High-Dose Vitamin D Intervention in Infants—Effects on Vitamin D Status, Calcium Homeostasis, and Bone Strength

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Context: Guidelines in Finland recommend 10 μg of vitamin D3 daily for all infants. Recent observations suggest that this may be insufficient to maintain optimal serum 25-hydroxyvitamin D (S-25-OHD).

Objective: The aim of the study was to evaluate effects of various vitamin D doses and determine a dose ensuring S-25-OHD of at least 80 nmol/liter in infants without signs of vitamin D excess.

Design: We conducted a randomized double-blind intervention study. Cord blood was obtained at birth for S-25-OHD; 113 infants were randomized to receive vitamin D3 10, 30, or 40 μg/d from age 2 wk to 3 months.

Setting: An investigator-initiated study was performed in a single maternity hospital in Helsinki, Finland.

Main Outcome Measures: S-25-OHD, calcium homeostasis, and skeletal characteristics were evaluated with peripheral quantitative computed tomography at age 3 months.

Results: Baseline S-25-OHD was similar in all three groups (median, 53 nmol/liter). At 3 months, the mean S-25-OHD values were 88, 124, and 153 nmol/liter, and the minimum values were 46, 57, and 86 nmol/liter in the groups receiving 10, 30, and 40 μg (ANOVA; $P < 0.001$). No hypercalcemia occurred; plasma calcium, serum PTH, and urine calcium excretion was similar between the groups. Peripheral quantitative computed tomography showed a trend toward larger tibial total bone and cortical bone area with higher vitamin D doses.

Conclusion: Vitamin D3 supplementation with up to 40 μg/d from age 2 wk to 3 months was safe and caused no hypercalcemia or hypercalciuria. The 40-μg dose maintained S-25-OHD above 80 nmol/liter in all infants. More extensive and longer intervention studies are necessary to assess long-term effects. **(*J Clin Endocrinol Metab* 97: 4139–4147, 2012)**

Vitamin D supplementation is widely used in Western countries, and several recommendations and guidelines have recently appeared, but the optimal serum 25-hydroxyvitamin D (S-25-OHD) concentration is still under debate. The American Academy of Pediatrics (AAP), the Institute of Medicine (IOM), and the 14th Vitamin D Workshop consensus on vitamin D nutritional guidelines defined the target S-25-OHD as above 50 nmol/liter (20 ng/ml) (1, 2), but many experts regard 80 nmol/liter (32 ng/ml) as the desirable lower level (3, 4). The IOM recently

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Abbreviations: ANCOVA, Analysis of covariance; BMD, bone mineral density; CTX-I, C-terminal cross-linked telopeptides of type I collagen; P-Ca, plasma calcium; PINP, procollagen I N-terminal propeptide; P-Pi, plasma phosphate; pQCT, peripheral quantitative computed tomography; S-25-OHD, serum 25-hydroxyvitamin D; S-PTH, serum PTH; U-Ca/Cr, urinary calcium/creatinine ratio.

also revised the dietary reference intakes for calcium and vitamin D and defined adequate vitamin D intake as 10 μg (400 IU) daily for infants up to age 12 months and the tolerable upper intake as 25 μg (1200 IU) daily for children younger than 6 months and 38 μg (1520 IU) for children aged 6 to 12 months (2).

In Finland, vitamin D supplementation has been recommended since the 1940s to all infants; the recommended daily dose until the 1960s was 100 or 50 μg (5). The present Finnish Nutritional Council guidelines recommend 10 μg daily for all infants between 2 wk and 2 yr of age (6). Despite these recommendations, vitamin D deficiency is common, suggesting that the recommended dose is inadequate. In our recent study, 20% of children at age 14 months had S-25-OHD below 50 nmol/liter despite a total daily vitamin D intake of 12 μg (7). Breastfeeding, common in Finland, is associated with vitamin D deficiency in the absence of supplementation (8). Maternal supplementation to improve the vitamin D content of breast milk, and thereby indirectly vitamin D status in the infant, requires doses exceeding 4000 IU (9, 10). Direct vitamin D supplementation of the child is a feasible and widely accepted way to provide vitamin D to infants independent of age and nutrition. The recommended dose of 10 μg has a historical background; one teaspoonful of cod liver oil contains approximately 10 μg of vitamin D, and this dose is sufficient to prevent rickets (11). However, this recommendation lacks a scientific basis, and intervention studies are essential to determine the optimal dose.

