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Treatment of Hypovitaminosis D in Infants and Toddlers

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Structured Abstract

Context: Hypovitaminosis D appears to be on the rise in young children, with implications for skeletal and overall health.

Objective: To compare the safety and efficacy of vitamin D2 daily, vitamin D2 weekly, and vitamin D3 daily, combined with supplemental calcium, in raising serum 25-hydroxyvitamin D (25(OH)D) and lowering parathyroid hormone (PTH) concentrations.

Design: Six-week randomized controlled trial.

Setting: Urban pediatric clinic in Boston.

Subjects: Forty otherwise healthy infants and toddlers with hypovitaminosis D (25(OH)D < 20 ng/mL).

Interventions: Participants were assigned to one of three regimens: 2000 IU oral vitamin D2 daily, 50,000 IU vitamin D2 weekly, or 2000 IU vitamin D3 daily. Each was also prescribed elemental calcium (50 mg/kg/day). Infants received treatment for 6 weeks.

Main Outcome Measures: Before and after treatment, serum measurements of 25(OH)D, PTH, calcium, and alkaline phosphatase.

Results: All treatments approximately tripled the 25(OH)D concentration. Pre-planned comparisons were non-significant: daily vitamin D2 vs weekly vitamin D2 (12% difference in effect, p=0.66) and daily D2 vs daily D3 (7%, p=0.82). The mean serum calcium change was small and similar in the three groups. There was no significant difference in PTH suppression.

Conclusions: Short-term Vitamin D2 2000 IU daily, vitamin D2 50,000 IU weekly, or vitamin D3 2000 IU daily yield equivalent outcomes in the treatment of hypovitaminosis D among young children. Therefore, pediatric providers can individualize the treatment regimen for a given patient to ensure compliance, given that no difference in efficacy or safety was noted between these three common treatment regimens.

Introduction

Vitamin D deficiency, or hypovitaminosis D, appears to be on the rise in young children, with an increased prevalence noted among African American breastfed infants residing in northern latitudes (1). This deficiency has been identified as the leading cause of rickets among infants, as breast milk contains inadequate amounts of vitamin D to support skeletal health in this age range (2,3). Furthermore, numerous sources of evidence now indicate that vitamin D (cholecalciferol) has several important physiologic effects beyond calcium absorption and bone maintenance (4,5), and early vitamin D repletion through supervised supplementation may have a positive impact on later neurologic health (6,7), immune function (8,9), and chronic disease risk (10,11). With the reemergence of hypovitaminosis D among infants and toddlers, questions regarding the most appropriate treatment regimen require clarification.

Multiple treatment regimens have been proposed to treat hypovitaminosis D in young children, including daily or weekly dosing for varying periods of time. Common recommendations include 200,000 IU vitamin D_3 every 3 months (12), 1,000 to 2,000 IU vitamin D_2 or vitamin D_3 daily for several weeks (13), or the administration of a single intramuscular injection of 600,000 IU of vitamin D_2, repeated after 12 weeks (14-16). A regimen that has been used commonly in treating adults is 5,000-15,000 IU of vitamin D_2 weekly for 4-8 weeks (17,18). Another effective method for treating hypovitaminosis D in adult patients is an oral dose of 50,000 IU of vitamin D_2 weekly for 8 weeks, with subsequent increases in serum 25(OH)D and decreases in PTH concentrations noted (19). Little information currently exists regarding the safety or efficacy of these vitamin D treatment doses in a pediatric population. The form of vitamin D administered during treatment also remains as an area of debate, and little information is again available in children. Recent reports citing data in adults suggest that vitamin D_3 may provide a more efficacious treatment regimen than vitamin D_2, both in terms of potency and duration of action (20-22). However, there is controversy surrounding this point, as one recent study in adults noted the two treatment methods to be equally effective (23). Additionally, a study of infants and their mothers showed that vitamin D_2 and vitamin D_3 accounted for a similar proportion of total 25(OH)D concentration in neonates (cord blood) and maternal serum (24).

The aim of the present study was to examine prospectively three common treatment short-term regimens for correction of hypovitaminosis D in infants and toddlers. We conducted a randomized clinical trial, treating participants with either daily low dose of vitamin D_2, a higher dose of vitamin D_2 once weekly, or a low dose of vitamin D_3 once daily. This study examined: 1) the efficacy of each treatment in raising serum 25(OH)D and lowering parathyroid hormone (PTH) concentrations; and, 2) the safety and tolerance of each regimen in infants and toddlers, as evaluated through documentation of hypo- or hypercalcemia and reported symptoms.

