97% of the children had s-25(OH)D levels ≥50 nmol/L, whereas vitamin D intakes were low in all groups (**Table 2**). Vitamin D intakes increased 10-fold during the intervention in the vitamin D groups, in which all children had s-25(OH)D levels ≥50 nmol/L at the endpoint. In contrast, 13% and 44% of children in the placebo groups had concentrations below 30 and 50 nmol/L, respectively, after the winter (**Supplemental**

TABLE 1 Baseline characteristics of all randomized children ($n = 200$) according to study group[1]

	Placebo tablets		Vitamin D tablets	
	HP yogurt	NP yogurt	HP yogurt	NP yogurt
n	50	51	50	49
Sex, girls, *n* (%)	24 (48%)	27 (53%)	22 (44%)	30 (61%)
Age, years	7.8 [7.0–8.5]	7.6 [7.0–8.2]	7.8 [7.3–8.2]	7.6 [7.1–8.2]
Puberty,[2] yes, *n* (%)	2 (4%)	0 (0%)	2 (4%)	4 (8%)
Parental education, *n* (%)				
≤Vocational or short academic	8 (16%)	10 (20%)	8 (16%)	5 (10%)
Bachelor's degree	18 (36%)	11 (22%)	14 (28%)	12 (24%)
≥Master's degree	24 (48%)	30 (59%)	28 (56%)	32 (65%)
Height, cm	129.3 ± 7.3	129.1 ± 7.5	130.5 ± 6.9	130.4 ± 6.3
Height-for-age *z*-score	0.74 ± 0.94	0.87 ± 0.99	0.92 ± 0.96	1.01 ± 0.84
Weight, kg	26.9 ± 5.6	26.6 ± 5.4	26.6 ± 4.1	28.2 ± 5.2
BMI-for-age *z*-score	0.02 ± 1.10	−0.02 ± 1.12	−0.15 ± 0.75	0.34 ± 1.04
Weight category,[3] *n* (%)				
Underweight	5 (10%)	6 (12%)	1 (2%)	4 (8%)
Normal weight	38 (76%)	37 (73%)	47 (94%)	37 (76%)
Overweight or obesity	7 (14%)	8 (16%)	2 (4%)	8 (16%)

[1] Values are means ± SDs, medians [25th–75th percentiles], or *n* (%). Abbreviations: HP, high-protein; NP, normal-protein.

[2] Puberty was evaluated by a self-administered 5-point scale (Tanner stages) of breast development for girls and pubic hair for boys. The first answer on the scale was defined as "no" and all answers from 2 to 5 were categorized as "yes."

[3] BMI was categorized by age- and sex-specific cutoffs defined to pass through BMIs of 18.5, 25.0, and 30.0 kg/m^2 at age 18 years.

Figure 1). Total protein intake remained at 15.9 ± 2.5 energy percentage (E%) in the NP groups and increased by 20 ± 25% to 18.3 ± 3.4 E% during the intervention in the HP groups, due to an increase in dairy protein intake at the expense of fat intake. The higher protein intake was not reflected in SUN, which did not differ between groups (Table 2). Adjustments for whether the dietary record was performed while the child was ill (yes/no) or during the COVID-19 lockdown (yes/no) did not change the results (data not shown).

Bone mineralization and linear growth

As expected during periods of growth, all groups increased in TBLH and lumbar spine BMC and BA during the intervention (all P values < 0.001). We found no interactions or main effects of vitamin D or HP dairy intake on the primary outcome of TBLH BMD or on linear growth (**Table 3**). However, vitamin D supplementation increased the lumbar spine BMD and BMD *z*-score, as well as the TBLH BMC, and tended to increase the BA compared to placebo (Table 3; **Figure 2**). Volume and size correction did not change these results (data not shown). Further adjustments for age, sex, puberty, and changes in BMI *z*-score and calcium intake did not change the results for spinal outcomes, but the effect on TBLH BMC was slightly attenuated when adjusting for BMI *z*-score ($P = 0.080$). Regarding the yogurt intervention, the HP groups had smaller increments in the lumbar spine BMD and BMD *z*-score, as well as the TBLH BMC and BA, than the NP groups (Table 3; **Figure 3**). Size, volume, and covariate adjustments did not change the results (data not shown).

Per-protocol analyses showed similar results and, in addition, a vitamin D–yogurt interaction on TBLH BA ($P_{interaction} = 0.042$), with the vitamin D–NP group having a higher BA increment than the other 3 groups ($P < 0.05$ for all post hoc comparisons), in line with the main effects of vitamin D and HP. Sex did not modify the effects on bone health or linear growth, and adjustments for changes in intakes of carbohydrate, sugar, fat, or SFAs did not change any of the results.

