Published in final edited form as:

J Bone Miner Metab. 2009 ; 27(4): . doi:10.1007/s00774-009-0065-7.

Standard multivitamin supplementation does not improve vitamin D insufficiency after burns

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Abstract

Children suffering severe burns develop progressive vitamin D deficiency because of inability of burned skin to produce normal quantities of vitamin D₃ and lack of vitamin D supplementation on discharge. Our study was designed to determine whether a daily supplement of a standard multivitamin tablet containing vitamin D₂ 400 IU (10 µg) for 6 months would raise serum levels of 25-hydroxyvitamin D [25(OH)D] to normal. We recruited eight burned children, ages 5–18, whose families were deemed reliable by the research staff. These children were given a daily multivitamin tablet in the hospital for 3 months in the presence of a member of the research staff and then given the remainder at home. At 6 months, the subjects returned for measurements of serum levels of 25(OH)D, 1,25-dihydroxyvitamin D [1,25(OH)₂D], intact parathyroid hormone (iPTH), Ca, P, albumin, and total protein as well as bone mass by dual energy X-ray absorptiometry. Serum 25(OH)D levels were compared to a group of seven age-matched burned children studied at an earlier date without the vitamin supplement but with the same method of determination of 25(OH)D at 6 months post-burn. In addition, the chewable vitamins were analyzed for vitamin D₂ content by high performance liquid chromatography. Serum concentration of 25(OH)D was 21 ± 11 (SD) ng/ml (sufficient range 30–100) with only one of the eight children having a value in the sufficient range. In comparison, the unsupplemented burn patients had mean serum 25(OH)D level of 16 ± 7 , $P = 0.33$ versus supplemented. Serum levels of 1,25(OH)₂D, iPTH, Ca, P, albumin, and total protein were all normal in the supplemented group. Vitamin D₂ content of the chewable tablets after being saponified and extracted was 460 ± 20 IU.

Bone mineral content of the total body and lumbar spine, as well as lumbar spine bone density, failed to increase as expected in the supplemented group. No correlations were found between serum 25(OH)D levels and age, length of stay, percent body surface area burn or third-degree burn. Supplementation of burned children with a standard multivitamin tablet stated to contain 400 IU of vitamin D₂ failed to correct the vitamin D insufficiency.

Keywords

Vitamin D insufficiency; Burns; Children; 25-hydroxyvitamin D

Introduction

Children suffering burns of at least 40% total body surface area (TBSA) lose bone due to the large endogenous glucocorticoid production resulting from the stress response and the bone-resorbing cytokines produced by the systemic inflammatory response [1]. Moreover, they develop hypocalcemic hypoparathyroidism with urinary calcium wasting [2], most likely due to up-regulation of the parathyroid calcium sensing receptor by the pro-inflammatory cytokines [3]. They have also been shown to develop a progressive vitamin D deficiency that has been detected as early as 14 months post-burn [4]. It is manifested by low serum concentrations of 25-hydroxyvitamin D [25(OH)D] on cross-sectional studies between 14 months and 7 years post-burn [5]. The progressive nature of the vitamin D deficiency is documented by the finding that, while at 2 years post-burn serum 25(OH)D levels are low and serum concentrations of 1,25-dihydroxyvitamin D [1,25(OH)₂D] levels are normal, at 7 years post-burn, the serum 25(OH)D levels remain low and approximately half the serum levels of 1,25(OH)₂D are low [5]. Moreover, there was a direct relationship between serum concentration of 25(OH)D and lumbar spine bone mineral density (BMD) Z scores [5]. The pathogenesis of this problem is not entirely clear. Following acute burn injury, serum levels of vitamin D metabolites may be low on measurement because both vitamin D binding protein [6] and albumin and total protein [6] are low. However, the children had been discharged from acute hospitalization without vitamin supplementation, and skin from previously burned children failed to synthesize vitamin D₃ in normal quantities following ultraviolet radiation exposure [4].

The aim of our study, therefore, was to determine whether daily administration of a standard multivitamin supplement containing vitamin D₂ (ergocalciferol) 400 international units (IU, 10 µg) could raise serum levels of 25(OH)D to what is now considered to be the sufficient range of 30–100 ng/ml [7]. Vitamin D deficiency is defined as a 25(OH)D level below 20 ng/ml, and vitamin D insufficiency is the range between 21–29 ng/ml [7]. The term vitamin D insufficiency denotes a range of values in serum at which serum parathyroid hormone levels remain incompletely suppressed and intestinal calcium transport suboptimal [7]. We have previously confirmed the existence of the inverse relationship between serum 25(OH)D and intact PTH concentrations in the sera of burned children [5]. Thus, while the insufficient range of serum 25(OH)D levels does not cause frank rickets or osteomalacia, it can still result in bone loss and perhaps compromise the achievement of peak bone mass in children.