Research Design and Methods

Subjects

A complete description of the referral sample for this treatment trial has been published previously (25). Briefly, during the cross-sectional screening portion of the study, 380 infants and toddlers, aged 8-24 months, were enrolled consecutively throughout the calendar year from the Children's Hospital Boston Primary Care Center between October 2005 and June 2007. Subjects completed a nutritional survey, and serum measurements of 25(OH)D, alkaline phosphatase, PTH, calcium, phosphorus, and magnesium were obtained. Skin pigmentation and sun sensitivity were evaluated by a research assistant using established methods (26,27). Exclusion criteria for the study included having a chronic disease (e.g. asthma, seizure disorder, sickle cell disease), or the use of oral glucocorticoid over the previous 3 months, or other therapy known to affect vitamin D metabolism. Patients found to be vitamin D deficient (25(OH)D $\leq$ 20 ng/mL [50 nmol/L]) were invited to participate in the

randomized clinical trial which included randomization to one of three vitamin D treatment regimens. The Committee on Clinical Investigation, Children's Hospital Boston, approved the study protocol, and parents or guardians of all participants provided written informed consent.

Treatment protocol

Patients identified to have hypovitaminosis D were randomly assigned to one treatment protocol. The randomization list was stratified by age at screening (9 mo or 18 mo) and blocked in randomly permuted sequences of 3 or 6, ensuring that no treatment would be disproportionately represented in any season or age group. The vitamin D treatments included one of three regimens: 2000 IU oral ergocalciferol (vitamin D_2) daily, 50,000 IU vitamin D_2 weekly, or 2000 IU cholecalciferol (vitamin D_3) daily. Each group was also prescribed 50 mg/kg/day of elemental calcium to prevent hypocalcemia associated with 'hungry bone' syndrome (14). Infants received the combined vitamin D and calcium treatment for a course of 6 weeks.

Vitamin D and calcium supplements were each provided in a liquid suspension that was administered orally from a vial directly onto the tongue. Parents were instructed to shake the vial before administration. The vitamin D_2 preparation (200 IU per drop or 0.025 mL) was manufactured by Sanofi-Synthelabo Inc. (Bridgewater, NJ), and doses were provided as 10 drops or 0.25 mL daily for the 2000 IU dose and 6.25 mL weekly for the 50,000 IU dose; for each vitamin D_2 dose, the suspension was administered via a provided dropper onto the tongue. The vitamin D_3 (2000 IU per drop, oil and water emulsion) was provided by Biotics Research Corporation (Rosenberg, TX) and one drop or 0.025 mL was administered daily from the vial directly onto the child's tongue. Assays of products ensured potency. The administration of the vitamin and mineral preparations in liquid form prevented problems with swallowing pills that may have presented a choking or compliance risk in our young patient population.

After approximately 3 weeks of treatment, a research assistant contacted each family to ensure that the child was receiving both the calcium and vitamin D without difficulty. Within 1 week after completing the treatment regimen, repeat serum 25(OH)D concentrations were measured, along with PTH, calcium, magnesium, phosphorus, and alkaline phosphatase. To assess compliance, the amount of vitamin D and calcium syrups remaining in the respective bottles was measured by a technician in the Central Pharmacy at Children's Hospital Boston. Outcome measures included changes in serum 25(OH)D, PTH, and alkaline phosphatase levels between baseline and follow-up. Two comparisons were formally designated as being of primary interest: daily vitamin D_2 vs weekly vitamin D_2, and daily vitamin D_2 vs daily vitamin D_3.

Laboratory measurements

During the baseline and follow-up visits, one blood sample (15 mL) was obtained from each subject. Laboratory samples were processed immediately at both Children's Hospital Boston and ARUP Laboratories (Salt Lake City, UT). Serum 25(OH)D levels were measured at ARUP Laboratories, using a Diasorin chemiluminescent assay (LIAISON®; DiaSorin Inc, Stillwater, MN). This assay accurately quantifies the sum of both 25(OH)D_3 and 25(OH)D_2. A multi-channel analyzer (Roche Diagnostics, Indianapolis) was used to measure serum calcium, phosphorus, magnesium, and alkaline phosphatase levels on site. Intact PTH was measured by a 2-site chemiluminescence immunoassay (Nichols Institute, San Clemente, CA).

