***d*- α -Tocopheryl Polyethylene Glycol-1000 Succinate Enhances the Absorption of Vitamin D in Chronic Cholestatic Liver Disease of Infancy and Childhood**

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ABSTRACT. Rickets and osteopenia, common problems in chronic childhood cholestasis, have been attributed to vitamin D malabsorption leading to reduced serum levels of 25(OH)-vitamin D. *d*- α -Tocopheryl polyethylene glycol-1000 succinate (TPGS), a water-soluble form of vitamin E, forms micelles at low concentration. We evaluated the potential role of TPGS in enhancing vitamin D absorption in eight children (aged 5 mo to 19 y) with severe chronic cholestasis (three extrahepatic biliary atresia, three non-syndromic intrahepatic cholestasis, and two Alagille syndrome). To evaluate vitamin D absorption, the subjects received vitamin D₃ 1000 IU/kg (maximum dose of 50 000 IU); they then received the same dose of vitamin D₃ mixed with TPGS (25 IU/kg). Serial serum vitamin D₃ levels and areas under the curve were measured. All patients had enhanced absorption of vitamin D when it was administered in a mixture with TPGS. Mean area under the curve for serum vitamin D₃ was 403.0 ± 83.1 nmol $\times$ h/L (155.6 ± 32.1 ng $\times$ h/mL), with a mean rise above baseline of 13.5 ± 1.8 nmol/L (5.2 ± 0.7 ng/mL) with vitamin D/TPGS compared with no rise when vitamin D was given alone (both $p < 0.001$). Seven patients have been followed for at least 3 mo while receiving the vitamin D/TPGS combination. Those with initially low serum 25(OH)-vitamin D levels (<37.5 nmol/L or 15 ng/mL) had normalization (range 37.5–146 nmol/L) within 1 mo, whereas those with initially normal levels remained normal. While the patients were receiving vitamin D/TPGS, serum vitamin E to total lipid ratio either normalized or remained normal. In conclusion, 1) TPGS enhances vitamin D absorption in infants and children with severe chronic cholestasis and 2) the enhanced absorption of both vitamins is sufficient to normalize or maintain adequate levels of serum 25(OH)-vitamin D and vitamin E to total lipid ratio. (*Pediatr Res* 31: 146–150, 1992)

Abbreviations

25(OH)D, 25-hydroxy-vitamin D

TPGS, *d*- α -tocopheryl polyethylene glycol-1000 succinate

Bone disease (rickets and osteopenia) is a common complication of chronic childhood cholestatic liver disease (1–3). Affected children have reduced levels of serum 25(OH)D secondary to poor intestinal absorption of fat-soluble vitamins because of reduced intraluminal bile acids, decreased sun exposure compounded by their chronic illness and multiple hospitalizations, and impaired hepatic hydroxylation of cholecalciferol to 25-OH-cholecalciferol in end-stage liver disease (1, 4–7). Despite the ingestion of massive doses of oral vitamin D₂ or D₃, metabolic bone disease may still develop. Various alternative modalities of therapy, including parenteral cholecalciferol and oral calcifediol and calcitriol, have been used in an attempt to normalize serum 25(OH)D levels (3–5, 8–10).

Similar to vitamin D, orally administered vitamin E is dependent upon bile salts for solubilization and facilitation of absorption. TPGS, a water-solubilized form of vitamin E, forms micelles at low concentrations (11). As early as 1970, evidence was available suggesting that vitamin E as TPGS was absorbed in infants with extrahepatic biliary atresia and cystic fibrosis (12). More recently, TPGS was shown to be an effective form of therapy for vitamin E deficiency in children with severe chronic cholestasis (13). The present study was designed to evaluate the potential role of TPGS in enhancing the absorption of vitamin D₃ from the gastrointestinal tract in infants and children with chronic cholestatic disease.

MATERIALS AND METHODS

Eight patients (one male, seven females), aged 5 mo to 19 y, with chronic cholestatic liver disease were enrolled. Three had extrahepatic biliary atresia, two had Alagille syndrome, and three had nonsyndromic intrahepatic cholestasis.