Vitamin D status during fetal development, childhood, and adolescence affects bone mineral density (BMD) and bone mass accrual (12–14). In addition to profound effects on bone, vitamin D also has several nonskeletal actions (15): it modifies immune responses (16–19) and has antimicrobial properties (20) and may thus influence the development of autoimmune diseases such as type 1 diabetes (21). A S-25-OHD plateau is achieved within 2 months of the start of vitamin D treatment in adults when dosing is less than 100 μg/d (22). Vitamin D3 is more potent than vitamin D2 in increasing S-25-OHD concentration (23), and a nonlinear relationship and interindividual variation between S-25-OHD and total intake of vitamin D can occur (2, 24). Hypercalcemia due to vitamin D intoxication has been estimated to occur only when S-25-OHD clearly exceeds 250 nmol/liter (22, 25, 26). In a 6-wk treatment trial of 40 vitamin D-deficient toddlers, a daily dose of 50 μg was safe, and the median S-25-OHD after treatment was 90 nmol/liter (27). In adolescents, weekly supplementation at a dose equivalent to 50 μg/d was safe during the 1-yr follow-up (28).

Among healthy newborns, no randomized studies seem to have defined the optimal supplemental vitamin D dose. Our aim was to evaluate the effect of a higher than currently recommended dose of vitamin D supplementation to determine a daily dose ensuring S-25-OHD concentration at or above 80 nmol/liter in infants, without ensuing signs of vitamin D excess.

Subjects and Methods

Study cohort

This was a double-blinded, randomized controlled study of 113 newborns born at Helsinki Maternity Hospital in Finland between September 2010 and February 2011. Families were recruited to the study during their routine prelabor hospital visit at gestational wk 33 to 36. The infants were eligible for this study if born at term (gestational wk from 37 + 0 to 42 + 0) and with a birth weight appropriate for gestational age. Parents gave their written informed consent, in agreement with the Declaration of Helsinki, before entering the study. Ethical approval was obtained from the Research Ethics Committee of the Hospital District of Helsinki and Uusimaa, and the study protocol was approved by the Finnish Medicines Agency (EudraCT 2009-015940-40) and Children's Hospital, Helsinki University Central Hospital. Each infant was randomized to receive 10, 30, or 40 μg vitamin D3 supplementation daily for 10 wk.

Methods

A cord blood sample was taken after birth, and S-25-OHD and serum PTH (S-PTH) concentrations were analyzed. Infants were randomized into three groups stratified by gender and received vitamin D3 10 μg (400 IU), 30 μg (1200 IU), or 40 μg (1600 IU) daily from age 2 wk to 3 months in a double-blinded fashion. Randomization was carried out by the Helsinki University Central Hospital Pharmacy. Before hospital discharge, families received written instructions on use of the study preparation and a calendar to record treatment compliance prospectively. A research questionnaire was mailed to the families 2 months after the child's birth. No other vitamin preparation was allowed simultaneously with the study product. No restrictions applied regarding infant feeding or the maternal diet. The study preparation was vitamin D3 dissolved in medium-chain triglyceride oil (Vitamin D3 Forte, 500 IU per drop; Renapharma, Uppsala, Sweden). The Helsinki University Central Hospital Pharmacy prepared the appropriate concentrations (10, 30, and 40 μg/ml) and carried out randomization after stratification by gender. The study was double-blinded; personnel responsible for the subjects' assessments remained blinded to the child's intervention group throughout the study. The Finnish Food Safety Authority Evira verified stability of the concentrations during the study period. Variation in vitamin D3 content in the study preparations was less than 10%.

At 3 months of age, the subjects made a follow-up visit. Weight, length, head circumference, length of the left tibia, and circumference of the left calf were measured. Tibia length was measured from the distal end of the medial malleolus to the proximal end of the medial tibial tuberosity. Circumference of the leg was measured at 65% of that distance. The research questionnaire was reviewed with the parents, and compliance with vitamin D, as prospectively recorded by the parents, was examined by review of the diaries and a count of the returned

bottles. Compliance was defined as "good" when the child received over 80% of the intended supplementation with the study preparation. The baby's nutrition, allergic symptoms, infections, and gastrointestinal symptoms, as well as possible symptoms of hypercalcemia (nausea, vomiting, poor feeding, or prolonged constipation) were evaluated in the research questionnaire, in addition to parental education (graded from 1 to 5, from comprehensive school to university education), maternal nutrition, and vitamin supplementation. Bone mineral content, BMD, and bone geometry of the left tibia were measured by peripheral quantitative computed tomography (pQCT) (XCT-2000; Stratec, Birkenfeld, Germany). A capillary blood sample from the heel underwent analysis for S-25-OHD and parameters of calcium homeostasis and blood count. Urine calcium, phosphate, and calcium/creatinine ratios were determined from a spot urine sample.

