

# Human Plasma Transport of Vitamin D after Its Endogenous Synthesis

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## Abstract

Transport of vitamin D<sub>3</sub> from its sites of cutaneous synthesis into the circulation has been assumed to be via the plasma vitamin D binding protein (DBP). We studied vitamin D transport from the skin in seven healthy volunteers who received whole body irradiation with 27 mJ/cm<sup>2</sup> dosage of ultraviolet B light (290–320 nm). Samples of venous blood were collected serially in EDTA and immediately chilled. In KBr, plasma samples were ultracentrifuged to provide a rapid separation of proteins of density < and > 1.3 g/ml. Upper and lower phases and serial fractions were analyzed for vitamin D<sub>3</sub> (extraction, HPLC), cholesterol (enzyme assay), and human DBP (hDBP) (radial immunodiffusion). Total plasma vitamin D (basal level < 1 ng/ml) increased by 10 h and peaked at 24 h (9 ± 1 ng/ml). 98% of the D<sub>3</sub> remained at the density > 1.3 layers for up to 7 d, whereas cholesterol (> 85%) was detected at density < 1.3 and all of the hDBP was at density > 1.3. In three volunteers who each ingested 1.25 mg of vitamin D<sub>2</sub>, the total plasma D<sub>2</sub> increased to 90 ± 32 ng/ml by 4 h, and the D<sub>2</sub> was evenly distributed between the upper and lower layers at 4, 8, and 24 h after the dose, indicating a continuing association of the vitamin with chylomicrons and lipoproteins, as well as with hDBP. Actin affinity chromatography removed D<sub>3</sub> from plasma of irradiated subjects, indicating the association of the D<sub>3</sub> with DBP. These findings indicate that endogenously synthesized vitamin D<sub>3</sub> travels in plasma almost exclusively on DBP, providing for a slower hepatic delivery of the vitamin D and the more sustained increase in plasma 25-hydroxycholecalciferol observed after depot, parenteral administration of vitamin D. In contrast, the association of orally administered vitamin D with chylomicrons and lipoproteins allows for receptor-mediated, rapid hepatic delivery of vitamin D, and the reported rapid but less-sustained increases in plasma 25-hydroxycalciferol. (*J. Clin. Invest.* 1993. 91:2552–2555.) Key words: vitamin D • transport • skin • plasma proteins

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## Introduction

Vitamin D is a precursor of a renal steroid hormone, 1,25-dihydroxyvitamin D (1). This precursor, however, cannot be provided by enzymatic synthesis. Its supply depends on ultraviolet (UV) irradiation of the skin or absorption from the diet. Since few natural foodstuffs contain much vitamin D (2), observers consider the endogenous, cutaneous production of cholecalciferol to be the physiological mechanism of precursor supply (2–5).

Distinct differences are recognized for the cutaneous synthesis vs. diet-derived supply of vitamin D (2, 6). A more efficient and sustained supply of vitamin D is associated with UV irradiation of the skin or parenteral administration of vitamin D (2, 6). In contrast, oral vitamin D consumption leads to rapid but less sustained availability of sterol and a similar pattern of increase in the hepatic metabolite, 25-hydroxycholecalciferol (2, 6–9).

Many studies have addressed the mode of vitamin D transport after oral consumption (6), but no direct studies of the plasma transport of cutaneously-derived vitamin D are reported. It has been assumed that vitamin D synthesized in skin enters the blood stream on the plasma binding protein for vitamin D and its metabolites (DBP)<sup>1</sup> and this assumption is based on in vitro studies of the relative potency of vitamin D in binding to DBP as compared with 7-dehydroxycholesterol, lumisterol, tachysterols<sub>3</sub> and pre-D<sub>3</sub> (10–12). However, other plasma carriers are recognized and some facilitate hepatic (13–15) and hepatocyte (16) entry of vitamin D, presumably via their receptors on hepatocyte plasma membranes. Our study addresses the nature of plasma vitamin D transport after UV irradiation of human volunteers.

