Normocalcemia in the Face of Marked Hypervitaminosis D: The Utility of Vitamin D Metabolite Profiling

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CASE DESCRIPTION

An 88-year-old female nursing home resident attended the endocrinology department for routine follow-up of primary hyperparathyroidism. Diagnosed 9 years earlier, she was deemed unsuitable for surgery and treated medically with cinacalcet, a calcimimetic that acts by allosteric activation of the calcium-sensing receptor reducing parathyroid hormone (PTH)[6] secretion by the parathyroid gland (*1*). Several years before her current outpatient attendance, she was diagnosed with vitamin D deficiency (*2*) [serum 25-hydroxyvitamin D (25(OH)D), 10.4 ng/mL (26 nmol/L)] (Table 1). The Endocrine Society Clinical Practice Guideline for the management of vitamin D deficiency considers a 25(OH)D concentration of ≥30 ng/mL (≥75 nmol/L) optimal for bone health (*2*). Vitamin D supplementation was recommended. Cholecalciferol (vitamin D_3) was prescribed (800 IU/day). At clinic, she was medically stable, well hydrated, and normotensive. Her estimated glomerular filtration rate (eGFR) was 30 mL/min/1.73 m^2, indicative of impaired renal function. The eGFR estimation [calculated using the Chronic Kidney Disease–Epidemiology Collaboration (CKD-EPI) formula] met the National Kidney Foundation criteria for chronic kidney disease (CKD) stage 3b, or moderate to severe loss of kidney function (*3*). Measurement of ionized calcium is not routinely available in the outpatient setting and was not requested in this case. However, she was normocalcemic with an adjusted calcium of 9.3 mg/dL (2.33 mmol/L) [reference interval (RI), 8.7–10.0 mg/dL (2.17–2.51 mmol/L)], despite an increased PTH concentration of 213 ng/L (RI, 15–65 ng/L). Total 25(OH)D was determined using LC-MS/MS. Vitamin 25(OH)D_2 and 25(OH)D_3 measured 235 ng/mL (588 nmol/L) and 18.8 ng/mL (47 nmol/L), respectively, providing for a markedly increased total 25(OH)D of 254 ng/mL (635 nmol/L). The hormonally active metabolite of vitamin D, 1,25-dihyroxycholecalciferol (1,25$(OH)_2$D), was borderline increased at 52 pg/mL with an RI of 17 to 50 pg/mL (125 pmol/L; RI, 40–120 pmol/L).

Following medication review, it was determined that 2 months before this clinic visit, the woman

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[6] **Nonstandard abbreviations:** PTH, parathyroid hormone; 25(OH)D, serum 25-hydroxyvitamin D; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; RI, reference interval; 1, 25$(OH)_2$D, 1,25-dihyroxycholecalciferol; UV, ultraviolet; DBP, vitamin D binding protein.

Table 1. Biochemistry from initial presentation in 2007 to 2018.[a]

Parameter	Total calcium	Adjusted calcium	Albumin	Intact PTH	Total 25(OH)D	ALP[b]	Creatinine	eGFR	Cinacalcet
Units	mg/dL	mg/dL	g/dL	ng/L	ng/mL	IU/L	mg/dL	mL/min/1.73 m^2	mg/day
RI	8.8–10.2	8.7–10.0	3.5–5.0	15–65	≥30	35–104	0.50–0.90	>90	NA
Month/year									
5/2007	11.7	11.6	4.6	260	—	129	1.20	43	30
4/2008	12.2	12.6	3.9	209	—	107	1.14	45	30
7/2008	12.0	12.4	3.9	185	—	128	1.04	51	30
8/2008	9.8	10.2	3.6	—	—	93	0.99	54	30
8/2008	10.2	10.7	3.7	—	—	98	1.05	50	30
8/2008	9.7	10.3	3.3	—	—	—	0.97	55	30
10/2008	9.7	9.8	4.3	132	—	157	1.11	47	30
12/2009	9.8	9.7	4.6	138	—	—	—	—	30
7/2011	9.7	9.7	4.4	—	—	109	1.19	42	60
7/2014	8.0	8.1	4.3	358	—	205	1.22	40	60
10/2014	8.9	8.8	4.6	187	10.4	221	1.19	41	30
6/2015	10.1	10.0	4.6	—	45	150	1.41	33	30
8/2015	11.4	11.5	4.4	264	—	142	1.32	36	30
3/2016	9.7	9.6	4.5	—	—	138	1.32	36	30
8/2016	9.3	9.3	4.4	213	254	128	1.54	30	60
1/2017	8.9	8.9	4.4	203	104	139	1.96	22	60
6/2017	9.3	9.2	4.5	343	76	162	1.56	29	60
10/2017	9.4	9.3	4.6	—	67	182	1.66	27	60
3/2018	9.1	9.3	4.1	—	60	—	1.47	31	60