Methods

Patient selection

After obtaining informed consent, patients were sequentially enrolled following discharge. The inclusion criteria included burn injury of ≥40% TBSA, age at least 5 years, and participation in an exercise program requiring in-hospital residence for 3 months before discharge. Children were excluded if they had any documented underlying intestinal

malabsorption, bone, or kidney disease. Furthermore, all families had to be evaluated by a member of the research nursing staff to determine whether they were likely to be compliant. Tanner staging was performed by a single individual under the supervision of a pediatric endocrinologist and with consent of the families. Results of serum 25(OH)D levels were compared against a group of similarly burned age-matched children previously studied who had not received the vitamin supplement but whose serum levels of 25(OH)D at 6 months post-burn were measured in the same laboratory by the same published methods as the levels for the supplemented patients at the 6-month time point.

Compliance monitoring

A chewable multivitamin tablet (Fruity Chews) containing 400 IU (10 μ g) vitamin D₂ was administered daily by a family member in the presence of a member of the research staff. Those families judged by experienced clinical research nurses to be compliant were discharged with a 3-month supply of the multivitamin, and patients and families returned to Shriners Burns Hospital at 6 months post-burn for determination of serum levels of 25(OH)D, 1,25(OH)₂D, intact parathyroid hormone (iPTH), and bone densitometry.

Competitive protein binding assays were performed to determine serum levels of 25(OH)D, with intra-assay and inter-assay coefficients of variation being 5 and 10%, respectively [8]. Thymus receptor binding assays were used to determine serum levels of 1,25(OH)₂D, with intra-assay and inter-assay coefficients of variation being 10 and 15%, respectively [9]. At the conclusion of the study, the chewable multivitamin tablets were analyzed for their actual vitamin D₂ content by grinding down the tablet followed by saponification, then extracting with organic solvent for 24 h, drying it down and chromatographing the reconstituted extract on a high performance liquid chromatograph [10, 11]. Serum concentration of iPTH was analyzed by ELISA kit (Diagnostic Systems Laboratories, Webster, TX). Bone densitometry measurements were carried out by use of a QDR4500A dual energy X-ray absorptiometer with pediatric software (Hologic, Waltham, MA).

Student *t*-test and rank correlation coefficient were used where appropriate. This study was approved by the Institutional Review Board of the University of Texas Medical Branch at Galveston and by the Shriners Hospitals for Children.

Results

Eight patients (6 M, 2F), ages 6–18 years, were sequentially enrolled. All had burns 40% TBSA. Patient demographics are shown in Table 1. These demographics are comparable to those we have published in our other studies and are typical of those seen in our patient population. Serum levels of 25(OH)D at 6 months post-burn were 21 ± 11 ng/ml, range 9–46, median 20, with 7 of the 8 values below the desired value of 30 ng/ml [12, 13] (Table 2).

There was no significant difference in serum levels of 25(OH)D in supplemented children compared with the seven unsupplemented burned children, 16 ± 7 ng/ml, range 5–23, $P = 0.33$ (Table 3). Ages were also not significantly different, 12 ± 3 and 8 ± 5 years in supplemented and unsupplemented groups respectively ($P = 0.07$) (Table 4). No correlations were found between serum levels of 25(OH)D, age, length of stay, percent TBSA burn, or percent third-degree burn.

Serum levels of 1,25(OH)₂D in supplemented children were 23 ± 12 pg/ml, all within the normal range, 15–60 pg/ml (Table 2). Serum iPTH, total Ca, P, total protein and albumin were normal as well at 6 months post-burn (Table 2). Mean serum iPTH levels rose from admission levels of 15 ± 11 to 21 ± 11 pg/ml. There was a trend toward an inverse

relationship between serum levels of 25(OH)D and iPTH, $R = -0.51$, though this did not reach significance, $P = 0.20$.