Power calculation was based on the S-25-OHD concentration, which was our main outcome measure. Group size of 35 was calculated to give 90% power with an α error of 0.05 when baseline S-25-OHD was expected to be 50 nmol/liter (15) and dose-response 1.4 nmol/liter·μg (7). Based on our previous study (29), a similar group size was estimated to provide 90% power with an α error of 0.05 for pQCT measurements.

Laboratory measurements

PTH, 25-OHD, intact procollagen I N-terminal propeptide (PINP), and C-terminal cross-linked telopeptides of type I collagen (CTX-I, CrossLaps) were measured in serum samples with an automated IDS-iSYS analyzer (IDS Ltd., Boldon, UK). The measurements were performed in the biomarker unit of Pharmatest Services Ltd. (Turku, Finland) (www.pharmatest.com); the intraassay coefficient of variation was less than 8%, and the interassay coefficient of variation was less than 10% for S-25-OHD. A S-PTH concentration below the detection limit (5 pg/ml) was recorded as 0 pg/ml and was included in the analysis. All specimens from the patients were analyzed in the same assay to avoid the effects of interassay variation.

Calcium (Ca), phosphate (Pi), and blood count were analyzed in the Central Laboratory of Helsinki University Central Hospital (HUSLAB). Plasma calcium (P-Ca) and phosphate (P-Pi) concentrations were analyzed by an accredited photometric assay (reference ranges, 2.22–2.82 and 1.5–2.5 mmol/liter, respectively). Hypercalciuria was defined by a urinary calcium/creatinine ratio (U-Ca/Cr) over 2.2 mmol/mmol (30). The reference ranges were: hemoglobin, 99 to 136 g/liter; white blood cells, 6 to 17.5 $\times$ 10^9/liter; and thrombocytes, 200 to 450 $\times$ 10^9/liter. Urine analyses were also performed in HUSLAB with an accredited photometric assay (mmol/liter).

Peripheral quantitative computed tomography

pQCT measures true volumetric BMD, bone strength, and bone-muscle ratio (31). Measurements were performed at 65 and 10% of the length of the tibia; values from the 65% site were included in final analysis. The pQCT measurements were recorded as "good" when no movement artifacts were present (n = 31) and "average" when movement artifacts were minor (n = 51). Altogether, 25 measurements inadequate in quality were omitted from analysis.

Statistical analysis

We conducted an intention-to-treat analysis, regardless of compliance; S-25-OHD and dose-responses were calculated also separately for those with greater than 80% compliance. All subjects with data at birth and at 3 months of age were included in repeated-measures analyses. The variables were checked for normality, and a natural logarithm transformation was used for skewed variables. ANOVA and Student's *t* test (continuous variables) or the χ^2 test (categorical variables) assessed differences between intervention groups or genders. Bivariate correlation was conducted with Pearson's correlation coefficient when normality was observed in at least one variable, but otherwise with Spearman's correlation coefficient. Repeated-measures analysis of covariance (ANCOVA), adjusted by gender, assessed the impact of intervention on S-25-OHD and S-PTH concentrations at age 3 months, in addition to within-subject change in concentrations over time. For 25-OHD, the analysis was conducted after logarithmic transformation. Statistical analyses were carried out by use of IBM SPSS Statistics 19 (IBM, Armonk, NY). Data are presented as mean and SD in *parentheses* unless otherwise indicated.

Results

Patients

This study included 113 children; in 93 subjects (82%), compliance with the study vitamin D3 preparation was