[a] Total 25(OH)D concentration considered optimal for bone health. Adjusted calcium mg/dL × 0.25 to convert to adjusted calcium mmol/L. Adjusted calcium (mg/dL) = total calcium (mg/dL) − [0.576 * albumin (g/dL)] + 2.56 (formula derived/validated locally and accredited to ISO15189); Creatinine mg/dL × 88.42 to convert to µmol/L; eGFR calculated using the CKD-EPI formula.
[b] ALP, alkaline phosphatase; RI, reference interval; NA, not applicable.

had been prescribed ergocalciferol (vitamin D_2) at a dose of 1.25 mg or 50000 IU/day in addition to cholecalciferol (vitamin D_3) at a dose of 20 µg or 800 IU/day.

Five months after cessation of all vitamin D supplementation she remained normocalcemic [adjusted calcium, 8.9 mg/dL (2.23 mmol/L)] with a concomitant PTH of 203 ng/L. Total 25(OH)D remained increased at 104 ng/mL (261 nmol/L), and $1,25(OH)_2D$ and $24,25(OH)_2D$ were raised at 66 pg/mL with an RI of 23 to 58 pg/mL (158 pmol/L; RI, 55–139 pmol/L) and 8.4 ng/mL with an RI of 0.5 to 5.6 (20.1 nmol/L; RI, 1.1–13.5 nmol/L), respectively. The ratio of 25(OH)D to $24,25(OH)_2D$ was 13 and within the RI (7–23) (*4*).

Informed consent was obtained from the patient's legal guardian.

DISCUSSION

Vitamin D refers to a group of fat-soluble secosteroids that play a central role in calcium and phosphate homeostasis and bone metabolism (*5*). There are 2 major forms of vitamin D—vitamin D_2 (ergocalciferol), the precursor form found in plants, and vitamin D_3 (cholecalciferol), the form synthesized on exposure of human and animal skin to ultraviolet (UV) light. To convert vitamin D to its hormonal form necessitates 2 hydroxylation steps. The first occurs in the liver where the