## Methods

**Materials.** KBr was purchased from Aldrich Chemical Co. (Milwaukee, WI). Affigel-15 resin was bought from Bio-Rad Laboratories (Richmond, CA). Crystalline vitamins D<sub>3</sub> and D<sub>2</sub> and a cholesterol enzyme assay kit for cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO).

**Volunteers.** The volunteer population was composed of 10 healthy subjects (3 women, 7 men, ages 22–54 y) who had no history of skin, hepatic, or renal disease. None were taking vitamin D, anticonvulsants, or glucocorticosteroids, and all subjects gave their informed consent to the institutional review boards at the University of Pennsylvania School of Medicine or the Jefferson Medical College.

**Cutaneous irradiation.** Seven subjects were exposed to 27 mJ/cm<sup>2</sup> of UV-B light (290–320 nm). Venous blood samples were taken at baseline, 10, 24, 72, and 168 h after the UV-B exposure, immediately

1. Abbreviation used in this paper: DBP, vitamin D binding protein.

transferred to tubes containing 0.2% (wt/vol) EDTA, and placed on ice. Irradiation experiments were done in January 1992.

**Oral dosage.** Three fasting subjects each swallowed a commercial 50,000 IU (1.25 mg) capsule of ergocalciferol, followed by 240 ml of distilled water. Venous blood samples were obtained at baseline, 4, 8, 48 h after the oral dose. Blood was immediately transferred to tubes containing EDTA and placed on ice.

**Sample preparation.** Blood from volunteers was centrifuged within 1 h of collection in a refrigerated centrifuge. Plasma aliquots for total plasma vitamin D analysis were stored at  $-20^{\circ}\text{C}$ . To carry out a rapid separation of plasma proteins of density less than and greater than 1.3 g/ml, the method of Chung et al. was used (17). In brief, unfrozen plasma aliquots (1.5 ml) were dispensed into tubes containing preweighed KBr (513 mg). After mixing, plasma was added to 3.5 ml of 0.15 M NaCl in ultracentrifuge tubes before heat sealing. Tubes were centrifuged at  $10^{\circ}\text{C}$  in a Ti 65.2 vertical rotor at 65,000 rpm in an ultracentrifuge (model L8M; Beckman Instruments, Inc., Fullerton, CA) for 45 min. Fractions were collected via bottom punctures, permitting the isolation of the lower (30% of volume, density  $> 1.3$  g/ml) and upper (70% of volume, density  $< 1.3$  g/ml) phases.

**Analyses.** Quantitation of vitamins  $\text{D}_3$  and  $\text{D}_2$  was carried out as previously reported (18). In brief, organic solvent extracts were applied to HPLC columns and the vitamin D fractions were quantitated by UV absorption spectrometry at 264 nm.

Cholesterol oxidase reagents were used to quantitate total cholesterol in ultracentrifuge fractions by visible spectrometry (Sigma kit).

DBP was quantitated in a radial immunodiffusion assay as reported earlier (19).

## Results

In pilot experiments to assess the validity and reproducibility of the plasma density separations in our laboratory, we analyzed total plasma content, as well as density less than and greater than 1.3 g/ml for DBP and cholesterol. For 16 separate human plasma samples, most of the DBP ( $89 \pm 2.1\%$ , SEM) and cholesterol ( $94 \pm 2.3\%$ , SEM) in plasma was recovered in the density gradient fractions. Table I displays the upper and lower phase distribution of the substances after centrifugation. Fig. 1 depicts the distribution of DBP and cholesterol in continuous ultracentrifuge fractions of fasting human plasma. The DBP is contained in the lower third of the gradient, whereas the cholesterol profile reflects its association with several carriers, including some binding to high-density lipoproteins and density  $> 1.3$  g/ml molecules (18).