Of seven patients available for follow-up after the tolerance test, while receiving the vitamin D/TPGS combination, five were on ursodeoxycholic acid (15 mg/kg/d). Four of these five had been on ursodeoxycholic acid for a mean duration of 6 mo (range 3–13 mo) before our study, whereas the remaining patient was started on ursodeoxycholic acid right after the tolerance tests for vitamin D (alone) and vitamin D/TPGS were performed. Four patients were receiving phenobarbital (2–6 mg/kg/d) before and during vitamin D/TPGS therapy.

Baseline evaluation. All patients were admitted to the Clinical Research Center and fasting blood samples were drawn for liver profile (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, albumin, prothrombin time, cholesterol), calcium, magnesium, phosphorus, electrolytes, urea nitrogen, creatinine, and osmolality. All were measured by standard chemistry methods used in the clinical laboratories of Children's Hospital Medical Center. Blood was similarly taken for

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determination of serum vitamin E level and total lipid and serum vitamin D and vitamin D metabolites. Plain x-rays of the wrist and/or knee and bone mineral content measurements were done.

Vitamin D absorption. After an overnight fast, vitamin D₃ (Duphar Nutrition, Inc., Waukegan, IL) 1000 IU/kg (maximum dose of 50 000 IU) was administered orally. Regular diet was resumed 2 h later. Serum vitamin D₃ levels were determined serially at 0, 4, 8, 12, and 24 h as outlined by Lo *et al.* (14). On the next day, after another overnight fast, a mixture of the same dose of vitamin D₃ and TPGS (Liqui-E; Twin Laboratories, Ronkonkoma, NY) 25 IU/kg was administered orally. Regular diet was again resumed 2 h after dosing. Serial measurements of serum vitamin D₃ and vitamin E levels were obtained at 0, 4, 8, 12, 24, and 48 h.

Analytical methods. Serum vitamin D and its metabolites were measured using methods of solid-phase extraction from the serum. Levels were determined by direct UV absorption after separation by HPLC (15). The normal range for total serum 25(OH)D for adults using this technique is 37.5–125 nmol/L (15–50 ng/mL); the normal range for serum 1,25-dihydroxyvitamin D is 60–120 pmol/L (25–50 pg/mL).

Serum vitamin E levels were determined by the fluorometric technique and serum total lipid was measured by colorimetry (16). The ratio of vitamin E to total serum lipid was calculated because vitamin E is known to partition from red cell membranes into serum containing elevated lipid concentrations (17–20). Normal values for serum vitamin E to total lipid ratio for patients less than 12 y old is 0.6 mg/g and for older children, 0.8 mg/g (18, 19).

Plain x-rays of the wrists and/or knees were taken. Bone mineral content was measured by direct single-beam photon absorptiometry of the distal one third of the left radius using a Lunar Bone Mineral (Lunar Corp., Madison, WI) analyzer designed for use in infants and children. A narrow band of low energy radiation (¹²⁵I) and a collimated scintillation detector were moved across the forearm. This method accurately indicates the ash weight of bone. Comparisons were made with age- and sex-specific norms (21).

Follow-up. The patients were discharged while receiving vitamin D₃ 1000 IU/kg mixed with TPGS 25 IU/kg (the same dose used for the tolerance test), given on a once-daily basis. They were followed monthly for the first 3 mo and thereafter every 3 mo, with monitoring of levels of vitamin D and its metabolites, serum levels of vitamin E and total lipid, calcium, magnesium, and phosphorus. Bone mineral content was determined every 6 mo, at which time the wrist and knee x-ray was repeated if a patient was found to have rickets at the start of the study.

Statistical analysis. Results are shown as mean $\pm$ SEM. Statistical analyses included two-tailed *t* test and analysis of variance. Areas under the curve were calculated using the linear/logarithmic trapezoidal rule and were computed using PKCALC (22).

This project was approved by the Institutional Review Board involving Human Subjects of the Children's Hospital Medical Center in Cincinnati. Written informed consent was obtained from parents or guardians and from patients when they were more than 11 y old.

RESULTS

The clinical profile of the study subjects is shown in Table 1. All patients were cholestatic with direct bilirubin from 1.0–27.2 μ mol/L. The patient with a conjugated bilirubin value of 1.0 μ mol/L had persistently high serum levels of cholestyramine (125–200 μ mol/L). Alkaline phosphatase values were all elevated. Serum bilirubin and alkaline phosphatase did not change significantly during the follow-up period, suggesting no significant alteration in the degree of cholestasis in our patients (no difference was noted even in those taking phenobarbital and/or ursodeoxycholic acid). All patients had prothrombin times <2.8 s above control. Five patients had reduced serum albumin; none

had ascites. Serum calcium levels were normal in all but one patient (2.0 mmol/L), who was also hypoalbuminemic (to 32 μ mol/L).