Biomarkers of bone turnover and growth

We found no interaction between the vitamin D and yogurt interventions on the bone biomarkers or growth factors, and no main effects of vitamin D (**Table 4**). However, in line with the effects of the yogurt intervention on the bone measures, osteocalcin increased less in the HP compared to NP groups. In addition, the change in IGFBP-3 tended to be lower with HP than NP, whereas there was no effect on IGF-I (Table 4). There were no main effects on PTH but there was a sex-specific vitamin D effect ($P_{vitamin\ D \times sex} = 0.031$) due to a reduction of –0.66 pmol/L (95% CI: –1.2 to –0.14 pmol/L) with vitamin D supplementation in boys only (girls, 0.14; 95% CI: −0.38 to 0.66 pmol/L). Results were unaffected by covariate adjustment, and the difference between HP and NP in IGFBP-3 became significant ($P = 0.034$) in the per-protocol analysis (HP compared with NP, −0.18; 95% CI: −0.34 to −0.014 mg/L).

Adverse events

No children had hypercalcemia at baseline or endpoint, and no serious adverse events were reported. During the intervention, 38 cases of symptoms were reported by the parents, with gastrointestinal discomfort (37%) and respiratory infections (26%) accounting for the majority. Eight cases of temporary gastrointestinal discomfort (5 of which were in the HP groups) and 1 case of parent-reported weight gain (placebo-NP group) were recorded as having a possible relation to the intervention. All other symptoms were assessed to be independent of the intervention.

TABLE 2 Dietary intake and nutrient biomarkers according to study group[1]

		Placebo tablets		Vitamin D tablets		Vitamin D	Yogurt
		HP yogurt	NP yogurt	HP yogurt	NP yogurt	Vitamin D vs. placebo	HP vs. NP
n		44	49	44	46	—	—
Nutrient intake:							
Energy,[2] kJ/d	Baseline	6272 ± 1443	6509 ± 1135	7289 ± 1487	6770 ± 1248	223 (−168, 615)	−122 (−504, 260)
	Endpoint	6172 ± 1506	6533 ± 1079	6985 ± 1672	6736 ± 1353	$P = 0.261$	$P = 0.529$
	Change	−101 ± 1561	24 ± 1424	−305 ± 1587	−34 ± 1417		
Protein,[2] E%	Baseline	15.4 ± 2.4	15.0 ± 2.2	15.7 ± 2.3	15.7 ± 2.6	0.56 (−0.3, 1.4)	2.4 (1.5, 3.2)
	Endpoint	17.7 ± 3.3	15.8 ± 2.7	19.0 ± 3.4	16.0 ± 2.2	$P = 0.197$	$P < 0.001$
	Change	2.3 ± 3.5	0.8 ± 2.6	3.3 ± 3.7	0.3 ± 3.0		
Dairy protein,[2] E%	Baseline	3.8 [2.5–5.4]	3.2 [2.5–4.7]	3.1 [2.5–4.7]	4.0 [2.8–5.1]	0.3 (−0.4, 1.0)	2.4 (1.6, 3.1)
	Endpoint	7.1 [3.0–8.7]	4.4 [3.3–5.7]	7.3 [5.6–8.8]	4.1 [3.1–5.2]	$P = 0.431$	$P < 0.001$
	Change	2.3 [1.0–4.3]	0.9 [–0.7–2.1]	3.7 [1.6–6.1]	0.0 [–0.8–1.1]		
Carbohydrate,[2] E%	Baseline	52.5 ± 4.7	54.6 ± 4.8	52.0 ± 4.6	54.5 ± 4.2	−2.4 (−3.8, −0.9)	0.2 (−1.3, 1.7)
	Endpoint	53.5 ± 5.6	52.9 ± 4.9	49.6 ± 5.1	51.7 ± 5.0	$P = 0.002$	$P = 0.836$
	Change	1.0 ± 5.5	−1.8 ± 6.0	−2.4 ± 5.1	−2.8 ± 6.0		
Sugar,[2,3] E%	Baseline	20.4 ± 4.7	20.1 ± 4.8	20.9 ± 4.7	21.8 ± 3.9	−1.5 (−2.8, −0.1)	−0.5 (−1.9, 0.9)
	Endpoint	21.0 ± 5.2	21.5 ± 4.7	19.8 ± 4.6	20.5 ± 4.6	$P = 0.032$	$P = 0.486$
	Change	0.6 ± 6.0	1.4 ± 5.4	−1.1 ± 5.0	−1.4 ± 5.2		
Fat,[2] E%	Baseline	32.1 ± 4.7	30.3 ± 4.5	32.4 ± 4.5	29.7 ± 4.3	1.8 (0.5, 3.1)	−2.5 (−3.8, −1.1)
	Endpoint	28.8 ± 4.9	31.3 ± 4.3	31.4 ± 4.6	32.3 ± 4.8	$P = 0.007$	$P < 0.001$
	Change	−3.3 ± 5.2	1.0 ± 5.4	−1.0 ± 4.6	2.5 ± 5.8		
Saturated fatty acids,[2] E%	Baseline	12.4 ± 3.0	12.0 ± 2.9	12.5 ± 2.5	11.4 ± 2.5	0.06 (−0.6, 0.7)	−1.8 (−2.5, 1.1)
	Endpoint	11.3 ± 2.3	13.4 ± 2.5	11.9 ± 2.5	12.8 ± 2.4	$P = 0.859$	$P < 0.001$
	Change	-1.1 ± 2.8	1.4 ± 3.3	-0.7 ± 2.3	1.4 ± 3.1		
Vitamin D,[4] μg/d	Baseline	2.5 [1.4–3.8]	2.3 [1.4–3.7]	2.3 [1.4–4.8]	2.6 [1.7–3.6]	23.2 (22.6, 23.8)	0.6 (–0.02, 1.2)
	Endpoint	1.9 [1.1–2.8]	1.8 [1.2–2.3]	26.4 [25.0–27.7]	25.4 [24.0–26.4]	$P < 0.001$	$P = 0.056$
	Change	−0.2 [−1.8–0.2]	−0.4 [−1.3–0.2]	23.1 [22.1–24.4]	22.0 [20.5–23.6]		
Calcium,[4] mg/d	Baseline	764 [527–1063]	906 [674–1291]	954 [736–1217]	917 [683–1272]	6 (−108, 122)	89 (−26, 205)
	Endpoint	874 [550–1115]	840 [625–999]	974 [692–1183]	799 [528–920]	$P = 0.912$	$P = 0.127$
	Change	−6 [−201–241]	−92 [−418–119]	−78 [−285–107]	−75 [−311–67]		