At baseline, total plasma cholecalciferol levels were  $< 2$  mg/ml or undetectable (Fig. 2 A). After irradiation, the total

Table I. Distribution of Cholesterol and hDBP in Ultracentrifuged Plasma

Experiment	Density $< 1.30$	Density $> 1.30$
No. 1		
Cholesterol	$87.6 \pm 0.6\%$	$12.5 \pm 0.6\%$
hDBP	$< 5\%$	$> 95\%$
No. 2		
Cholesterol	$85.3 \pm 1.2\%$	$14.7 \pm 1.2\%$
hDBP	$< 5\%$	$> 95\%$

Data are expressed as mean  $\pm$  SEM for eight plasma samples in each experiment.

hDBP, human plasma protein for vitamin D and its metabolites.

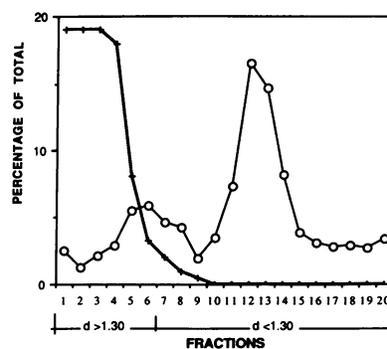


Figure 1. The distribution of plasma cholesterol ( $\circ$ ) and DBP ( $+$ ) in ultracentrifuge density gradient fractions.

plasma cholecalciferol levels increased by 10 h, peaked at 24 h, and decreased by 3 and 7 d.

Fig. 2 B depicts the distribution of cholecalciferol in plasma before and after cutaneous irradiation. As shown, almost all of the baseline and postirradiation plasma cholecalciferol was associated with the fractions of density  $> 1.3$  g/ml fractions.

After the oral dosage of vitamin  $\text{D}_2$ , large increases in plasma vitamin  $\text{D}_2$  were observed (Fig. 3 A). Baseline plasma  $\text{D}_2$  levels were undetectable in two subjects and 1 ng/ml plasma in the other subject. In contrast to the cutaneous irradiation supply of vitamin  $\text{D}_3$ , the plasma vitamin  $\text{D}_2$  levels declined briskly toward baseline by 2 d.

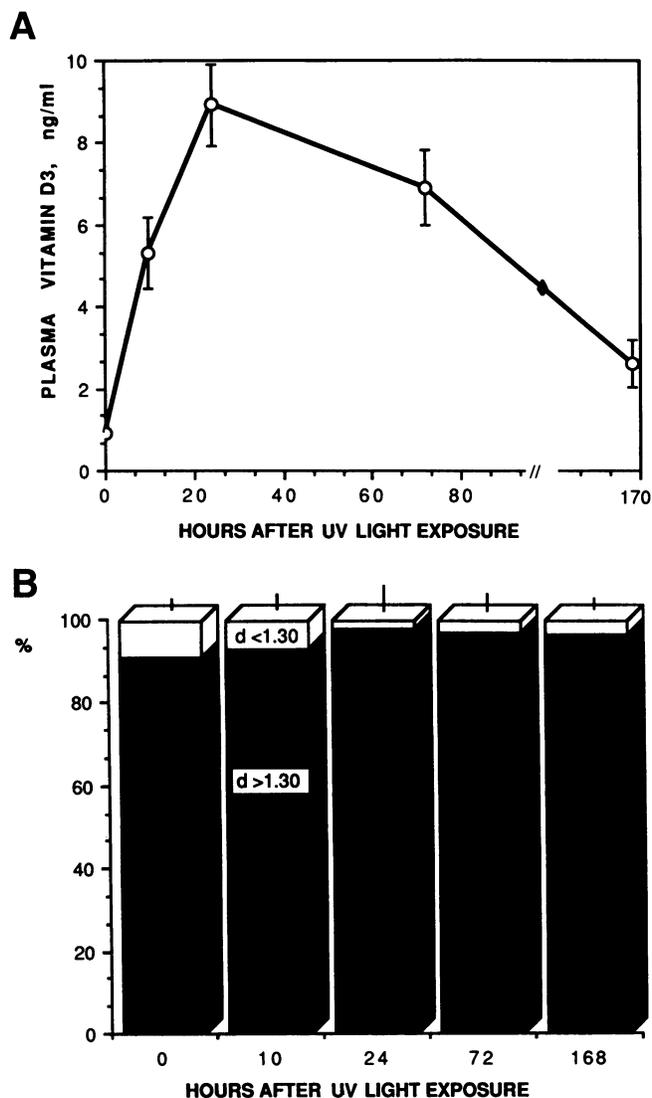
The distribution of orally administered vitamin  $\text{D}_2$  in plasma was very different from that observed after cutaneous production of vitamin  $\text{D}_3$ . As shown in Fig. 3 B, 41% of the plasma  $\text{D}_2$  was found in the fractions of density  $< 1.3$  g/ml at 4 h after the dose. This distribution in the lower density fractions decreased slightly by 8 and 24 h. After this large dose, it is clear that the vitamin is associated with low- and high-density carriers.