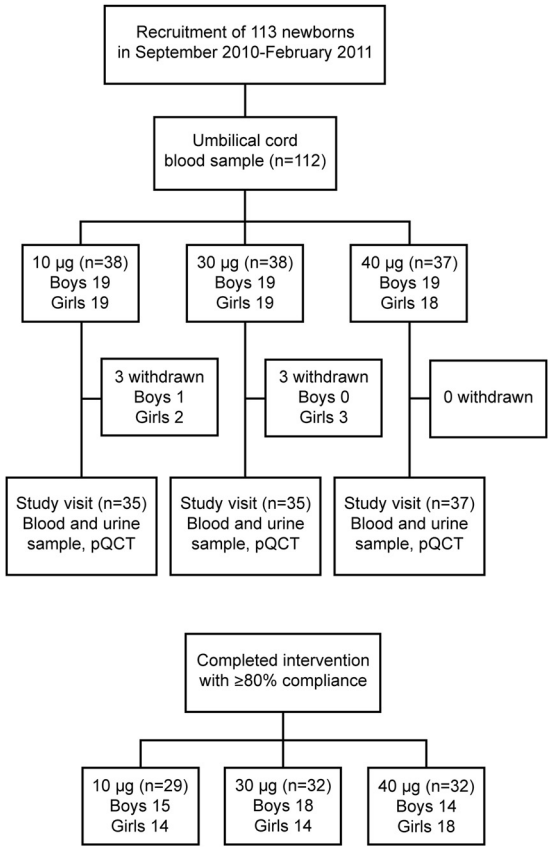

FIG. 1. Flowchart of recruitment and follow-up with numbers of withdrawn subjects and subjects who completed the study with good compliance.

above 80%, as evaluated at 3 months. No differences in compliance were evident between genders or intervention groups. The most commonly reported reason for poor compliance was abdominal symptoms; the noncompliant families discontinued use of the study preparation in favor of other commercially available vitamin D products. At the follow-up visit, all subjects had some ongoing supplementation with vitamin D. The intervention period was on average 10.7 wk. The follow-up visit at 3 months occurred for 107 (95%) subjects (Fig. 1). No adverse effects suggestive of hypercalcemia emerged in any subjects. For baseline characteristics and anthropometric data at 3 months, see Tables 1 and 2.

Serum 25-OHD concentration

Between the three groups, baseline S-25-OHD concentration did not differ. In contrast, at 3 months, S-25-OHD showed significant variation across intervention groups (Fig. 2). Maternal intake of supplemental vitamin D3 correlated weakly with baseline S-25-OHD concentration (Pearson r = 0.215; $P = 0.024$), whereas neither gestational weeks nor birth weight correlated with baseline S-25-OHD. Mean (±SD) maternal daily vitamin D3 supplementation during late pregnancy (self-reported) was 11.1 (±6.9) μg. In repeated-measures ANCOVA using gender as a covariate, the S-25-OHD concentration increased with time ($P < 0.001$), and the response between intervention groups differed ($P < 0.001$). In compliance-based analysis, including only subjects with greater than 80% compliance, the mean (±SD) increment in S-25-OHD was 39 (±21), 74 (±29), and 105 (±39) nmol/liter (ANOVA, $P < 0.001$), with corresponding dose-responses of 3.9, 2.5, and 2.6 nmol/liter·μg vitamin D3 (ANOVA, $P < 0.001$) for the groups receiving 10, 30, or 40 μg of vitamin D daily. Baseline S-25-OHD, birth length, and weight gain in 3 months had an inverse correlation with dose-response effect (Spearman's r = −0.298, $P = 0.002$; r = −0.257, $P = 0.008$;and r = −0.417, $P < 0.001$). Treatment compliance correlated positively with the dose-response effect (Spearman's r = 0.339; $P < 0.001$). The minimum S-25-OHD concentrations in the intervention groups were 49, 57, and 86 nmol/liter; the corresponding values in those with good (≥80%) compliance were 70, 86, and 91 nmol/liter. The maximum S-25-OHD concentrations in the intervention groups were 125, 198, and 230 nmol/liter, all measured in subjects with good compliance. All children, regardless of vitamin D dosing, achieved a final S-25-OHD concentration above 50 nmol/liter (IOM recommendation) when compliance was greater than 80%. The Endocrine Society's recommendation (S-25-OHD ≥ 75 nmol/liter) was achieved by 100% with 30- and 40-μg dosing, whereas 93% of those with compliance greater than 80% reached this with 10-μg dosing.