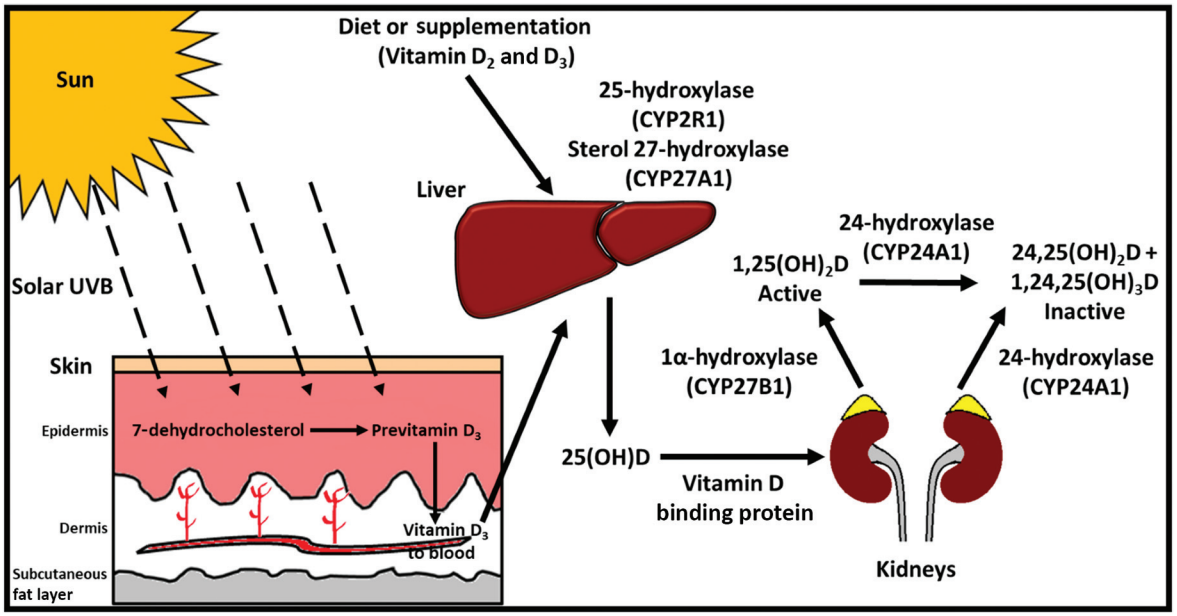

Fig. 1. Vitamin D metabolism.
UVB radiation penetrates the skin, converting 7-dehydrocholesterol to pre-vitamin D_3, which is rapidly converted to vitamin D_3. Vitamin D_3 is transported through the circulation to the liver. Dietary vitamin D_2 and D_3 are transported from the intestine to the liver by chylomicrons (plasma and lymph). In the liver, vitamin D is hydroxylated to 25(OH)D, mediated by CYP2R1 [cytochrome P450 (CYP) enzyme]. Once released into the circulation, 25(OH)D binds to vitamin D binding protein and is transported to the kidneys and other tissues. In the proximal tubules of the kidney, 1α-hydroxylation (CYP27B1) of 25(OH)D results in the production of the active vitamin calcitriol ($1,25(OH)_2D$). $1,25(OH)_2D$ induces the expression of the enzyme 24-hydroxylase encoded by the CYP24A1 gene, which catalyzes the conversion of 25(OH)D and $1,25(OH)_2D$ to the inactive 24-hydroxylated products, $24,25(OH)_2D$ and $1,24,25(OH)_3D$, respectively. Adapted with permission from Griffin et al. (*6*).

enzyme 25-hydroxylase (CYP2R1) converts vitamin D_2 to $25(OH)D_2$ and vitamin D_3 to $25(OH)D_3$. The second occurs in the proximal tubules of the kidney where the enzyme 25(OH)D-1α-hydroxylase (CYP27B1) converts $25(OH)D_2$ and $25(OH)D_3$ to their respective biologically active metabolites, $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$. This physiologically active form of vitamin D binds to the vitamin D receptor in target tissues and regulates gene transcription (*5*). It promotes enterocyte differentiation and intestinal calcium absorption. Together with PTH it induces bone resorption and improves the efficiency of renal calcium reabsorption. The production of $1,25(OH)_2D$ is tightly regulated through the concentrations of calcium, phosphorus, PTH, and $1,25(OH)_2D$ itself. The mitochondrial enzyme 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) is the key regulator in preventing the development of high levels of $1,25(OH)_2D$ (Fig. 1) (*6*). It catalyzes the conversion of 25(OH)D and $1,25(OH)_2D$ into 24-hydroxylated excretion products, thus preventing the activation of 25(OH)D to $1,25(OH)_2D$ and/or degrading $1,25(OH)_2D$ within its target cells to terminate its biological activity (*7*).

In the circulation, 85% to 90% of 25(OH)D is tightly bound to vitamin D binding protein (DBP) and 10% to 15% is loosely bound to albumin with <1% of the total concentration of vitamin D and its metabolites in the free form. For hormones that are ligands for nuclear receptors, there is good evidence to suggest that free or bioavailable hormones are physiologically more relevant than their

total concentration (*8*). For example, in liver disease, free 25(OH)D metabolite levels were found to be normal despite low total 25(OH)D concentrations. Therefore, valid estimates or direct measurement of free 25(OH)D metabolites may provide a better measure of the true vitamin D status than measuring total concentrations. However, this necessitates robust, precise, and standardized methods for the measurement of serum DBP, free, and total 25(OH)D metabolites. Thus far, assays for DBP and free 25(OH)D metabolites have proven to be technically challenging and lack standardization (*8*).

Availability of newly developed high-throughput direct free 25(OH)D assays (e.g., Future Diagnostics Solutions) has the potential to change future laboratory practice with respect to assessing vitamin D status (*8*).

The accumulated data on free vitamin D from the mid 1980s to date are contradictory and of questionable clinical value because of the myriad of different techniques used to measure DBP, total 25(OH)D, and the heterogeneity of the study populations used to assess clinical utility. Notwithstanding, free vitamin D is strongly correlated to total 25(OH)D in most normal populations and inversely related to PTH (*8*).

In contrast, there are well-validated and approved methods for the measurement of total 25(OH)D and $1,25(OH)_2D$ (*9*). The half-life of 25(OH)D is 2 to 3 weeks as compared with 4 to 6 h for $1,25(OH)_2D$ and better reflects the contribution of diet and cutaneous synthesis. Hence, the measurement of the major circulating form of vitamin D, 25(OH)D, is still considered the best biochemical indicator of vitamin D nutritional status. Accurate assessment of a patient's vitamin D status mandates the use of an assay with equimolar recognition and recovery of both the D_2 and D_3 metabolites. In our laboratory, this is achieved using LC-MS/MS to measure total 25(OH)D concentration (the sum of the individually measured $25(OH)D_2$ and $25(OH)D_3$ metabolites).