(*Continued*)

TABLE 2 (*Continued*)

		Placebo tablets		Vitamin D tablets		Vitamin D	Yogurt
		HP yogurt	NP yogurt	HP yogurt	NP yogurt	Vitamin D vs. placebo	HP vs. NP
Nutrient biomarkers:							
Serum 25(OH)D,[5] nmol/L	Baseline	80.1 ± 19.6	79.6 ± 17.6	80.8 ± 14.4	82.6 ± 17.6	40.3 (35.7, 44.9)	−1.7 (−6.2, 2.9)
	Endpoint	43.4 ± 17.8	51.1 ± 16.4	90.8 ± 16.0	87.4 ± 18.0	$P < 0.001$	$P = 0.473$
	Change	−36.7 ± 16.8	−28.5 ± 17.4	10.0 ± 11.6	4.8 ± 15.7		
Serum calcium,[5,6] mmol/L	Baseline	2.26 ± 0.08	2.28 ± 0.09	2.23 ± 0.18	2.30 ± 0.09	0.0013 (−0.022, 0.025)	−0.0068 (−0.031, 0.017)
	Endpoint	2.31 ± 0.07	2.31 ± 0.07	2.30 ± 0.06	2.33 ± 0.09	$P = 0.913$	$P = 0.581$
	Change	0.051 ± 0.097	0.037 ± 0.094	0.066 ± 0.191	0.024 ± 0.097		
SUN,[5] mmol/L	Baseline	3.83 ± 0.81	3.79 ± 0.68	4.08 ± 0.92	4.09 ± 0.57	0.04 (−0.22, 0.30)	0.07 (−0.19, 0.33)
	Endpoint	3.90 ± 0.84	4.15 ± 0.89	4.39 ± 0.80	4.00 ± 0.73	$P = 0.778$	$P = 0.586$
	Change	0.072 ± 0.832	0.367 ± 0.931	0.307 ± 0.979	−0.086 ± 0.837		

[1]Based on 4-day dietary records,[2] FFQ,[4] and fasting blood samples.[5] Children with available data from both baseline and endpoint for the specific parameter are included. Group values are presented as means ± SDs or medians [25th–75th percentiles]. Main effects of vitamin D and yogurt are presented as estimated between-group differences (95% CI) from the same, additive 2-way ANCOVA models adjusted for baseline; a P value < 0.05 was considered statistically significant. Abbreviations: COVID-19, coronavirus disease 2019; E%, energy percentage; FFQ, food frequency questionnaire; HP, high-protein; NP, normal-protein; SUN, serum urea nitrogen; 25(OH)D, 25-dihydroxyvitamin D.

[2]From 4-day dietary recording, $n = 179$ ($n = 44$, $n = 48$, $n = 42$, and $n = 45$ in the placebo-HP, placebo-NP, vitamin D-HP, and vitamin D-NP groups, respectively).

[3]Includes all sugar, including naturally occurring sugar from dairy, fruit, and so forth.

[4]From the FFQ. For vitamin D, the intervention tablets (25 μg/tablet) are included at endpoint.

[5]From fasting blood samples. Missing data are due to unsuccessful or insufficient blood sampling and the COVID-19 lockdown; $n = 148$–150 ($n = 33$–34, $n = 39$–40, $n = 35$, and $n = 41$ in the placebo-HP, placebo-NP, vitamin D-HP, and vitamin D-NP groups, respectively).

[6]Corrected for serum albumin.