Inter-assay coefficients of variation were 5.4 - 7% for PTH, 8.6-10.0% for 25(OH)D, 0.67% for alkaline phosphatase, and 1.5 - 2.2% for the cations. The definition of hypovitaminosis D correlated with the lowest end of the normal reference range as provided by the manufacturer (Diasorin, Inc.). Identification of severe hypovitaminosis D was consistent with the 25(OH)D Diasorin LIAISON® assay sensitivity (7 ng/mL).

Statistics

We conducted an intention-to-treat analysis, attributing the assigned treatment to all randomized subjects regardless of compliance. To compare baseline characteristics among the three trial arms, we used Fisher's extract test for dichotomous variables and one-way analysis of variance for continuous measures, the latter corroborated in cases of skewed distribution by the Kruskal-Wallis test.

Changes in 25(OH)D, PTH, alkaline phosphatase, and cation levels were assessed and compared among trial arms by repeated-measures analysis of covariance (ANCOVA), adjusted for age, weight, sex, skin tone, and sun sensitivity. All concentrations showed mildly skewed distributions and were log-transformed for analysis. For reporting, levels were re-transformed to concentration units. Mean changes on the log scale were constructed from parameters of the fitted ANCOVA and expressed as percentages; e.g., 100% x (exp(ΔlogPTH) – 1). The two contrasts of primary interest, daily D2 – weekly D_2 and daily D_2 – daily D_3, were constructed from parameters of the ANCOVA and compared to zero with a two-sided model, Bonferroni-adjusted critical p-value of 0.025.

Pre-trial power calculations for the two primary comparisons indicated that a sample of 15 subjects per arm would provide 80% power to detect a difference between treatments on the order of 60% for the 6-wk change in 25(OH)D. These calculations were based on cross-sectional data from our adolescent clinic (28) and an estimated intra-class correlation of 0.5 (not obtainable from the cross-sectional data).

Statistical computations and generation of the randomization list were performed with SAS software version 9.1 (SAS Institute, Cary, NC).

Results

Subjects

Within our clinical sample of 380 infants and toddlers (25), we identified 40 infants and toddlers to have hypovitaminosis D (25(OH)D ≤ 20 ng/mL [50 nmol/L]). Within this sample of 40 participants, 35 completed the course of therapy (87.5%). The baseline characteristics of participants in the three treatment arms are illustrated in Table 1. There were no significant differences between groups with respect to gender, skin pigmentation, skin sensitivity, or season of year at baseline, prior to randomization. Biochemically, participants were also similar, and weight and age did not significantly differ across treatment groups.

Treatment effects on serum 25(OH)D

All three treatments virtually tripled the 25(OH)D concentration in these vitamin D-deficient children (Fig. 1). The greatest effect was attained with weekly vitamin D_2: from 13.8 to 44.0 ng/mL, an increase of 220%. The next greatest was the effect of D_3 (13.7 to 41.2 ng/mL, 202%), followed by daily vitamin D_2 (15.7 to 43.9 ng/mL, 182%). The pre-planned comparisons were non-significant: daily vitamin D_2 vs weekly vitamin D_2 (12% differences in effect, p=0.66) and daily D_2 vs daily D_3 (7%, p=0.82). All participants achieved 25(OH)D concentrations ≥ 20 ng/mL except for 3 participants. Within this subgroup, one participant was receiving vitamin D_3 daily; the other two, vitamin D_2 weekly. For each case, the compliance of the family had been questioned at the follow-up visit.

The two pre-planned contrasts were small in comparison to the 200% pre-post change, and both were non-significant. Daily vitamin D_2 showed an effect 12% lower than weekly vitamin D_2 (p=0.66) and 7% lower than daily D_3 (p=0.82). Post-hoc power calculations using the attained sample size and standard errors showed that the conjectured 60% difference between arms was detectable with only 22% power, partly due to a lower correlation than anticipated (0.2 vs 0.5) between baseline and the greatly increased post-treatment levels (Figure 2).