Vitamin D was not detectable in the serum of any of the patients at baseline. Four were taking oral vitamin D, three were receiving 25(OH)D (calcifediol), and one was receiving 1,25-dihydroxyvitamin D (calcitriol). Serum 25(OH)D levels were low (<37.5 nmol/L or 15 ng/mL) in five of the eight patients (Table 2). Of the five, three were receiving vitamin D doses of 400–850 IU/kg, one was receiving calcifediol (with a serum level of 21.2 nmol/L or 8.5 ng/mL), and one was receiving calcitriol. Among the three patients with normal 25(OH)D levels, two were receiving calcifediol and one was receiving vitamin D₃ 97 IU/kg.

Of the eight patients in the study, four were receiving TPGS and had serum vitamin E/total lipid from 0.73 to 1.4 mg/g (Table 3). Of the remaining four subjects, three were being treated with Aquasol E (Rorer Pharmaceutical, Fort Washington, PA) and all had extremely low ratios (0.15–0.21 mg/g). One patient, who had a low serum 25(OH)D level while taking calcifediol, had a low vitamin E to total lipid ratio (0.16 mg/g) while taking TPGS.

Radiographically, seven of the eight patients were osteopenic, although none had changes that were unequivocally consistent with rickets. One patient had a normal bone mineral content, whereas the rest had bone mineral content from 1 to 5 SD below the age-specific norms, with values ranging from 32 to 64% of normal (Table 2). Four of the five subjects with low serum 25(OH)D levels were also osteopenic.

Absorption studies. All patients have previously been shown to be unable to absorb vitamin D₃ by oral vitamin D tolerance test with a dose of 1000 IU/kg. Administration of 1000 IU/kg of vitamin D₃ mixed with TPGS 25 IU/kg resulted in a mean peak rise of serum vitamin D₃ level from baseline of 13.5 $\pm$ 1.8 nmol/L (5.2 $\pm$ 0.7 ng/mL), with a range of 6.0–19.4 nmol/L (2.3–7.5 ng/mL). The rise above baseline with the vitamin D/TPGS mixture was significantly different ($p < 0.001$) from the rise noted when vitamin D₃ was administered alone. Peak concentrations were reached at 4 h in one subject, at 8 h in two, at 12 h in four, and at 24 h in one. The mean vitamin D₃ level was 4.7 $\pm$ 1.3 nmol/L (1.8 $\pm$ 0.5 ng/mL) at 4 h, 10.9 $\pm$ 1.3 nmol/L (4.2 $\pm$ 0.5 ng/mL) at 8 h, 12.4 $\pm$ 2.0 nmol/L (4.8 $\pm$ 0.8 ng/mL) at 12 h, 10.4 $\pm$ 2.0 nmol/L (4.0 $\pm$ 0.8 ng/mL) at 24 h, and 5.2 $\pm$ 2.0 nmol/L (2.0 $\pm$ 0.8 ng/mL) at 48 h (Fig. 1). The mean area under the curve of serum vitamin D₃ was 403.0 $\pm$ 83.1 nmol $\times$ h/L after the patients received the vitamin D/TPGS mixture, which was significantly different from 0 when vitamin D₃ was administered alone ($p < 0.001$). We have previously shown that administration of 1000 IU/kg of vitamin D to noncholestatic controls resulted in a peak rise from baseline of serum vitamin D of 158.0 $\pm$ 25.4 nmol/L or 61.0 $\pm$ 9.8 ng/mL (range 103.6–253.3 nmol/L or 40.0–97.8 ng/mL) at 8–12 h. In one control subject, the peak was reached at 4 h. The area under the curve for the vitamin D tolerance test was 4921 $\pm$ 474 nmol $\times$ h/L (1900 $\pm$ 183 ng $\times$ h/mL) (10).