TABLE 3 Bone and linear growth outcomes according to study group[1]

		Placebo tablets		Vitamin D tablets		Vitamin D × Yogurt	Vitamin D	Yogurt
		HP yogurt	NP yogurt	HP yogurt	NP yogurt	P[2]	Vitamin D vs. placebo[3]	HP vs. NP[3]
n		45	49	44	46	—	—	—
TBLH BMD, g/cm^2	Baseline	0.633 ± 0.064	0.635 ± 0.059	0.646 ± 0.058	0.657 ± 0.068	0.154	0.0005 (−0.0039, 0.0049)	0.0014 (−0.0029, 0.0057)
	Endpoint	0.649 ± 0.064	0.652 ± 0.064	0.666 ± 0.063	0.673 ± 0.071		$P = 0.820$	$P = 0.528$
	Change	0.015 ± 0.015	0.017 ± 0.015	0.019 ± 0.014	0.015 ± 0.015			
TBLH BMD z-score	Baseline	0.284 ± 0.953	0.343 ± 0.802	0.393 ± 0.862	0.596 ± 0.956	0.126	0.014 (−0.052, 0.079)	0.026 (−0.040, 0.091)
	Endpoint	0.168 ± 0.906	0.249 ± 0.819	0.339 ± 0.914	0.459 ± 0.951		$P = 0.678$	$P = 0.437$
	Change	−0.116 ± 0.241	−0.094 ± 0.231	−0.053 ± 0.191	−0.136 ± 0.225			
TBLH BMC, g	Baseline	679.1 ± 131.2	688.8 ± 140.6	710.8 ± 127.1	724.8 ± 133.3	0.506	6.5 (0.39–12.5)	−9.3 (−15.4, −3.3)
	Endpoint	716.7 ± 135.6	734.2 ± 144.7	754.3 ± 140.2	780.3 ± 143.1		$P = 0.037$	$P = 0.003$
	Change	37.6 ± 20.2	45.4 ± 20.3	43.4 ± 20.3	55.5 ± 25.0			
TBLH BA, cm^2	Baseline	1064 ± 114	1076 ± 132	1094 ± 116	1095 ± 106	0.126	9.2 (−0.71, 19.1)	−17.0 (−26.9, −7.2)
	Endpoint	1097 ± 113	1118 ± 125	1126 ± 123	1152 ± 115		$P = 0.069$	$P = 0.001$
	Change	32.4 ± 32.2	41.5 ± 36.4	32.7 ± 29.8	57.6 ± 36.4			
L1–L4 BMD, g/cm^2	Baseline	0.681 ± 0.074	0.682 ± 0.084	0.691 ± 0.078	0.679 ± 0.074	0.425	0.0070 (0.0011, 0.013)	−0.0068 (−0.013,
	Endpoint	0.683 ± 0.076	0.692 ± 0.082	0.702 ± 0.086	0.695 ± 0.078		$P = 0.021$	−0.00080)
	Change	0.0014 ± 0.0171	0.0105 ± 0.0188	0.0110 ± 0.0202	0.0152 ± 0.0249			$P = 0.027$
L1–L4 BMD z-score	Baseline	0.056 ± 0.807	0.077 ± 0.955	0.152 ± 0.918	0.022 ± 0.836	0.488	0.084 (0.014, 0.15)	−0.080 (−0.15, −0.0096)
	Endpoint	−0.059 ± 0.809	0.066 ± 0.908	0.145 ± 0.980	0.073 ± 0.852		$P = 0.019$	$P = 0.026$
	Change	−0.114 ± 0.210	−0.011 ± 0.221	−0.007 ± 0.233	0.051 ± 0.297			
L1–L4 BMC, g	Baseline	21.5 ± 4.4	21.8 ± 4.2	22.4 ± 4.6	22.3 ± 4.1	0.283	0.14 (−0.18, 0.45)	−0.011 (−0.32, 0.30)
	Endpoint	22.6 ± 4.4	23.2 ± 4.3	23.8 ± 5.2	23.6 ± 4.5		$P = 0.393$	$P = 0.944$
	Change	1.13 ± 0.91	1.32 ± 1.09	1.47 ± 1.10	1.30 ± 1.20			
L1–L4 BA, cm^2	Baseline	31.3 ± 4.2	31.9 ± 3.7	32.2 ± 3.8	32.7 ± 3.4	0.502	−0.13 (−0.52, 0.26)	0.30 (−0.092, 0.69)
	Endpoint	32.9 ± 4.1	33.3 ± 3.8	33.8 ± 4.3	33.8 ± 3.6		$P = 0.514$	$P = 0.133$
	Change	1.60 ± 1.29	1.41 ± 1.55	1.58 ± 1.39	1.13 ± 1.11			

(*Continued*)

TABLE 3 (*Continued*)

		Placebo tablets		Vitamin D tablets		Vitamin D × Yogurt	Vitamin D	Yogurt
		HP yogurt	NP yogurt	HP yogurt	NP yogurt	P^2	Vitamin D vs. placebo[3]	HP vs. NP[3]
Height, cm	Baseline	128.4 ± 6.8	129.3 ± 7.5	130.6 ± 7.2	130.5 ± 6.5	0.597		
	Endpoint	131.1 ± 7.0	132.0 ± 7.6	133.3 ± 7.4	133.1 ± 6.5		−0.028 (−0.20, 0.14)	0.011 (−0.16, 0.18)
	Change	2.6 ± 0.6	2.7 ± 0.6	2.7 ± 0.5	2.6 ± 0.6		$P = 0.741$	$P = 0.900$
Height-for-age *z*-score	Baseline	0.68 ± 0.90	0.90 ± 1.00	0.92 ± 1.00	1.05 ± 0.85	0.440		
	Endpoint	0.67 ± 0.92	0.90 ± 1.00	0.92 ± 1.02	1.03 ± 0.86		−0.0062 (−0.035, 0.022)	0.0073 (−0.021, 0.036)
	Change	−0.013 ± 0.102	−0.008 ± 0.093	−0.006 ± 0.088	−0.024 ± 0.106		$P = 0.670$	$P = 0.615$