When plasma from UV light-treated subjects (24-h samples) was subjected to actin affinity chromatography to remove DBP (20), all of the vitamin D was removed from these plasmas, indicating that the vitamin D was bound to DBP.

## Discussion

Our results indicate that high density or nonlipoprotein carriers are responsible for the blood transport of recently synthesized, endogenous vitamin  $\text{D}_3$ . Since the vitamin  $\text{D}_3$  was removed from these plasmas by actin affinity chromatography, the cutaneously produced vitamin  $\text{D}_3$  was bound to DBP as previously assumed (10–12). Our data conform to the recognized, gradual egress of cutaneously synthesized vitamin  $\text{D}_3$  (2, 6, 21, 22), and underscore the differences between the cutaneous and dietary sources of the vitamin (2).

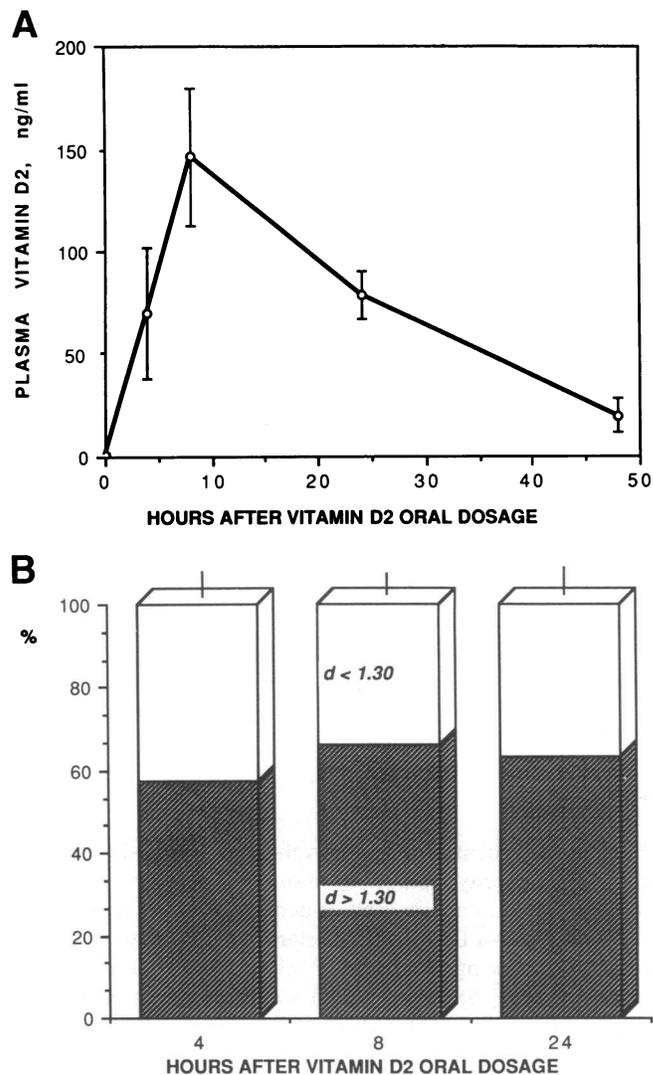
Diet-derived vitamin D requires the components of lipid absorption, as indicated by the association of absorbed vitamin D with chylomicron carriers in chyle (23). When absorbed vitamin D reaches the subclavian vein, some of it is presented to the liver on the chylomicron remnants via specific receptor-mediated uptake and some redistributes to other plasma carriers such as DBP, lipoproteins, and albumin (6, 13, 24–26). Since DBP is present in lymph and chyle, some of the absorbed vitamin D redistributes to DBP during chyle transport (26, 27). The hepatic ingress of vitamin D is modulated by its plasma carriers, and experiments with liver perfusions (14, 15)