25(OH)D. One patient underwent orthotopic liver transplantation 2 wk after the absorption study. Seven patients have been followed for at least 3 mo while receiving the vitamin D/TPGS combination. Five of these seven patients, who initially had serum 25(OH)D levels below 37.5 nmol/L (15 ng/mL), were found to have normalized their levels to a range of 37.5–146 nmol/L (15–58 ng/mL) by 1 mo (Table 4). The other two patients, both of whom were taking calcifediol and had normal serum 25(OH)D, maintained normal levels (70–100 nmol/L) after 6 mo of therapy. Six patients available for follow-up have maintained normal serum 25(OH)D concentrations (range 65–134 nmol/L) for at least 6 mo. None of the patients required a decrease in vitamin D dose during the course of therapy with vitamin D₃/TPGS because of high levels of serum 25(OH)D.

Serum vitamin E/total lipid. On follow-up of the three patients previously taking Aquasol E with initial ratios of 0.11–0.30 mg/

Table 1. Patient profile*

Patient	Age (y)	Sex	Diagnosis	Direct bilirubin ($\mu\text{mol/L}$)	Alkaline phosphatase (katal/L)	Calcium (mmol/L)	Albumin ($\mu\text{mol/L}$)	Prothrombin time/control (s)
1	8.5	F	Alagille	7.4	12.9×10^{-6}	2.4	60	14.7/12.9
2†	1.2	F	EHBA	19.0	26.3×10^{-6}	2.5	47	11.0/13.0
3	3.5	F	Alagille	27.2	13.5×10^{-6}	2.2	44	11.9/12.9
4	1.7	F	NSIHC	3.3	6.2×10^{-6}	2.4	57	11.6/12.9
5	19	M	NSIHC	1.0	3.8×10^{-6}	2.4	66	13.9/13.2
6‡	0.4	F	EHBA	3.8	10.5×10^{-6}	2.2	39	15.4/12.9
7§	9.5	F	NSIHC	21.8	6.8×10^{-6}	2.0	32	15.8/13.0
8†	0.8	F	EHBA	20.6	13.5×10^{-6}	2.4	39	12.0/13.0

* Reference values: Direct bilirubin: $<0.34 \mu\text{mol/L}$. Alkaline phosphatase: $2.3\text{--}3.9 \times 10^{-6}$ kat/L, 0–2 y; $3.9\text{--}12.3 \times 10^{-6}$ kat/L, 2–17 y; $0.5\text{--}2.0 \times 10^{-6}$ kat/L, adult. Calcium: $2.1\text{--}2.7$ mmol/L. Albumin: $51\text{--}75 \mu\text{mol/L}$. EHBA, extrahepatic biliary atresia; NSIHC, nonsyndromic intrahepatic cholestasis.

† Transplanted after 6 mo.

‡ Transplanted after 4 mo.

§ Transplanted after 2 wk.

Table 2. Vitamin D profile

Patient	Vitamin D form (dosage)	Serum 25(OH)D (nmol/L)*	Bone mineral content		Rickets
			SD below	% of normal†	
1	D ₂ (400 IU/kg)	28.7	2–4	64	±
2	25(OH)D (2 $\mu\text{g/kg}$)	185.0	4–5	38	–
3	25(OH)D (3.8 $\mu\text{g/kg}$)	21.2	2–3	42	±
4	1,25di(OH)D (0.05 $\mu\text{g/kg}$)	22.5	Normal	Normal	–
5	25(OH)D (1 $\mu\text{g/kg}$)	166.5	2–4	63	–
6	D ₃ (470 IU/kg)	5.0	1–2	32	–
7	D ₃ (97 IU/kg)	41.7	3	52	–
8	D ₂ (850 IU/kg)	19.5	2–3	42	±

* Conversion factor for serum 25(OH)D level: ng/mL (conventional unit) $\times 2.496 = \text{nmol/L}$ (SI unit).

† Values compared with age-specific norms.

Table 3. Vitamin E profile

Patient	Vitamin E form (dosage)	Serum vitamin E/total lipid (mg/g)
1	TPGS (16 IU/kg)	0.79
2	TPGS (20 IU/kg)	0.73
3	TPGS (45 IU/kg)	0.16
4	Aquasol E (48 IU/kg)	0.15
5	TPGS (32 IU/kg)	0.87
6	Aquasol E (75 IU/kg)	0.30
7	TPGS (15 IU/kg)	1.40
8	Aquasol E (69 IU/kg)	0.11

g, serum vitamin E/total lipid values were found to have normalized to $0.6\text{--}1.7$ mg/g at 3 mo (Table 4). Three patients previously receiving TPGS with normal serum vitamin E to total lipid ratios on admission into the study maintained normal ratios. The patient who started with a low ratio (0.16 mg/g) despite previous therapy with TPGS subsequently normalized her ratio (to 0.83 mg/g) at 3 mo. The initially low levels of serum 25(OH)D and vitamin E to total lipid ratio in this patient were attributed to poor compliance (see Table 4, patient 3). Six patients have maintained normal serum vitamin E/total lipid for at least 6 mo.