Calcium homeostasis and bone turnover

Urinary and plasma Ca and Pi concentrations and urinary creatinine concentration were measured at 3 months to monitor safety (Fig. 3). No hypercalcemia appeared, even in subjects with S-25-OHD values exceeding 150 nmol/liter (Table 3). P-Ca showed no difference between intervention groups (ANOVA; $P = 0.373$) and no correlation with S-25-OHD (Pearson; $P = 0.114$). P-Pi concentrations were normal in all subjects; no difference emerged between intervention groups or any correlation with S-25-

TABLE 1. Baseline characteristics of the mothers and infants in the three intervention groups

	Intervention group			
	10 μg	30 μg	40 μg	ANOVA *P*
Mother (n)	38	38	37	
Age at delivery (yr)	31.3 (3.9)	31.7 (3.5)	30.5 (3.5)	0.368
BMI before gestation (kg/m^2)	23 (3)	22 (3)	23 (3)	0.275
Duration of gestation (d)	283 (7)	281 (6)	284 (9)	0.182
Fractures (n)	0.8 (1.3)	0.4 (0.7)	0.3 (0.6)	0.158[a]
Education (1–5)	4.1 (1.0)	4.4 (0.9)	4.4 (0.8)	0.403[b]
Smokers (n)	4	2	3	0.551[b]
Vitamin D3 supplementation (μg/d)	9.8 (5.4)	11.3 (7.3)	12.2 (7.9)	0.311
Child				
Girls (n)	19	19	18	
Boys (n)	19	19	19	
Weight at birth (kg)	3.5 (0.4)	3.4 (0.3)	3.6 (0.4)	0.156
Length at birth (cm)	50.3 (1.6)	50.1 (1.8)	50.8 (1.7)	0.172
Head circumference at birth (cm)	35.3 (1.4)	35.5 (1.3)	35.7 (1.3)	0.524
Apgar (1 min)	9 (1)	9 (2)	9 (1)	0.928
Umbilical artery blood pH	7.24 (0.08)	7.25 (0.07)	7.24 (0.10)	0.803

Values are given as mean (SD). BMI, Body mass index.

[a] Kruskal-Wallis test.

[b] χ^2 test.

TABLE 2. Patient characteristics in the three intervention groups at age 3 months

	Intervention group			
	10 μg	30 μg	40 μg	ANOVA *P*
n	35	35	37	
Boys/girls (n)	18/17	19/16	19/18	0.961[a]
Age at follow-up (d)	90 (5.5)	87 (12.7)	88 (4.1)	0.284
Weight (kg)	6.1 (1.0)	6.1 (0.6)	6.1 (0.6)	0.992
Δ Weight (kg)	2.7 (0.9)	2.7 (0.6)	2.5 (0.6)	0.563
Length (cm)	60.9 (2.2)	61.0 (2.1)	61.4 (1.7)	0.525
Δ Length (cm)	10.7 (1.5)	11.0 (1.6)	10.6 (1.5)	0.515
Head circumference (cm)	41.1 (1.3)	40.9 (1.0)	41.1 (1.3)	0.647
Δ Head circumference (cm)	5.8 (1.5)	5.3 (1.1)	5.4 (1.0)	0.191
Leg length (cm)	10.4 (0.5)	10.4 (0.5)	10.4 (0.4)	0.754
Leg circumference (cm)	16.2 (1.6)	16.3 (0.9)	16.2 (1.3)	0.852
Compliance (%)	85 (24)	89 (17)	89 (19)	0.614
Breast-feeding (n)	34	32	36	0.412[a]
Formula (liters/wk)	2.0 (2.1)	2.8 (2.7)	2.7 (2.0)	0.748
Cord blood S-25-OHD (nmol/liter)[b]	52 (14)	54 (15)	54 (13)	0.772
Final S-25-OHD (nmol/liter), ITT[c]	88 (18)	124 (30)	153 (40)	<0.001
Final S-25-OHD (nmol/liter), CB[c]	93 (16)	128 (27)	157 (38)	<0.001
Cord blood PTH (pg/ml)	4 (18) (n = 37)	5 (24) (n = 38)	4 (9) (n = 37)	0.910
S-PTH at 3 months (pg/ml)	13 (7) (n = 28)	15 (14) (n = 29)	20 (21) (n = 29)	0.856

Values are given as means (SD). Δ, Difference between baseline and 3-month values; ITT, intention-to-treat; CB, compliance-based, including only subjects with greater than 80% compliance.

[a] χ^2 test.

[b] n = 37, 38, and 37 in the three groups.

[c] n = 34, 35, and 37 in the three groups.

OHD. Hypercalciuria occurred in 39% of all subjects, but U-Ca/Cr level was the same between intervention groups (ANOVA, $P = 0.623$) with no correlation with S-25-OHD concentration at 3 months (Pearson r = 0.137; $P = 0.160$). P-Ca and U-Ca/Cr correlated positively (Pearson r = 0.276; $P = 0.004$).