[1] Values are presented as means ± SDs. All complete cases are included. Abbreviations: BA, bone area; BMC, bone mineral content; BMD, bone mineral density; HP, high-protein; L1–L4, lumbar spine vertebrae 1–4; NP, normal-protein; TBLH, total body less head.
[2] *P* values are from the treatment interaction term in 2-way ANCOVA models adjusted for baseline; a *P* value < 0.05 was considered statistically significant.
[3] Main effects of vitamin D and yogurt are presented as estimated between-group differences (95% CIs) obtained from the same, additive 2-way ANCOVA models adjusted for baseline; a *P* value < 0.05 was considered statistically significant.

Discussion

Vitamin D and/or high dairy protein intakes did not affect the whole-body bone density, but daily supplementation of 20 μg of vitamin D_3 increased the lumbar spine bone density and whole-body bone mass during extended winter. In addition, intake of HP yogurt resulted in smaller increments of change in spinal bone density, whole-body bone mass and size, and osteocalcin than intake of regular yogurt, in contrast with our hypothesis.

Our finding that vitamin D supplementation increased spinal bone density was consistent across analyses and is in line with results of the most recent meta-analysis of randomized trials in children (8). In that meta-analysis, Winzenberg et al. (8) showed a standardized mean difference in the spine BMD of 0.15 (95% CI: −0.01 to 0.31) with vitamin D supplementation compared to placebo, whereas the effect in our study was twice as large, perhaps due to the prevalence of vitamin D insufficiency and deficiency in the placebo groups after winter. Although these effects may appear small, a 1-SD reduction in BMD at different body sites has been associated with >1.5-fold increased risks of fractures in children (39) and adults (40). Furthermore, Winzenberg et al. (8) found higher whole-body BMCs after vitamin D supplementation in studies where baseline s-25(OH)D level was below 35 nmol/L. Although we were not able to assess status-specific effects due to the low proportion of children with a low vitamin D status at baseline, we found an overall effect on BMC. Recent trials in children and adolescents showed no effects of providing 5–25 μg/d of vitamin D on the whole-body or spinal BMD or BMC (9–11), which may partially be due to intervention across seasons (10), lower doses of 5 or 10 μg/d (9, 10), or a shorter study duration of 3 months (11).

One of the likely mechanisms by which vitamin D increases bone mineralization is by decreasing PTH synthesis, but a systematic review showed that circulating PTH does not respond to vitamin D supplementation in subjects receiving adequate calcium (41). This may explain why we found no overall effect on PTH, although an effect was seen in boys. Another potential mechanism is vitamin D–stimulated IGF-I synthesis (15). The lack of effect on IGF-I in our study may indicate that the vitamin D effect on bone mineralization was mediated through other mechanisms than IGF-I, and is consistent with no effect on linear growth.

Several of the bone outcomes were lower after consumption of HP than NP yogurt, and this was supported by lower osteocalcin with HP. To our knowledge, no trial in children has investigated effects of dairy protein intake per se on bones. One study in malnourished 7- to 13-year-old New Guinean children (6) showed an increased skeletal maturation score after supplementation with skim milk powder corresponding to 10 or 20 g of protein/d compared to no supplementation. In addition, milk and dairy supplementation in children and adolescents has been shown to increase BMC in a systematic review (3), indicating that dairy as a whole induces effects on bones. Consistent with our findings, protein compared to carbohydrate supplementation resulted in lower increases in whole-body BA in adolescents and young adults participating in a strength and conditioning program (7). Further, observational studies in children showed no (42) or inverse (43) associations between protein intake and BMD and BMC accretion, respectively, and a cross-sectional study in 8-year-old boys showed an inverse