Bone mineral content. We repeated the bone mineral content determination on six patients at 6 mo and on two patients again at 12 mo. None of them had deterioration (*i.e.* decrease in percentage of normal) in bone density while receiving therapy compared with age-matched control subjects.

DISCUSSION

The aqueous environment of the intestinal lumen poses a major obstacle to the absorption of fat and fat-soluble vitamins.

Formation of mixed micelles is accomplished by interaction of bile acids with lipids, including fat-soluble vitamins. This step is a prerequisite for solubilization of lipophilic substances in an aqueous phase, from which they can be absorbed by enterocytes (23). When micelles carry the lipid-soluble substances toward the absorptive microvillus cell membrane, their collision with the cell membrane results in partitioning of fat-soluble vitamins (but not bile salts) into the monomeric phase, allowing penetration of the lipid cell membrane (24, 25).

Vitamin D is absorbed by enterocytes by a nonsaturable, passive bile salt-dependent diffusion process (26–29). Studies using serial serum measurements of vitamin D after an oral load, as performed in the present study, have shown that normal adults and children have a predictable rise in serum vitamin D, whereas adults and children with malabsorption syndromes, including cholestatic diseases, have reduced postdose serum rises (6, 10, 14). In patients with chronic cholestatic liver disease, intraluminal bile acid deficiency results in malabsorption of fat and fat-soluble substances, including vitamin D. Malabsorption is the primary cause of reduced serum 25(OH)D in adults with primary biliary cirrhosis (30–32) and in children with chronic cholestasis (6). In comparing two groups of patients with extrahepatic biliary atresia, we showed that good postoperative bile flow was associated with normal vitamin D absorption and serum 25(OH)D levels (10). Only one of six patients studied with good postoperative bile flow had a baseline serum 25(OH)D below 37.5 nmol/L (15 ng/mL). These data suggest that significant malabsorption of vitamin D accompanies cholestatic liver disease in most, but not all, pediatric patients. In the present study, all eight patients with severe cholestasis had no measurable rise in serum vitamin D after an oral challenge, with accompanying low levels of serum 25(OH)D and varying degrees of bone undermineralization. It is important to remember, however, that tolerance tests, as carried

Fig. 1. Vitamin D absorption studies. Serum vitamin D concentrations (mean $\pm$ SEM) after an oral load of vitamin D 1000 IU/kg in five control patients (A) and after a test dose of vitamin D 1000 IU/kg mixed with TPGS 25 IU/kg in eight infants and children with chronic cholestasis (B). Broken line in B shows the inability of these eight severely cholestatic patients to absorb vitamin D when it was administered alone. Note difference in scale between the two graphs; peak rise from baseline is 57 ng/mL (147.6 nmol/L) in A, as opposed to 4.8 ng/mL (12.4 nmol/L) in B. Conversion factor for serum vitamin D level: ng/mL (conventional unit) $\times$ 2.59 = nmol/L (SI unit).

out in our study, are a mere reflection of absorption and will therefore be limited by their inability to take into consideration factors beyond their scope, such as tissue distribution and metabolism.

Like vitamin D, vitamin E absorption is dependent upon intraluminal bile acid concentrations exceeding the critical micellar concentration (33). Solubilization of tocopherol by bile salts is a function of micellar size and the less polar bile salts form micelles with vitamin E more easily (34). Recent studies have led to the recognition of a novel form of vitamin E with aqueous solubility. TPGS can form micelles (with a critical micellar concentration of 0.04–0.06 mM), which facilitate tocopherol delivery to the intestinal mucosal cell (11, 13). These critical micellar concentrations are well below those found in the commercially available form, Liqui-E. Results of studies on the role of TPGS in the treatment of vitamin E deficiency in children

with chronic cholestasis have concluded that oral TPGS is safe and efficacious for prolonged usage, especially in the low doses used (13). Experience on its use now includes about 65 patients who have been followed for up to 4–5 y (RJ Sokol, personal communication).