Recently, the measurement of the metabolite $24,25(OH)_2D$ and the use of the ratio of serum 25(OH)D to $24,25(OH)_2D$ concentration have been promulgated as useful biochemical tools to aid the assessment of vitamin D catabolic status. For example, in the case of persistent hypercalcemia, suppressed PTH, and increased $1,25(OH)_2D$ levels, a raised $25(OH)D/24,25(OH)_2D$ ratio would be consistent with a CYP24A1 loss of function mutation (genetic cause). In contrast, these same data with a normal $25(OH)D/24,25(OH)_2D$ ratio would be indicative of an iatrogenic cause.

In vitamin D-deficient patients, the required intake of vitamin D depends on the shortfall of exposure to effective UV radiation. It is not known what the safe upper value for 25(OH)D is for avoiding hypercalcemia. The Institute of Medicine considers supplementation of 4000 IU of vitamin D per day for children older than 9 years and all adults as the safe upper limit for healthy persons (*2*, *10*). It is advised that persons receiving in excess of 5000 IU/day have their 25(OH)D levels measured at 3 months after initiation of supplementation.

Excess vitamin D can cause severe and life-threatening hypercalcemia consequent to increased intestinal calcium absorption and induction of bone resorption. However, vitamin D toxicity is considered a rare event invariably caused by inadvertent or intentional ingestion of excessively high amounts of vitamin D. The clinical history, medical examination, and biochemical results for our patient were unremarkable for vitamin D toxicity despite the reported total concentration of 25(OH)D of 254 ng/mL (635 nmol/L) being 5-fold the 25(OH)D level of 50 ng/mL (125 nmol/L) at which it is suggested that toxicity can occur (*10*). As the patient was normocalcemic when vitamin D was measured, no active treatment was undertaken. However, all vitamin D supplementation was immediately discontinued. The laboratory findings of normocalcemia, marked hypervitaminosis D, together with the normal $25(OH)D/24,25(OH)_2D$ ratio, prompted careful

review of the patient's medications and supplements, both prescribed and over-the-counter. This found that her daily vitamin D intake was 50 800 IU for the preceding 2 months. The highest daily intake of vitamin D considered to cause no side effects in humans when used indefinitely without medical supervision is 4000 IU/day. The No Observed Adverse Effects Level (NOAEL) is set at 10 000 IU/day, as vitamin D doses <10 000 IU/day are not usually associated with toxicity, whereas doses ≥50 000 IU/day for several weeks or months are frequently associated with toxic side effects, including documented hypercalcemia (*11*).

Multidisciplinary discussions found that the likely mechanisms preventing hypercalcemia developing in this patient were multifactorial: upregulation of CYP24A1 (the enzyme that degrades 25(OH)D and 1,25$(OH)_2$D) as part of the normal physiological protective response to prevent vitamin D toxicity, the dose of the calcimimetic drug cinacalcet, and renal impairment (CKD stage 3b) leading to reduced α-hydroxylation of 25(OH)D to its metabolically active form, 1,25$(OH)_2$D. Many authorities advise use of alfacalcidol in CKD at stage 3 severity. The use of ergocalciferol, even in toxic doses as in this case, may have produced an attenuated metabolic response because of the coexistence of moderate stage 3b CKD. Moreover, a possible contributor to the inadvertent prescription of high doses of vitamin D_2 was relatively unclear advice in the relevant prescribing information. Ultimately, greater attention should be paid to dosing units (i.e., μg vs mg) and dose selection, especially for elderly patients who may have decreased hepatic, renal, and cardiac function. In this case, vitamin D metabolite profiling was a useful adjunct in determining the cause of this patient's hypervitaminosis D.

TAKEAWAYS

- Vitamin D metabolism is complex.
- 25(OH)D is the best indicator of vitamin D status.
- Accurate 25(OH)D measurement requires equimolar recovery of D_2 and D_3 metabolites.
- Consider vitamin D metabolite profiling as a diagnostic tool.
- Consider the risks of medication errors when prescribing vitamin D supplementation. Specifically paying greater attention to dosing units (i.e., μg vs mg) and dose selection especially for elderly patients who may have decreased hepatic, renal, and cardiac function.

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