At baseline, over 80% of S-PTH concentrations (n = 112) were below the detection limit. S-PTH concentration increased over time (ANCOVA, $P < 0.001$), but at 3 months S-PTH did not differ between intervention groups (Kruskal-Wallis; $P = 0.940$). No correlation between S-PTH and S-25-OHD was evident. S-PTH and U-Ca/Cr were inversely correlated (Spearman's r = −0.319; $P = 0.003$). In repeated-measures ANCOVA using gender as a covariate, no difference existed between intervention groups (n = 85; $P = 0.387$).

No differences in bone turnover markers occurred at 3 months between the intervention groups (Fig. 3).

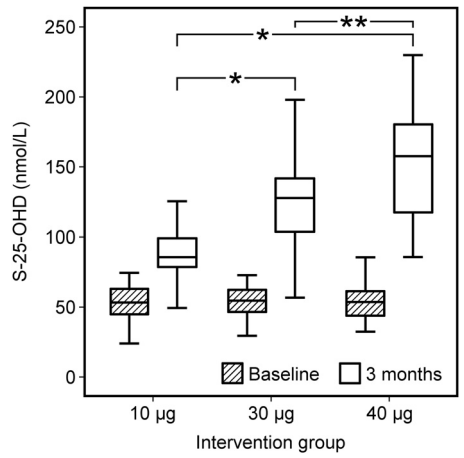

FIG. 2. S-25-OHD concentration at birth and at 3 months. Baseline S-25-OHD concentration was evaluated from a umbilical cord blood sample (n = 112) and was similar in all intervention groups (ANOVA; $P = 0.772$). The mean S-25-OHD concentration at 3 months (n = 106) differed significantly between intervention groups (ANOVA after logarithmic transformation; $P < 0.001$; *post hoc* Tukey values, * at $P < 0.001$, and ** at $P = 0.002$).

Peripheral quantitative computed tomography

No significant correlations between pQCT parameters and S-25-OHD, S-PTH, P-Ca, or U-Ca/Cr appeared in partial correlation analysis, controlling correlations for gender and quality of pQCT measurement (data not shown). In multivariate ANCOVA, a trend toward better stress and strain index and larger total bone and cortical bone area was noted with higher vitamin D doses, when gender and quality of pQCT measurement were used as covariates ($P = 0.070$, $P = 0.069$, and $P = 0.053$, respectively) (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Discussion

This study evaluated short-term effects and safety of higher than currently recommended vitamin D supple-

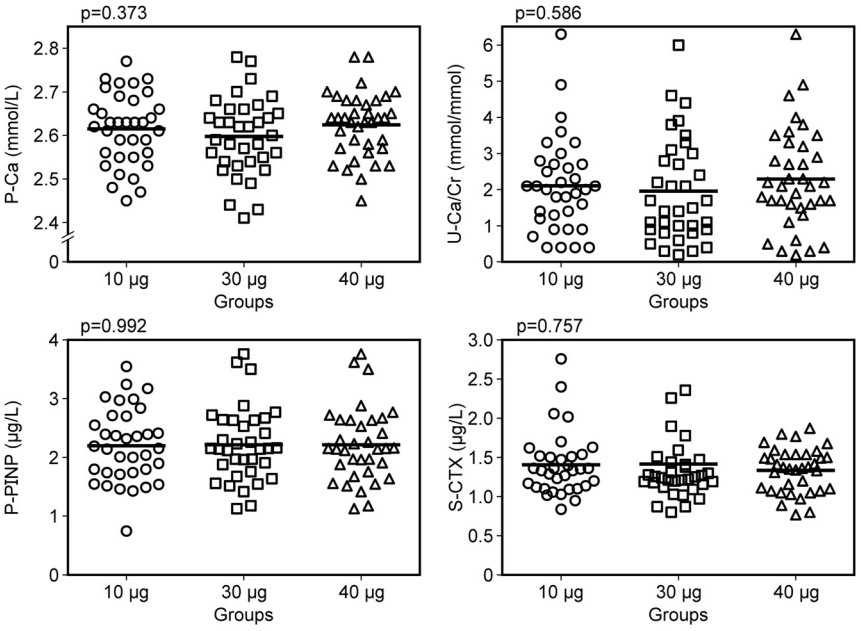

FIG. 3. P-Ca, U-Ca/Cr, and bone formation (P-PINP) and resorption (S-CTX) markers at 3 months in the three vitamin D intervention groups receiving 10, 30, and 40 μg of vitamin D3 daily. No statistically significant differences between the groups appeared in any of these parameters.

mentation in infants. In this 3-month intervention study, we show that daily supplementation with 40 μg vitamin D3 from age 2 wk to 3 months is associated with neither hypercalcemia nor increased rate of hypercalciuria and results in S-25-OHD concentrations within the normal range and consistently above 80 nmol/liter. No adverse effects appeared during the 10-wk treatment.