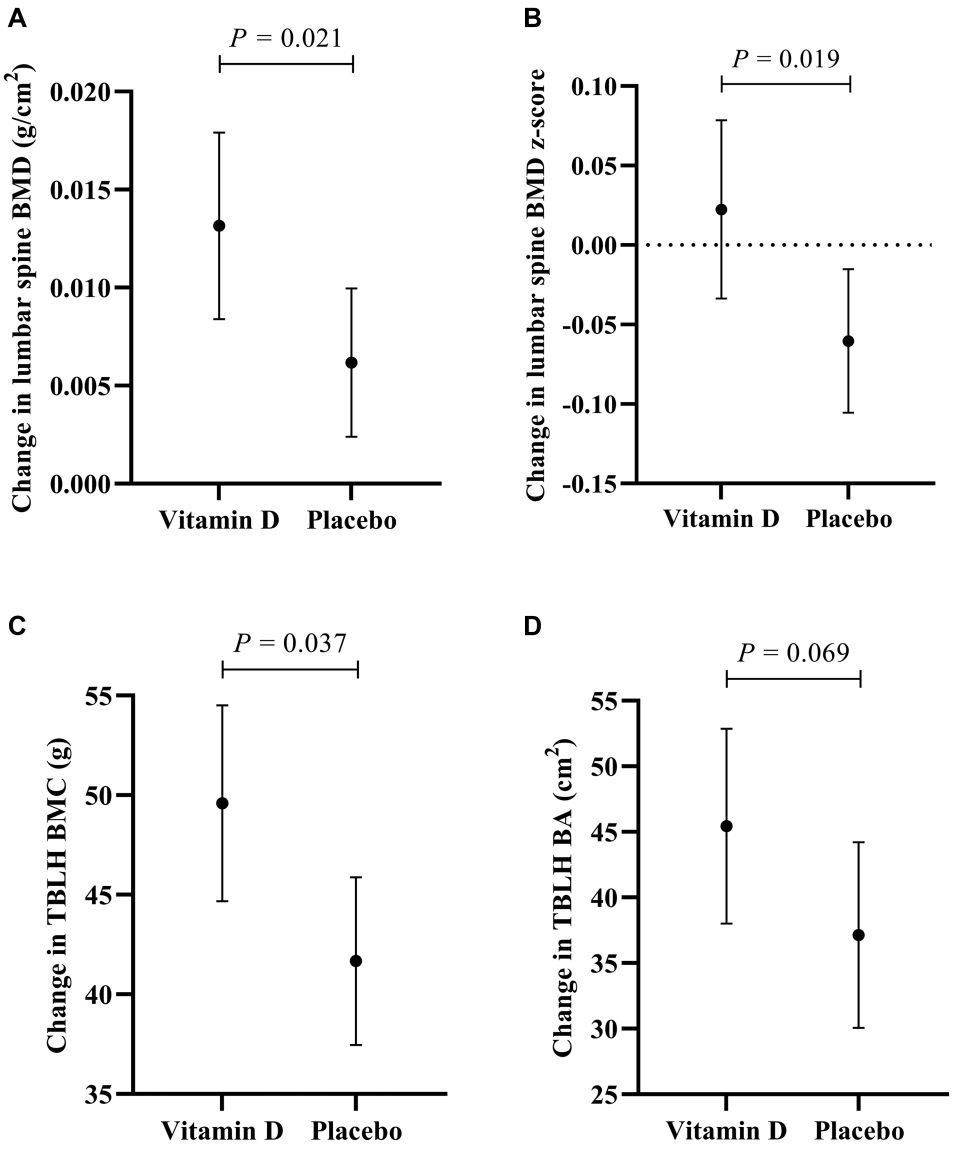

FIGURE 2 Mean (95% CI) changes in (A) lumbar spine BMD, (B) lumbar spine BMD *z*-score, (C) TBLH BMC, and (D) TBLH BA in the vitamin D and placebo groups, as well as *P* values for between-group differences (additive ANCOVA adjusted for baseline and yogurt group). For all, $n = 184$. Abbreviations: BA, bone area; BMD, bone mineral density; BMC, bone mineral content; TBLH, total body less head.

association between dairy protein intake and osteocalcin (44). Other studies showed positive associations between intakes of protein (22–24), dairy protein (24), and milk protein (45) and bone mineralization at different body sites, and a meta-analysis showed a small protective effect of total protein intake on the lumbar spine BMD in adults (46). Bone metabolisms are, however, different in children and adults, as periods of growth are characterized by high bone turnover where formation exceeds resorption, whereas resorption exceeds formation beginning in middle-aged adulthood (1).

The inconsistent evidence may be due to the fact that proteins have been shown to increase both the dietary acid load and calcium excretion, and thereby bone resorption, but also to increase calcium absorption and IGF-I, and thereby bone formation (25). Although the protein intake of 2.5 ± 0.8 g/kg/d in the HP groups seems high compared to the RDA of 0.9 g/kg body weight/d specified by the Food and Agriculture Organization of the United Nations, World Health Organization, and United Nations University (47), this was simply achieved by substitution with common HP dairy products on the market. No upper protein intake level has been established, and the baseline intake in our study population of 15 ± 2 E% fell well within children's actual (48, 49) and recommended protein intakes of 10–20 E% in the Nordic countries (50) and 10–30 E% in the United States (17). Nonetheless, we speculate whether HP contributed to a higher dietary acid load in our study that was not counteracted by IGF-I, and therefore resulted in lower bone mineralization than NP. Further, this might be different in children with a different calcium intake, as calcium has been shown to influence the effect of dietary protein on bones in the elderly (51). Overall, we hypothesize that potential bone mineralizing effects of dietary protein are only seen up to a certain

TABLE 4 Biomarkers for bone and growth according to study group[1]