Calcium

We examined serum calcium concentrations from the larger cross-sectional sample of healthy infants and toddlers from which the trial participants were derived. Baseline calcium concentrations were compared to the current trial participants, each with

hypovitaminosis D, to 329 vitamin D replete subjects. The median calcium level was slightly higher in the vitamin D replete subjects (10.50 vs 10.35, p=0.04 by Wilcoxon test).

The mean change in serum calcium levels was small and similar in the three treatment groups (-3% for vitamin D_2 daily, +3% vitamin D_2 weekly, +1% vitamin D_3 daily).

Parathyroid Hormone (PTH)

Eight participants (20%) presented with elevated PTH at baseline (reference range 10-65 pg/mL). All cases returned to normal limits following treatment. As illustrated in Fig. 1, the largest change in PTH was observed in the group receiving vitamin D_2 weekly (down 40%, from 32.1 to 19.2 pg/mL, adjusted for covariates), as compared to patients in the other treatment arms (vitamin D_2 daily, down 20% from 38.5 to 30.8 pg/mL, and vitamin D_3 daily, down 36% from 40.9 to 26.3 pg/mL). There was no significant difference in PTH suppression among the three groups (p=0.74).

Alkaline phosphatase

There was no significant impact of treatment on alkaline phosphatase concentrations (Fig. 1).

Compliance

To assess compliance, we submitted vials containing the remaining vitamin D and calcium liquid preparations to the Children's Hospital Boston Central Pharmacy. The amount remaining in the vials was compared to the expected amount consumed. No appreciable difference was noted in compliance among the 3 treatment groups.

Discussion

To our knowledge, this study is the first to compare the efficacy and safety of three common short-term treatment regimens to correct hypovitaminosis D in infants and toddlers. We report no difference in outcome between vitamin D_2 daily, vitamin D_2 weekly, or vitamin D_3 daily for a sample of young children. These findings are consistent with one previous report in adults suggesting that these two formulations contribute equally to circulating 25(OH)D levels (23).

Three previous reports citing data from adults have advocated strongly for supplementation with vitamin D_3 (cholecalciferol) over D_2 (ergocalciferol) as the preferred treatment method for vitamin D deficiency (20-22). However, our study showed that each treatment regimen was equally effective, as well as safe. These data are reassuring to providers, as vitamin D_2 daily or weekly, or vitamin D_3 daily, combined with elemental calcium, appears to provide an effective and well-tolerated treatment for correcting hypovitaminosis D in infants and toddlers. Furthermore, the consistency of these data across the treatment arms will allow practitioners to tailor their specific treatment regimens to meet an individual patient's needs, preferences, and probability of compliance.

In this study, we sought to examine the differences between PTH at baseline and following replacement therapy in each treatment group, as suboptimal serum 25(OH)D levels can be associated with a secondary or compensatory hyperparathyroidism. Interestingly, the largest change in PTH between baseline and 7 weeks was observed in the group receiving vitamin D_2 weekly. At baseline, we found elevated PTH concentrations in 8 subjects among the cohort, and all participants' levels decreased to the normal range following treatment. These findings further support the similarities between these three treatment arms in reversing the secondary effects that associated biochemical markers may have on vitamin D homeostasis.

Our data provide clinical guidance regarding the appropriate dosage range of vitamin D to treat deficiency in this young population. Among infants, hypercalcemia has been reported with the administration of single high-dose therapy of 300,000 IU (29) or 600,000 IU (30), as well as daily doses exceeding 10,000 IU daily (31). While a single 600,000 IU dose has been strongly advocated by one group as a safe regimen and one that eliminates the problem of noncompliance (12), this recommendation has been met with controversy and, specifically, concerns about hypercalcemia

(29,32), especially in an outpatient setting. In our study, we report a surprising higher overall incidence of mild hypercalcemia at baseline in contrast to after treatment. All subjects were asymptomatic. There was no statistically significant correlation between the presence of hypercalcemia at baseline and following each tested course of treatment. Therefore, these more conservative regimens of vitamin D_2 daily, vitamin D_2 weekly, or vitamin D_3 daily may provide the necessary treatment without the increased risk of hypercalcemia commonly associated with single large dose therapies (also known as *stosstherapy*) (12). The potential toxicity associated with stosstherapy is further underscored by a recent report that showed hypercalcemia in an infant treated with the equivalent of 4 daily stosstherapy doses (33).