In the present study, we have shown that TPGS, an agent that solubilizes vitamin D by incorporation into micelles, enhances its absorption in the face of intraluminal bile acid deficiency. Although vitamin D absorption was not normalized by solubilizing with TPGS, it was enhanced sufficiently to result in a measurable postdose rise in serum vitamin D level and normalization of serum 25(OH)D concentrations within a month.

Numerous investigations have been conducted in search of better therapy for vitamin D deficiency states in infants and children with chronic cholestasis. Several reports have claimed success in preventing or treating bone disease in cholestatic patients, in whom oral vitamin D was ineffective, with the use of oral 25(OH)-vitamin D (8, 9, 35). We have previously recommended the use of 25(OH)D to prevent bone disease in patients with "failed" portoenterostomy while awaiting orthotopic liver transplantation (10). These patients face the additional risk of decreased formation of skin cholecalciferol because of lack of sun exposure as a result of recurrent hospital admissions for biliary tract infections, sepsis, and/or nutritional complications, all occurring at a crucial time. Therapy with calcifediol may result in a normal serum level of 25(OH)D, but in the case of two patients in our study, was not enough to prevent osteopenia. Furthermore, its use is limited by its cost and dosage preparation (20- and 50- μ g capsules). Roberts *et al.* (3) reported improved bone density in eight of 12 children with cholestatic liver disease when treated with 0.05–0.1 μ g/kg/d of calcitriol. Problems with dosage preparation (0.25- and 0.5- μ g capsules), extremely high cost, and potential toxicity compromise its utility. The availability of an alternate form of therapy, such as vitamin D/TPGS combination, will reduce patients' expenses and allow the use of a liquid compound that can be dispensed and modified from patient to patient. We have shown that changing from calcifediol to this form of vitamin D administration in three subjects allowed them to maintain serum 25(OH)D levels within the normal range and normalize serum 25(OH)D levels in those patients who previously had low levels while receiving vitamin D alone. Follow-up testing of six patients on vitamin D/TPGS therapy did not reveal any fluctuations in serum 25(OH)D level that might lead us to consider seasonal variation as a factor behind this improvement (7, 36).

Based upon our studies, we believe that TPGS forms micellar particles with vitamin D. The solubilized vitamins traverse the unstirred water layer and are released into monomeric phase as the micellar particle comes in contact with the absorbing enterocyte, thereby facilitating their absorption. In the presence of intraluminal bile acid deficiency, we have found that TPGS may play an important role in enhancing the absorption of vitamin D and other fat-soluble vitamins and compounds. Studies have already suggested its utility in enhancing the absorption of cyclo-

Table 4. Follow-up serum 25(OH)D and vitamin E/lipid levels at baseline and on therapy with vitamin D/TPGS*

Patient	Serum 25(OH)D (nmol/L)							Serum vitamin E/lipid (mg/g)						
	0 mo	1 mo	2 mo	3 mo	6 mo	9 mo	12 mo	0 mo	1 mo	2 mo	3 mo	6 mo	9 mo	12 mo
1	28.7	58.2	88.1	149.8	111.8	112.6	93.6	0.79	0.73	0.98	1.03	0.54	1.08	0.79
2†	185.0	114.8	ND	99.8	70.4			0.73	0.42	ND	0.63	0.84		
3	21.2	ND	77.4	128.5	121.8	ND	65.6	0.16	ND	0.49	0.83	0.56	ND	0.54
4	22.5	146.0	118.3	99.1	133.8	105.3	105.8	0.15	1.66	1.43	1.69	1.24	1.26	1.24
5	166.5	103.3	ND	99.8	99.8	94.1	96.8	0.87	1.0	ND	0.75	1.45	0.60	0.68
6‡	5.0	37.5	33.9	103.3				0.30	1.77	1.20	1.61			
8†	19.5	67.4	44.7	59.9	93.6			0.11	0.70	0.45	0.67	0.71		

* See Table 2 for conversion of serum 25(OH)D level from conventional to SI unit. ND, not determined.

† Underwent liver transplantation after 6 mo.

‡ Underwent liver transplantation after 4 mo.

sporin A, a highly lipophilic compound, in the presence of cholestasis or with a long Roux-En-Y loop after liver transplantation (37).

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