Furthermore, lumbar spine bone mineral content (BMC) and bone mineral density (BMD) and total body BMC failed to increase between discharge and 6 months following burn injury (Fig. 1). There were no significant differences between discharge and 6-month DXA values. This pattern contrasts to what is normally seen in growing children, who increase total body BMC annually by 3.7–16.2% at the 50th percentile and lumbar spine BMD annually by 2.5–12.4%, depending on age and pubertal status [14]. Table 5 lists the age, sex, Tanner stage, and total body BMC from discharge to 6 months post-burn in the eight supplemented patients. Rank correlation coefficient for percent change in total body BMC and Tanner staging was significant with $R^2 = 0.69$ ($P < 0.025$). In contrast there was no significant relationship between serum 25(OH)D and change in BMC, $R^2 = 0.25$, $P = \text{NS}$.

Analysis of the Fruity Chews multivitamin tablets ($n = 4$) revealed that the average vitamin D₂ content was 460 ± 20 IU.

Discussion

Our data show that despite receiving a daily multivitamin supplement containing approximately 400 IU of vitamin D₂ for 6 months, serum levels of 25(OH)D remained insufficient and not different from unsupplemented burn patients at 6 months post-burn. Moreover, BMC of the whole body and BMC and BMD of the lumbar spine failed to improve, possibly due to inadequate blood levels of 25(OH)D or other metabolic factors that remain to be identified. It should also be noted that while pubertal stage appeared in this limited sample to be more important than circulating 25(OH)D levels in the improvement of total body BMC from hospital discharge, the body is still in a hypermetabolic state for the first year post-burn. Therefore, many of the hormones present in older burn patients may play a role in modifying bone loss post-burn. Moreover, the studies relating serum 25(OH)D levels to bone mass were performed at 2 and 7 years post-burn after the hypermetabolic period resolved. Therefore, serum levels of 25(OH)D may play a more important role in improving bone mass after the disappearance of the catabolic response and the reduction of such hormones as endogenous glucocorticoids.

While in hospital, patients received nutrition consisting of daily multivitamin intake 400 IU vitamin D₃ (cholecalciferol, Thera Plus, Hi-Tech Pharmacal, Amityville, NY) in addition to varying amounts of vitamin D₃-containing foods such as formulas (Vivonex TEN, 200 IU vitamin D₃, 5 µg/l), Pediasure (120 IU vitamin D₃, 3 µg/234 ml), whole milk (100 IU vitamin D₃, 2.5 µg/234 ml), or yogurt (60 IU vitamin D₃, 1.5 µg/4 oz). It is not clear whether patients were vitamin D deficient in the hospital, because both vitamin D binding protein [6] and albumin [6] were low. However, by 6 months after burn, serum albumin and total protein were normal, and it was assumed that the D-binding protein was also normal, considering that 85% of serum 1,25-dihydroxyvitamin D is bound to D-binding protein [15] and circulating 1,25-dihydroxyvitamin D was normal in our supplemented patients. Because it was not measured, constitutive protein insufficiency cannot be completely discounted as a contributor to the inadequate circulating levels of 25(OH) D at 6 months post-burn.

Remaining possibilities for failure to reach vitamin D sufficiency include occult fat malabsorption that has not been detected in these burn patients or an increased vitamin D requirement. A greater vitamin D requirement likely exists than previously appreciated. It has been shown previously that in healthy adults, supplementation of orange juice with 1,000 IU of vitamin D₃ resulted in a 150% rise in serum 25(OH)D levels over 12 weeks [16]. It is possible that burn patients have a requirement that is closer to this range.

Furthermore, inasmuch as Holick et al. [17] have recently showed that vitamin D₂ and vitamin D₃ are equally effective in maintaining serum levels of 25(OH)D, our use of ergocalciferol is not likely a factor in the failure to achieve higher levels.

Regardless of the etiology of the problem, standard multivitamin supplementation does not correct it. Pre-and post-treatment values of total body and lumbar spine bone mass failed to improve with the vitamin D supplementation, and while serum iPTH levels normalized in these patients at 6 months post-burn, the factors that influence this improvement are not certain, and adequate vitamin D supplementation should lower serum iPTH levels, not raise them. Serum levels of 25(OH)D and 1,25(OH)₂D were not measured in hospital because of the initially low serum levels of constitutive proteins, including vitamin D binding protein and albumin, as previously mentioned. Adequate levels of serum 25(OH)D are expected to increase BMC because in several instances, including our own patients, serum concentrations of 25(OH)D correlate directly with various parameters of bone density [5].

This problem highlights an additional issue that needs to be emphasized: the daily requirements for vitamin D may be different in sick children compared to well children. The amount of vitamin D required is likely to be dependent on both the severity and nature of the condition in question. However, future consideration must be paid to the realization that giving sick children the amount of vitamin D recommended for well children may underestimate their requirements.