Limitations of this study deserve acknowledgment and consideration. First, the sample size was small and power limited. The observed differences among the three treatment regimens in improvement of 25(OH)D level, although consistently small in comparison to the gross change achieved by treatment, were not precisely determined and thus admit the possibility of larger underlying effects. Our negative finding may, therefore, be due to the small sample size, although likely not to variability in age or weight, for which we controlled in our analyses. Second, it is more difficult to assess compliance among young infants and toddlers, as they are unable to swallow pills, necessitating administration of a liquid vitamin preparation. Therefore, measurements of the exact amount of remaining vitamin D suspension administered during treatment were more difficult to assess compared to pill counts that would be possible in an older patient population. In order to standardize our data acquisition, a single pharmacy technician completed all measurements throughout the course of the study. However, the potential inaccuracy of viscous liquid (versus pill) measurement deserves acknowledgment, including the fact that the measurement involved extraction of the suspension from a vial. Lastly, it is possible that the participants' parents provided increased amounts of dietary vitamin D (e.g., vitamin D-fortified milk, salmon, eggs), in addition to the supplementation upon hearing of their child's deficiency. However, such low-potency dietary modification is not likely to have significantly affected one treatment group's results as compared to another, and not in a way that would change the observed consistency noted among the treatment groups.

In summary, we demonstrate that 2000 IU daily vitamin D_2, 50,000 IU vitamin D_2 weekly, or 2000 IU daily vitamin D_3 yield equivalent outcomes in the short-term treatment of hypovitaminosis D among otherwise healthy infants and toddlers. These results indicate that pediatric providers can determine the appropriate method of treatment for a given patient or family to ensure compliance, given that no difference in efficacy or safety was noted. The argument favoring large dose depot therapies for correcting hypovitaminosis D must be reevaluated, as more conservative lower dose therapies may provide a safer method of treatment, especially in the outpatient setting, without the associated risk of hypercalcemia. In addition, the case for vitamin D_3 as the most effective treatment method must be reconsidered for young children, as a weekly dose of vitamin D_2 may yield a similar outcome without the inconvenience of a daily treatment. We recommend early treatment with one of these three treatment regimens, or subtle variations to the dosages studied, to prevent the potential skeletal and extraskeletal problems associated with hypovitaminosis D. Lastly, we do not endorse the use of the current relatively high doses of vitamin D for the long-term prevention of hypovitaminosis D in infants and young children. Further research is needed to clarify the appropriate daily vitamin D supplementation dose for the pediatric age group.

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Table 1: Baseline characteristics of participants, compared across randomly assigned treatment groups.

	D_2 daily (12)	D_2 weekly (14)	D_3 daily (14)	p*
	n (%)			
Gender: Male	4 (33)	6 (43)	8 (57)	0.45
Female	8 (67)	8 (57)	6 (43)	
Skin pigmentation:				0.56
1 (heaviest)	7 (58)	9 (64)	9 (64)	
2	4 (33)	3 (21)	3 (21)	
3	1 (8)	2 (14)	0 (0)	
4 (lightest)	0 (0)	0 (0)	2 (14)	
Skin sensitivity:				0.35
1 (burn easily)	0 (0)	0 (0)	1 (7)	
2 (burn always)	0 (0)	0 (0)	0 (0)	
3 (burn moderately)	0 (0)	2 (14)	2 (14)	
4 (burn minimally)	3 (25)	2 (14)	2 (14)	
5 (burn rarely)	7 (58)	6 (43)	9 (64)	
6 (never burn)	2 (17)	4 (29)	0 (0)	
Month of enrollment:				0.94
Darkest (Nov–Jan)	5 (42)	4 (29)	3 (21)	
Intermediate (Feb–Apr)	2 (17)	2 (14)	3 (21)	
Lightest (May–Jul)	2 (17)	4 (29)	5 (36)	
Intermediate (Aug–Oct)	3 (25)	4 (29)	3 (21)	
	Median (QD)†, *minimum–maximum*			
Age, mo	10.0 (3.5), 9.0–21.6	9.8 (1.5), 7.6–22.6	10.1 (1.9), 8.0–22.9	0.63
Weight, kg	10.1 (0.8), 8.2–12.0	9.2 (0.6), 7.5–11.4	9.5 (1.0), 7.2–12.4	0.41
25(OH)D, ng/mL	18 (3), 7–20	17 (4), 7–20	17 (4), 7–20	0.75
PTH, pg/mL	33 (16), 12–166	27 (45), 7–508	37 (14), 16–72	0.22
Alkaline phosphatase, U/L	267 (51), 144–553	329 (97), 192–708	241 (58), 172–537	0.14
Calcium, mg/dL	10.4 (0.2), 9.7–11.2	10.4 (0.3), 7.0–11.1	10.3 (0.3), 9.9–10.8	0.59
Magnesium, mg/dL	2.5 (0.1), 2.2–2.7	2.3 (0.2), 2.0–2.7	2.3 (0.2), 2.0–2.8	0.30
Phosphorus, mg/dL	5.6 (0.5), 3.7–6.8	5.9 (0.5), 2.5–7.0	5.8 (0.5), 4.3–6.4	0.80