The maximum S-25-OHD values achieved with 30 and 40 μg were 198 and 230 nmol/liter, but no adverse effects were observed. Furthermore, all parameters of calcium homeostasis remained within normal limits with no differences among the three groups. It is likely that these concentrations represent peak values and that during prolonged supplementation S-25-OHD would plateau and gradually decrease with the child's growth after 10-wk treatment (22); relative vitamin D dose will decrease with normal growth (32). In our cohort, parental educational status, breast-feeding frequency, maternal vitamin D supplementation, and treatment compliance were high, and these partly explain the high maximum S-25-OHD concentrations at 3 months. These families were possibly more health-oriented than the Finnish population in general, so the dose-responses in the general population, with poorer compliance, are likely to remain lower.

Regarding safety measurements, we measured plasma calcium and urine calcium/creatinine ratios at 3 months. None of the children developed hypercalcemia, and calcium values did not differ between intervention groups and did not correlate with S-25-OHD. Vanstone *et al.* (33) recently reported hypervitaminosis D and hypercalcemia in an infant who received 1400 IU (35 μg) of vitamin D3 daily for 2 months. However, the peak S-25-OHD was 84 ng/ml (210 nmol/liter), and the peak serum calcium was 11.0 mg/dl (2.75 mmol/liter), well within the age-specific reference range for our hospital; it therefore remains uncertain whether any evidence of vitamin D toxicity existed. Hypercalciuria occurred in 39% of our study subjects, suggesting merely that the reference values are inappropriate for this age group. Urine calcium excretion correlated with P-Ca but not with S-25-OHD or vitamin D dosing. Unfortunately, the volume of blood that could be drawn from the subjects was limited, and 1,25-dihydroxyvitamin D was not measured. Determining hypercalciuria by measuring the U-Ca/Cr ratio in a random spot sample has its limitations (34). Urinary calcium excretion is best measured in a 24-h collection, which in this age group is extremely challenging. Individual diet, diurnal variation, physical activity, genetic factors, and muscle mass may influence U-Ca/Cr values. Although U-Ca/Cr is not an ideal method for measuring hypercalciuria, in most clinical situations it is considered reliable (34). Nephrocalcinosis, a potential complication of hypercalciuria, can be excluded with renal ultrasound, but this was not included in our study protocol. Importantly, the results clearly show that the degree of urinary calcium excretion was a function of neither vitamin D intake nor its concentration but was regulated by other factors. However, because the duration of our study was only 3 months, the long-term safety of the higher vitamin D doses needs evaluation in future studies.

Maternal vitamin D status affects bone growth in early childhood (30). In our cohort, 88% of the mothers received regular supplementation of vitamin D during late pregnancy (mean, 11 μg/d). The mean S-25-OHD at birth, measured from umbilical cord blood, was 53 nmol/liter, and only four children had cord blood S-25-OHD above 80 nmol/liter. In Finland, pregnant and lactating women are advised to take 10 μg of supplemental vitamin D daily. Our results confirm that current recommendations are insufficient to ensure adequate vitamin D status in newborns, a finding also of our earlier study (30). Recently, Hollis *et al.* (35) showed that maternal daily supplementation with 400, 2000, and 4000 IU throughout pregnancy resulted in S-25-OHD of 46, 57, and 66 nmol/liter in the infants at birth. Differences in maternal

TABLE 3. Vitamin D dose and parameters of calcium homeostasis at the end of intervention in subjects with S-25-OHD concentration greater than 150 nmol/liter at 3 months