		Placebo tablets		Vitamin D tablets		Vitamin D × Yogurt	Vitamin D	Yogurt
		HP yogurt	NP yogurt	HP yogurt	NP yogurt	P[3]	Vitamin D vs. placebo[4]	HP vs. NP[4]
n[2]		33–34	39–41	36	38–41	—	—	—
Osteocalcin, μg/L	Baseline	38.3 ± 9.1	37.1 ± 10.8	38.1 ± 11.9	37.1 ± 9.5	0.130	−0.26 (−2.9, 2.3)	−3.18 (−5.79, −0.57)
	Endpoint	37.8 ± 12.9	38.2 ± 10.0	35.3 ± 8.5	39.8 ± 9.8		$P = 0.846$	$P = 0.017$
	Change	− 0.5 ± 11.9	1.1 ± 7.3	− 2.8 ± 8.8	2.7 ± 7.6			
PTH, pmol/L	Baseline	2.95 ± 0.98	2.88 ± 0.97	2.80 ± 0.95	2.70 ± 1.13	0.075	−0.22 (−0.59, 0.14)	−0.027 (−0.39, 0.34)
	Endpoint	3.57 ± 1.50	3.22 ± 1.09	2.91 ± 1.06	3.18 ± 1.39		$P = 0.224$	$P = 0.884$
	Change	0.62 ± 1.25	0.34 ± 1.20	0.10 ± 0.87	0.49 ± 1.23			
IGF-I, μg/L	Baseline	115 ± 40	122 ± 33	126 ± 40	136 ± 37	0.481	−0.54 (−7.9, 6.8)	−4.6 (−12.0, 2.7)
	Endpoint	123 ± 41	131 ± 40	130 ± 38	145 ± 42		$P = 0.885$	$P = 0.217$
	Change	8 ± 25	9 ± 21	4 ± 25	10 ± 20			
IGFBP-3, mg/L	Baseline	3.83 ± 0.84	4.18 ± 0.79	3.83 ± 0.67	4.01 ± 0.70	0.547	0.035 (−0.13, 0.20)	−0.15 (−0.31, 0.018)
	Endpoint	3.90 ± 0.93	4.35 ± 0.72	3.98 ± 0.68	4.22 ± 0.69		$P = 0.670$	$P = 0.081$
	Change	0.06 ± 0.68	0.17 ± 0.47	0.15 ± 0.56	0.20 ± 0.40			
IGF-I:IGFBP-3	Baseline	0.12 ± 0.03	0.12 ± 0.02	0.13 ± 0.03	0.14 ± 0.03	0.093	−0.0012 (−0.0066, 0.0043)	0.00040 (−0.0048, 0.0056)
	Endpoint	0.13 ± 0.03	0.12 ± 0.02	0.13 ± 0.03	0.14 ± 0.03		$P = 0.668$	$P = 0.881$
	Change	0.007 ± 0.015	0.003 ± 0.014	− 0.002 ± 0.021	0.001 ± 0.018			

[1]Values are presented as means ± SDs. Children with available data from both baseline and endpoint for the specific outcome are included. Abbreviations: COVID-19, coronavirus disease 2019; HP, high protein; IGF-I, insulin-like growth factor I; IGFBP-3, insulin-like growth factor binding protein-3; NP, normal protein; PTH, parathyroid hormone.

[2]Missing data due to unsuccessful or insufficient blood sampling and the COVID-19 lockdown.

[3]P values are from the treatment interaction term in 2-way ANCOVA models adjusted for baseline; a P value < 0.05 was considered statistically significant.

[4]Main effects of vitamin D and yogurt are presented as estimated between-group differences (95% CI) obtained from the same, additive 2-way ANCOVA models adjusted for baseline; a P value < 0.05 was considered statistically significant.