Acknowledgments

This study was presented in part at the Fourth International Conference on Children's Bone Health, Montreal, 21–24 June 2007. Funding was provided by NIH 1P50 GM 60338 and by NIDRR H133A020102-05. We are grateful for the technical assistance of Z Lu, Boston University, and Mary Kelly and Joanna Huddleston of Shriners Burns Hospital in Galveston.

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Fig. 1.

The mean and standard deviation of bone mineral content (*BMC*) of the total body (*T*) and lumbar spine (*LS*) and bone mineral density of the lumbar spine (*LS-BMD*) expressed as percent change from hospital discharge in study patients receiving standard vitamin D supplementation from time of discharge

Table 1

Patient demographics: supplemented patients

Patient	Age (years)	% TBSA burn/% 3rd degree	Length of stay (days)
1	9.1	60/60	57
2	6.6	87/87	117
3	14.1	71/71	26
4	12.1	42/42	20
5	15.3	48/41	19
6	9.8	53/34	32
7	15.1	56/56	17
8	14.7	54/54	32
Mean $\pm$ SD	12.1 $\pm$ 3.3	59 $\pm$ 14/56 $\pm$ 17	40 $\pm$ 34

TBSA Total body surface area

Serum levels of parathyroid hormone (PTH), total Ca, P, albumin, total protein, and vitamin D metabolites at 6 months post-burn: supplemented patients

Table 2

Patient	Total Ca (mg/dl)	Phosphorus (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Intact PTH (pg/ml)	25(OH)D (ng/ml)	1,25(OH) ₂ D (pg/ml)
1	8.8	5.5	6.6	3.6	17.3	24	30
2	8.8	4.8	6.3	3.7	18	13	NA
3	9.2	5.2	7.0	4.1	10.1	46	NA
4	8.4	3.8	6.3	3.2	42.7	16	37
5	9.0	5.6	7.1	4.2	31.9	9	33
6	9.8	5.4	6.3	3.7	17	20	25
7	9.3	4.7	6.2	3.6	8.9	20	18
8	8.5	4.7	6.2	3.6	36.9	20	27
Mean ± SD	9.0 ± 0.4	5.0 ± 0.5	6.6 ± 0.4	3.7 ± 0.3	22.9 ± 12.6	21 ± 11	23 ± 12
Normal range	8.8–10.3	3.5–5.5	5.3–8.0	3.3–5.8	10–65	20–100	15–60

NA Not assessed

Table 3

Biochemistry and bone mineral content: unsupplemented patients

Patient	Ionized Ca (mg/dl)	P (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Intact PTH (pg/ml)	Total body BMC (% discharge value)	25(OH)D (ng/ml)
1	NA	NA	NA	NA	NA	75.9	<5
2	4.1	5.1	5.8	2.9	3.8	NA	21
3	3.9	5.1	5.9	2.4	6.6	146.5	14
4	NA	5.8	6.9	3.7	<1	66.4	23
5	4.2	5.2	6.7	3.9	NA	58.3	8
6	NA	5.5	6.9	4.2	47.8	170	21
7	NA	5.7	7.7	4.4	3.5	NA	20
Mean $\pm$ SD		5.4 $\pm$ 0.3	6.7 $\pm$ 0.6	3.6 $\pm$ 0.7	12.5 $\pm$ 17.7		16 $\pm$ 7
Normal range	4.5–5.5	3.5–5.5	5.3–8	3.3–5.8	10–65		20–100

PTH Parathyroid hormone, BMC bone mineral content, NA not assessed

Table 4

Patient demographics: unsupplemented patients

Patient	Age (years)	Gender	TBSA (%)/% 3rd	Length of stay (days)
1	10	F	54/50	41
2	6	F	40/35	5
3	3	M	60/50	21
4	11	M	55/45	32
5	12	M	71/52	19
6	2	M	40/28.5	23
7	13	M	58.5/5	22
Mean $\pm$ SD	8.1 $\pm$ 4.1		54 $\pm$ 10/38 $\pm$ 16	23 $\pm$ 10

TBSA Total body surface area

Table 5

Tanner stages, age, sex, and bone density of supplemented burn patients

Patient	Age	Gender	Tanner	Total body BMC (% of discharge value)
1	9.1	F	1	88.4
2	6.6	M	NA	86.9
3	14.1	M	2	88.1
4	12.1	M	NA	87.6
5	15.3	M	5	109
6	9.8	F	2	90.2
7	15.1	M	4	97.3
8	14.7	M	4	107.8

NA Not assessed, *BMC* bone mineral content