* Testing for equal distribution in the three treatment arms. For binary and polytomous variables, Fisher exact test; for continuous measures, one-way analysis of variance, corroborated by Kruskal-Wallis test in cases of skewed distribution.

† QD equals half the interquartile range (75th percentile minus 25th percentile), analogous to standard deviation.

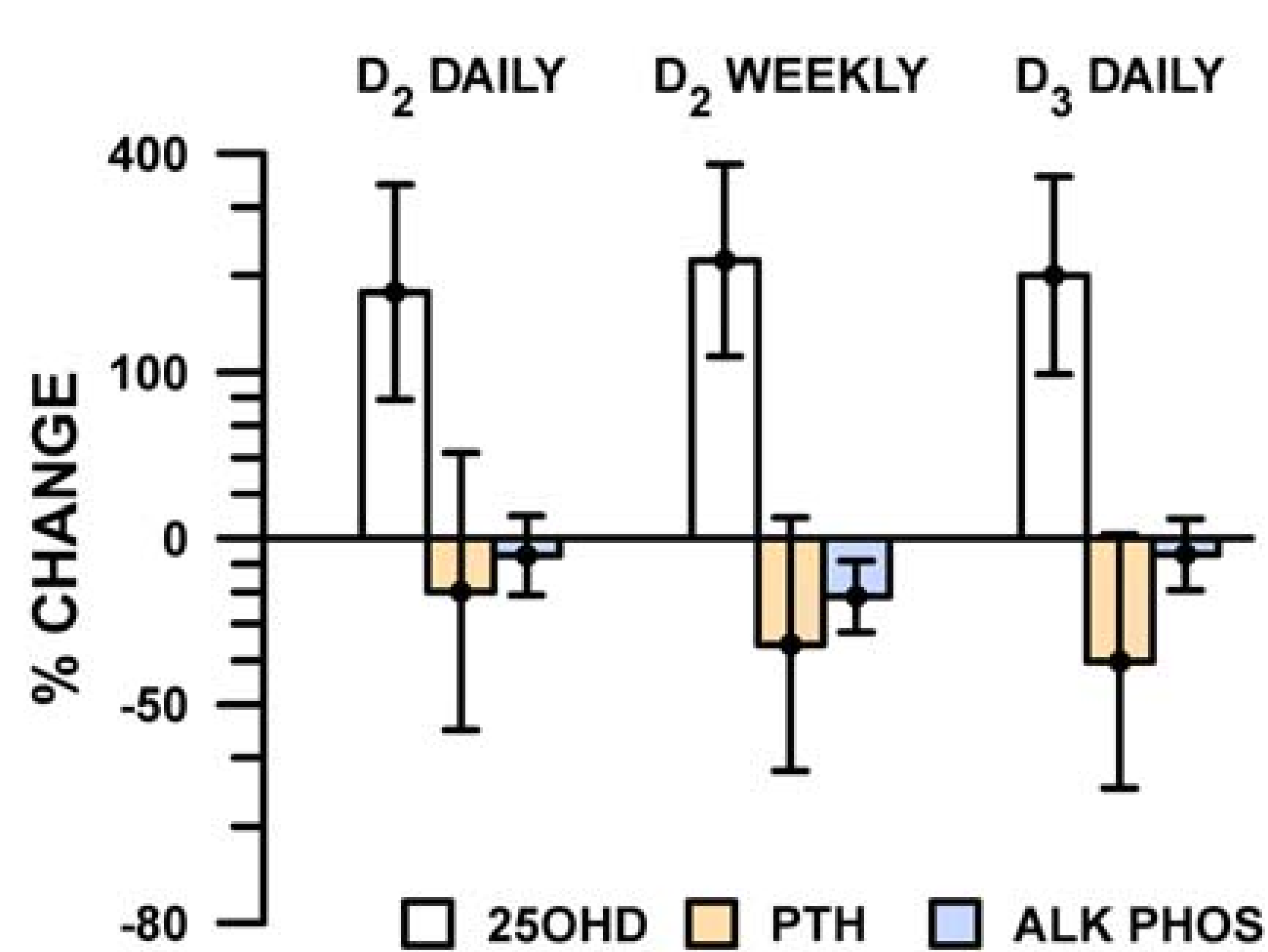
Table 2. Serum levels of 25(OH) vitamin and related biochemical measurements (before and after treatment, all participants)