**Figure 2.** (A) Total plasma vitamin D<sub>3</sub> concentrations before and after whole body UV-B light irradiation of seven subjects. Mean values and SEM are shown. Vitamin D<sub>2</sub> was not detected (< 1 ng/ml) in any of the samples. (B) The distribution of vitamin D<sub>3</sub> in density gradient fractions of plasma obtained before and after UV irradiation. Vertical bars depict the SEM.

and hepatocytes (16) indicate that carriers with liver membrane receptors (chylomicron remnants, LDL) greatly facilitate hepatic vitamin D uptake. In contrast, albumin and HDL are less facilitative, and vitamin D bound to DBP is least apt to enter the liver or hepatocytes (15, 16).

Many previous reports of vitamin D's disposition in vivo are difficult to reconcile, and this is probably due to the various modes of parenteral vitamin D administration, including organic solvents (15, 28), intralipid (13), plasma (29), and oily depot injections (9). In addition, although DBP is the only stereoselective plasma carrier of vitamin D, most of the other lower affinity carriers are in high concentration in plasma. When simply mixed with plasma directly, vitamin D does associate, to some extent, with non-DBP carriers (15). It seems likely, therefore, that administration of vitamin D intrave-



**Figure 3.** (A) Total plasma vitamin D<sub>2</sub> concentrations after oral dosage to three volunteers. Mean values and SEM are shown. Basal concentrations were undetectable in two of the subjects. (B) The distribution of vitamin D<sub>2</sub> in density gradient fractions of plasma obtained before and after oral dosage of vitamin D<sub>2</sub>. Vertical bars depict the SEM.

nously in solvents (e.g., propylene glycol, ethanol) will result in the vitamin's association with non-DBP carriers to some extent. Since non-DBP transport results in more rapid hepatic uptake of vitamin D (14, 15), the plasma kinetics of administered vitamin D and the subsequent appearance of 25(OH)D in plasma are clearly affected by the modes of plasma transport and the prevailing stores of vitamin D (2, 6, 22).

Comparisons of the bioavailability of vitamin D administered by various routes have been made. Whole body irradiation with effective UV light has resulted in plasma 25(OH)D levels comparable to those observed during oral consumption of 0.25 mg of vitamin D daily (30). Single, intravenous doses of large amounts of vitamin D result in relatively rapid increases of plasma 25(OH)D not unlike results seen after a single, large, oral dose (9). It appears probable that these brisk

25(OH)D responses are due to the initial association of administered vitamin D with non-DBP carriers. Oily depot (intramuscular or subcutaneous) injections of large doses of vitamin D result in a more gradual egress from depot sites and more gradual increases and sustained plasma 25(OH)D levels (9, 31).

Since cutaneous production of vitamin D<sub>3</sub> results in a gradual and more sustained plasma availability of vitamin D and its bioactive product, 25-hydroxyvitamin D, this source is physiologically attuned to the recognized, intense economy of vitamin D stores (2, 6). Rapid hepatic delivery of vitamin D can result in its waste with the appearance of inactive forms, including water-soluble conjugates (2). Our present results clearly indicate that DBP plays a central role in the physiological economy of cutaneously derived vitamin D<sub>3</sub>.

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