Patient no.	D3 (μg)	Sex	S-25-OHD (nmol/liter)	S-PTH (pg/ml)	P-Ca (mmol/liter)	P-Pi (mmol/liter)	U-Ca/Cr (mmol/mmol)
1	40	F	229.9		2.59	2.01	1.31
2	40	M	228.1	0	2.68	1.69	1.72
3	40	F	209.7	0	2.78	1.97	1.74
4	40	F	208.8	15	2.65	2.07	2.84
5	40	M	198.1	7	2.64	1.77	3.99
6	30	F	197.8	12	2.50	1.82	1.39
7	40	F	190.3	16	2.56	1.88	3.60
8	40	F	189.4	6	2.78	1.90	2.72
9	40	M	188.9	0	2.68	1.63	6.34
10	40	F	185.6	10	2.63	1.94	2.31
11	40	M	180.5	7	2.63	1.73	1.13
12	30	F	179.2	7	2.64	1.87	2.10
13	40	F	178.7	11	2.57	1.89	3.48
14	40	F	171.6	14	2.53	1.94	0.31
15	40	F	171.4	30	2.63	1.70	2.15
16	40	M	169.4	23	2.50	2.09	2.94
17	40	F	166.8	19	2.61	1.87	4.55
18	40	F	165.4	13	2.69	1.87	0.45
19	40	F	165.1		2.64	1.89	1.55
20	30	M	164.5	11	2.58	2.04	2.73
21	40	F	164.5	63	2.70	1.95	1.68
22	30	M	158.8	7	2.44	1.89	2.18
23	40	M	157.7	10	2.69	1.85	4.94
24	30	M	157.0	24	2.66	1.78	3.80
25	30	M	154.5	8	2.59	1.77	1.08
26	40	M	152.4		2.62	1.95	3.81
27	30	F	151.0		2.77	1.93	4.57

P-Pi, Plasma Pi; F, female; M, male.

intake of vitamin D are less likely to explain our baseline differences in 25-OHD. One limitation was that we did not analyze the impact of maternal dietary intake of vitamin D on vitamin D status of the newborn or on its content in breast milk during the 3-month intervention. The vitamin D content of breast milk is, however, low and probably has a minor effect on the vitamin D status of the breast-fed child (3, 10).

The recommendation for supplemental vitamin D for children is well followed in Finland during the first year of life, with 86% receiving regular supplementation (36). After the first year, the compliance declines, and less than 50% of 3 yr olds receive regular vitamin D. It is a challenge to improve supplementation of vitamin D in older children in Finland. Compliance with our study preparation was good (mean, 88%), with an overall compliance higher than in the Finnish population in general. The Finnish National Nutrition Council revised in the beginning of 2011 its guidelines for vitamin D supplementation for children and adolescents: the recommended daily dose for children under age 2 yr was 10 μg and for children aged 2 to 18 yr was 7.5 μg throughout the year, regardless of diet. We showed earlier in 1-yr-old children that a mean total daily vitamin D intake of 12 μg was insufficient in achieving an adequate S-25-OHD (7). Interestingly, in the present study the dose-response effect differed between intervention groups, regardless of a similar baseline S-25-OHD, the 10-μg dose having the highest dose-response. In earlier studies, a wide range of variation has been observable in S-25-OHD increment after vitamin D treatment, also in a dose-dependent fashion (37) as occurred here. In our recent study, a better response to supplementation emerged in subjects with initially lower vitamin D status (7). Conversion of vitamin D3 to 25-OHD varies with vitamin D intake and may in part be due to saturation of hepatic 25-hydroxylase (38) or to individual differences in calcium status (39).

BMD reflects bone remodeling activity, so that young bone matrix is less mineralized, and BMD is therefore lower (40). However, newly formed primary bone is more dense, and during rapid periosteal bone formation, cortical BMD is high. During the postnatal period, a redistribution occurs of bone tissue from the endocortical to the periosteal surface; external bone diameter increases and BMD declines, partly due to decreasing relative cortical area. pQCT measurement was technically difficult in 3-month-old children; no sedation was used and movement artifacts were common. Although less than one third of the measurements succeeded optimally, we could include more than 75% of the measurements in the analysis.

Because motion artifacts were present in a significant proportion, it is possible that they affected pQCT results. In addition, 10 wk of treatment is a short period for changes in bone architecture and mineral accrual. Despite these limitations, we could observe a trend toward better bone growth, and hence, better bone strength with higher doses of vitamin D. These observations need to be confirmed in a larger cohort with longer follow-up. However, these findings are well in line with our previous study in which we showed that skeletal status of the newborn, as analyzed by pQCT, is influenced by maternal vitamin D status, and these skeletal effects remained evident until age 1 yr (7, 29).

In conclusion, this intervention study shows that vitamin D3 supplementation with daily doses of 10, 30, or 40 μg from age 2 wk to 3 months is safe; even higher doses do not cause hypercalcemia or any increased rate of hypercalciuria. Furthermore, the higher doses may have beneficial effects on bone strength. The 40-μg dose maintained S-25-OHD above 80 nmol/liter in all infants. A more extensive and longer intervention study should assess long-term effects.

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