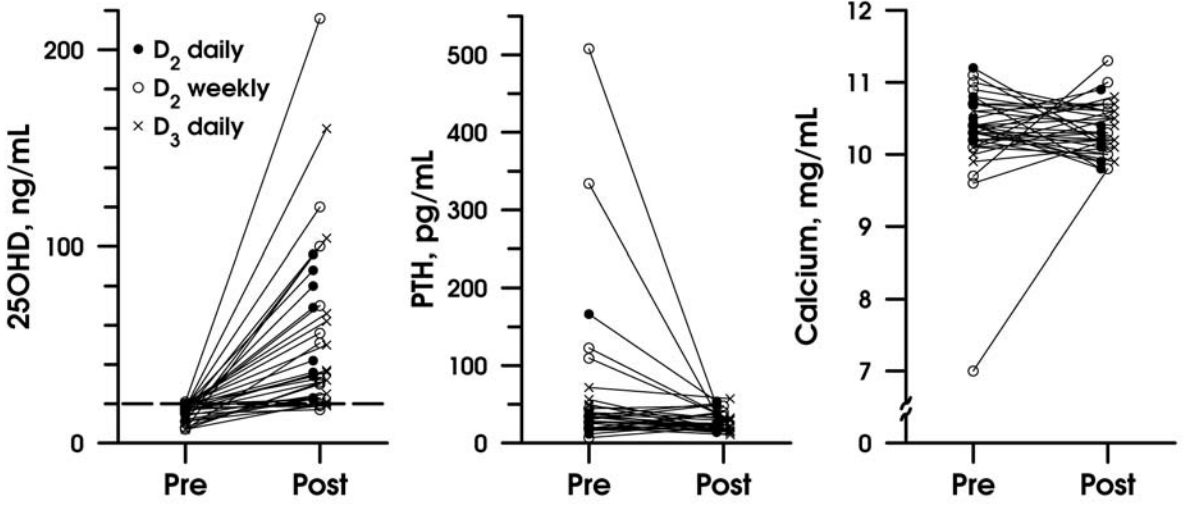
	Median (25th percentile–75th percentile)	
	Baseline	Post-treatment
25(OH)D, ng/mL	17 (11–19)	36 (23–70)
PTH, pg/mL	34 (20–50)	24 (18–35)
Alkaline phosphatase, U/L	283 (232–383)	269 (211–350)
Calcium, mg/dL	10.4 (10.1–10.7)	10.3 (10.1–10.6)
Magnesium, mg/dL	2.4 (2.2–2.5)	2.3 (2.2–2.4)

Figure Legends

Figure 1: Change in serum 25OH-D and related markers in infants and toddlers diagnosed with rickets (25OHD≤20 ng/dL), after six weeks of treatment with randomly assigned treatment regimens. Mean and 95% confidence interval from repeated-measures regression analysis of log-transformed concentration measures.

Figure 2: Absolute changes in serum 25(OH) D, PTH, and calcium are depicted from the baseline and follow-up visits for the 3 treatment groups: vitamin D_2 (2000 IU) daily (closed circle) and (50,000 IU) weekly (open circle), and vitamin D_3 (2000 IU) daily (x).


D2 DAILY
D2 WEEKLY
D3 DAILY
400
100
0
-50
-80
% CHANGE
25OHD
PTH
ALK PHOS


25OHD, ng/mL
200
100
0
D2 daily
D2 weekly
D3 daily
Pre
Post
PTH, pg/mL
500
400
300
200
100
0
Pre
Post
Calcium, mg/mL
12
11
10
9
8
7
0
Pre
Post