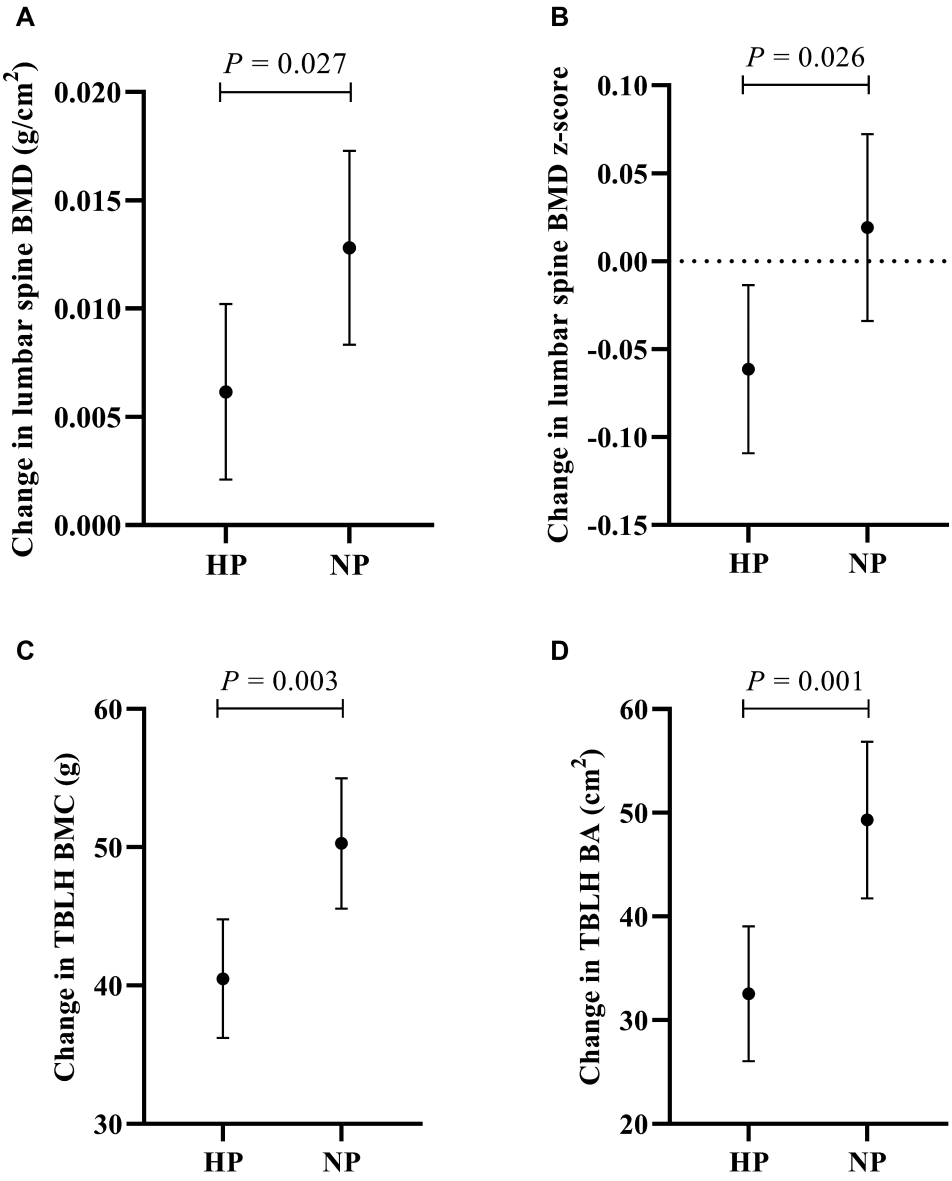

FIGURE 3 Mean (95% CI) changes in (A) lumbar spine BMD, (B) lumbar spine BMD *z*-score, (C) TBLH BMC, and (D) TBLH BA in the HP and NP groups, as well as *P* values for between-group differences (additive ANCOVA adjusted for baseline and tablet group). For all, $n = 184$. Abbreviations: BA, bone area; BMD, bone mineral density; BMC, bone mineral content; HP, high-protein yogurt, NP, normal-protein yogurt; TBLH, total body less head.

level of protein intake, or that they may require higher calcium intakes.

The strengths of our study include the randomized and factorial design, which allowed us to investigate combined and separate effects of vitamin D and high dairy protein intake with additional statistical power when collapsing the groups. Also, the extended winter design allowed us to examine the effects of vitamin D supplementation without substantial sun-induced vitamin D synthesis. Bone mineralization was evaluated using DXA scans of the TBLH and lumbar spine, which are the recommended method and sites for assessments of BMC and areal BMD in children (52). Moreover, the results were overall consistent across the different outcomes and analyses, both before and after adjustments. We only adjusted for multiple comparisons in pairwise post hoc analyses because the outcomes were strongly correlated. Although blinding of the yogurts was not possible, the outcomes were objectively measured and the investigators were blinded prior to the data analysis. To allow for decay, the tablets contained an extra 5 μg of vitamin D. That the content was shown to be stable over time, however, ensured that 98% of vitamin D–supplemented children had total vitamin D intakes of at least 20 μg/d. Due to the short recruitment period and the highly demanding dietary regimen, slightly fewer children than planned participated. The study may thus have had insufficient power for the interaction analyses, but was well powered for investigating the main effects. Apart from differences in protein content, the HP and NP yogurts differed in sugar and saturated fat contents, neither of which are considered important for bone mineralization (1). Further, the yogurts were matched in energy and calcium contents. Thus, the observed effects on bone mineralization were

most likely caused by the difference in protein intake rather than other nutrients. Although the groups differed in carbohydrate and fat intakes, this did not confound the results. There were no differences in SUN between the groups despite the good compliance estimated from recording sheets and dietary records, perhaps because nitrogen excretion is mainly a biomarker of short-term protein intake (53).

In conclusion, vitamin D supplementation and high dairy protein intake did not affect whole-body bone density or linear growth in healthy, Danish, 6- to 8-year-old children. However, vitamin D supplementation of 20 μg/d, which maintained vitamin D sufficiency during winter, increased the bone density of the lumbar spine and the whole-body bone mass. Intake of high compared to normal dairy protein through yogurt did not benefit bone mineralization, and the HP groups even showed smaller increments of change in bone density of the lumbar spine, as well as the whole-body bone mass and size, compared to the NP groups. The results provide evidence that supports a recommended vitamin D intake of about 20 μg/d and do not support recommendations of HP dairy products for optimizing bone mineralization in otherwise well-nourished, healthy children. However, more studies are needed to evaluate possible long-term effects of repeated winter vitamin D supplementation on bones.

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The authors' responsibilities were as follows—CTD, CM, AL, and MH: designed the research; NGS, LT, and JWC: conducted the research; KDC: analyzed and interpreted serum 25-hydroxyvitamin D; NGS: analyzed data; NGS and CTD: wrote the paper and had primary responsibility for the final content; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

Data described in the manuscript will not be made available because data are not anonymized and therefore considered as "personal data."

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